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Synthesis of novel cyanine dyes containing carbamoylethyl component – Noncovalent labels for nucleic acids detection

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Abstract

Fourteen novel mono-, di- and tricationic 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 carbamoylethyl substituents. All derivatives absorb in the region 428–515 nm and have a molar absorptivity of 58 000–91 000 l. mol^{-1}. cm^{-1}. The reaction products were characterized by ^{1}H-NMR spectroscopy and elemental analysis.

Keywords: Monomeric; Cationic monomethine cyanine dyes; Carbamoylethyl substituents; Nucleic acids

1. Introduction

Modification of proteins, DNA and other biopolymers by labeling them with reporter molecules has become a very powerful research tool in molecular biology. In addition, there are a growing number of commercial applications of these modified biomolecules including clinical immunoassay, DNA hybridization tests, gene fusion detection tests, etc. 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 [1,2]. The growing scientific and commercial interest in the field of synthesis and application of cyanine dyes as bio-probes [3–6] and our investigation of this area [7–12] have prompted us to search for new intermediates [13,14] and cyanine dyes [15].

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 in MeOH). 4,7-Dichloroquinoline, acrylamide, acetonitryl, diacetone alcohol, cyclohexanole, 1,3-dibromopropane, *N*-ethyldiisopropylamine, triethylamine, pyridine, 2-methylbenzothiazole and 1,4-diazabicyclo[2.2.2]octane (**DABCO**) are commercially obtained products.

2.1. Synthesis of dyes *8a–8l*

Appropriate amounts of **3a–3d** (Table 1, Schemes 5 and 6), and of intermediates **5a–5g** (for the preparation of dyes **8a–8i**) or **7a–7c** (for dyes **8j–8l**) were finely powdered together and suspended in 8–30 ml methanol in a reaction vessel, equipped with electromagnetic stirrer. Double excess of *N*-ethyldiisopropylamine was added dropwise for about 1 min and the reaction mixture was stirred at room temperature

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Table 1
Reaction conditions, melting points and yields of dyes **8a**–**8l**, **9a** and **9b**

Product no	Starting compounds (mmol/no)	Solvent (ml)/net $(iPr)_2$ (mmol)	Reaction time	Yield (%)	M.p. (°C)
8a	2.50/**3a** and 2.50/**5a**	10 MeOH/5.00	2 h (rt)	67	266–268
8b	3.32/**3a** and 3.65/**5c**	20 MeOH/6.64	2 h (rt)	89	273–275
8c	3.32/**3a** and 3.65/**5e**	15 MeOH/6.64	2 h (rt)	56	241–243
8d	6.85/**3b** and 7.5/**5f**	15 MeOH/13.7	2 h (rt)	51	199–200
8e	2.48/**3c** and 2.73/**5g**	10 MeOH/4.96	1 h (rt)	62	241–243
8f	3.72/**3c** and 4.50/**5b**	15 MeOH/7.44	2 h (rt)	63	271–272
8g	7.45/**3c** and 9.68/**4e**	30 MeOH/14.9	1 h (rt)	72	229–230
8h	2.4/**3d** and 2.86/**5b**	25 MeOH/4.8	1 h (rt)	74	202–204
8i	3.5/**3b** and 4.2/**5d**	10 MeOH/7.1	2 h (rt)	71	220–223
8j	10.7/**3a** and 12.8/**7a**	15 MeOH/21.4	2 h (rt)	52	242–244
8k	1.2/**3a** and 1.2/**7b**	8 MeOH/2.40	30 min (rt)	93	259–261
8l	6.94/**3a** and 8.7/**7c**	15 MeOH/13.9	1 h (rt)	77	
9a	1.1/**8i** and 8.0/ **DABCO**	8 $HO(CH_2)_2OH$ and 10 $CH_3O(CH_2)_2OH$	6 h (reflux)	54	204–206
9b	2.6/**8i** and 3.43/mono-methylated **DABCO** (Scheme 7)	8 $HO(CH_2)_2OH$ and 10 $CH_3O(CH_2)_2OH$	6 h (reflux)	60	185–187

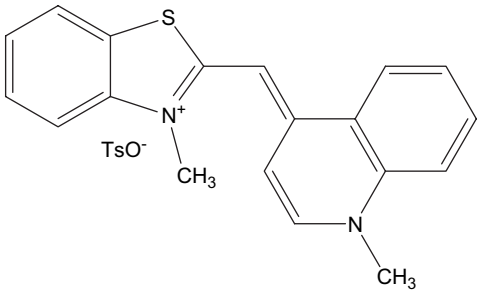
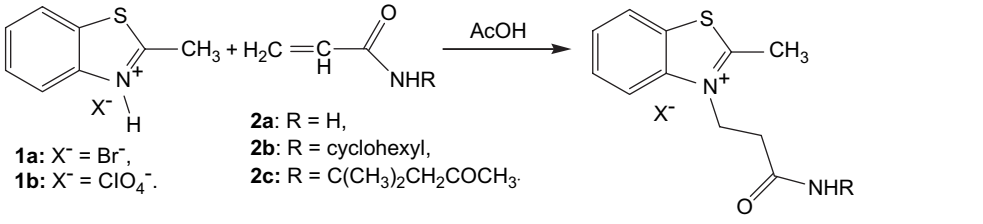


Fig. 1. Molecular structure of the asymmetric monomethine cyanine dye Thiazole Orange.

for 2–7 h. The formed precipitate was suction filtered, washed with methanol and air-dried. Dyes **5a**–**5i** and **8** were recrystallized several times from methanol.

2.2. Synthesis of dyes *9a* and *9b*

Appropriate amounts of **8i** and **DABCO** or 1-methyl-4-aza-1-azonia-bicyclo[2.2.2]octane were finely powdered together. The mixture was suspended in ethyleneglycol/methoxyethanol – 8/10 ml (boiling at 140–145 °C) (Table 1) – in the reaction vessel, equipped with reflux condenser and mechanical stirrer. The reaction mixture was vigorously stirred and refluxed for 6 h (TLC monitoring). After cooling to room temperature, 40 ml diethyl ether was added and the precipitated dye was suction filtered and air-dried. Dyes **9a** and **9b** were recrystallized several times from ethanol.

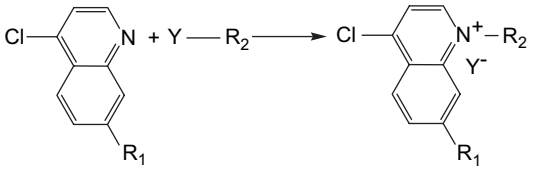


1a: X^- = Br^-,
1b: X^- = ClO_4^-.

2a: R = H,
2b: R = cyclohexyl,
2c: R = $C(CH_3)_2CH_2COCH_3$.

3a: R = H, X^- = Br^-,
3b: R = H, X^- = ClO_4^-,
3c: R = cyclohexyl, X^- = ClO_4^-,
3d: R = $C(CH_3)_2CH_2COCH_3$, X^- = ClO_4^-.

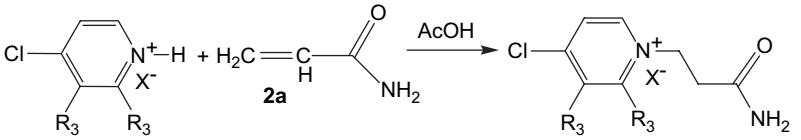
Scheme 1. Synthesis of starting compounds **3a**–**3d**.



4a: R_1 = H,
4b: R_1 = Cl.

5a: R_1 = H, R_2 = CH_3, Y = $CH_3SO_4^-$,
5b: R_1 = Cl, R_2 = CH_3, Y = $CH_3SO_4^-$,
5c: R_1 = H, R_2 = CH_2Ph, Y = Br^-,
5d: R_1 = H, R_2 = $(CH_2)_3Br$, Y = Br^-.

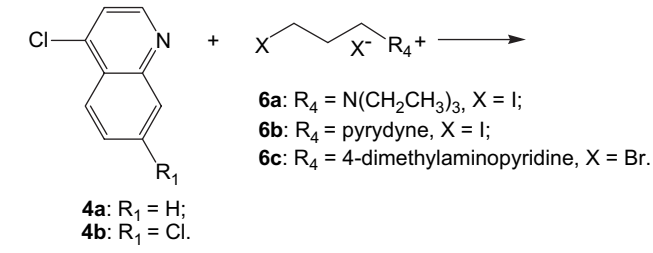
Scheme 2. Synthesis of intermediates **5a**–**5d**.



4a: R_3 = CH=CH-CH=CH, X^- = ClO_4^-;
4b: R_3 = CH=CH-CCl=CH, X^- = ClO_4^-;
4c: R_3 = H, X^- = Cl^-.

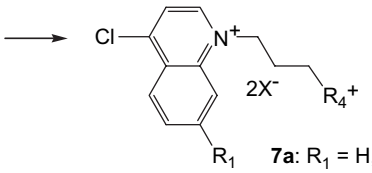
5e: R_3 = CH=CH-CH=CH, X^- = ClO_4^-;
5f: R_3 = CH=CH-CCl=CH, X^- = ClO_4^-;
5g: R_3 = H, X^- = Cl.

Scheme 3. Synthesis of intermediates **5e**–**5g**.



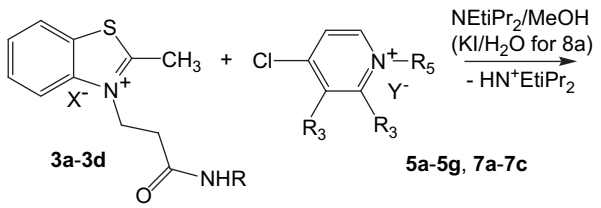
6a: R_4 = $N(CH_2CH_3)_3$, X = I;
6b: R_4 = pyrydyne, X = I;
6c: R_4 = 4-dimethylaminopyridine, X = Br.

4a: R_1 = H;
4b: R_1 = Cl.



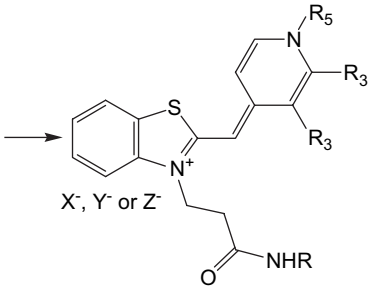
7a: R_1 = H, R_4 = $N(CH_2CH_3)_3$, X = I;
7b: R_1 = H, R_4 = pyridine, X = I;
7c: R_1 = C1, R_4 = 4-dimethylaminopyridine, X = Br.

Scheme 4. Synthesis of starting compounds **7a**–**7c**.



3a-3d

5a-5g, **7a-7c**



8a: R = H, R_3 = -$(CH=CH)_2$-, R_5 = CH_3, Z = I;
8b: R = H, R_3 = -$(CH=CH)_2$-, R_5 = CH_2Ph, X = Br;
8c: R = H, R_3 = -$(CH=CH)_2$-, R_5 = $(CH_2)_2CONH_2$, X = ClO_4^-;
8d: R = H, R_3 = CH=CH-CCl=CH, R_5 = $(CH_2)_2CONH_2$, X = ClO_4^-;
8e: R = cyclohexyl, R_3 = H, R_5 = $(CH_2)_2CONH_2$, X = ClO_4^-;
8f: R = cyclohexyl, R_3 = CH=CH-CCl=CH, R_5 = CH_3, X = ClO_4^-;
8g: R = cyclohexyl, R_3 = -$(CH=CH)_2$-, R_5 = $(CH_2)_2CONH_2$, X = ClO_4^-;
8h: R = $C(CH_3)_2CH_2COCH_3$, R_3 = CH=CH-CCl=CH, R_5 = CH_3, X = ClO_4^-;
8i: R = H, R_3 = -$(CH=CH)_2$-, R_5 = $(CH_2)_3Br$, X = ClO_4^-.

Scheme 5. Preparation of dyes **8a**–**8i**.

Table 2
Structures, names and ^{1}H NMR spectra of dyes **8a–8l**, **9a** and **9b**

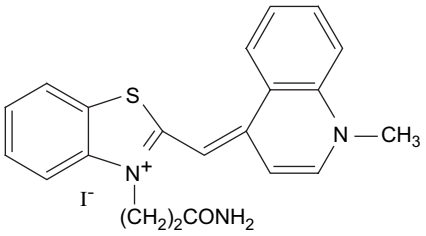
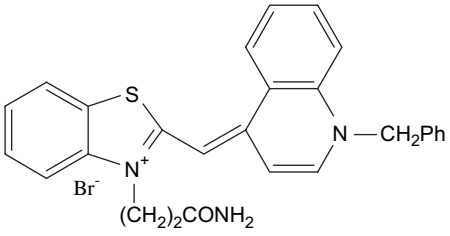
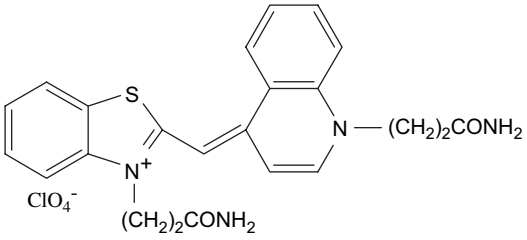
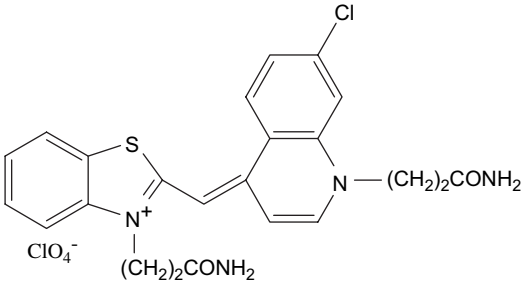
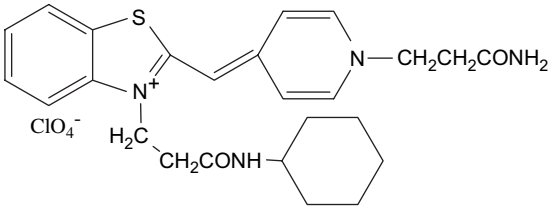
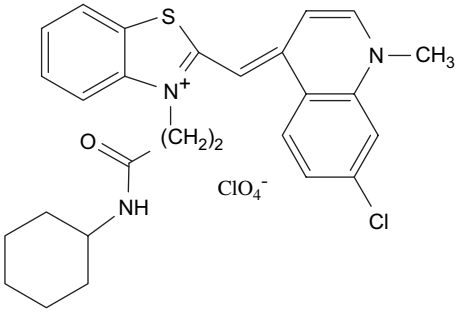
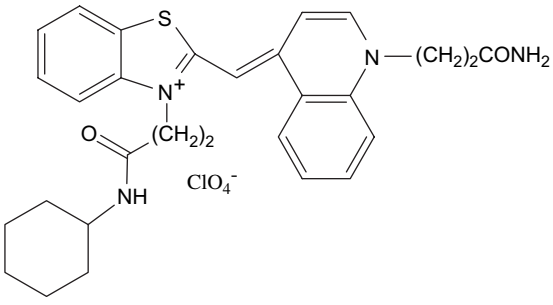
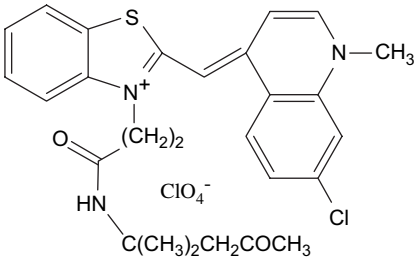
Product no	Structure/name	^{1}H NMR (DMSO-d_6, δ (ppm))
8a		2.72 t (2H, CH_2CO), 4.18 s (3H, N^+CH_3), 4.79 t (2H, N^+CH_2), 7.13 s (2H, CH + NH), 7.36–8.81 m (11H, Ar + NH)
	1-Methyl-4-[(3-(2-carbamoylethyl)-3*H*-benzothiazol-2-ylidene) methyl]-quinolinium iodide	
8b		2.73 t (2H, CH_2CO), 4.84 t (2H, N^+CH_2), 5.90 s (2H, CH_2Ph), 7.19 s (1H, CH), 7.39 d (2H, NH_2), 7.31–8.85 m (15H, Ar)
	1-Benzyl-4-[(3-(2-carbamoylethyl)-3*H*-benzothiazol-2-ylidene) methyl]-quinolinium bromide	
8c		2.69–2.77 m (4H, 2 × CH_2CO), 4.81 br s (4H, 2 × N^+CH_2), 7.13 s (1H, CH), 7.30 d (2H, NH_2), 7.34–8.80 m (10H, Ar)
	1-(2-Carbamoylethyl)-4-[(3-(2-carbamoylethyl)-3*H*-benzothiazol-2-ylidene)methyl]-quinolinium perchlorate	
8d		2.71–2.77 m (4H, 2 × CH_2CO), 4.75–4.83 m (4H, 2 × CH_2), 7.12 s (1H, CH), 7.29 (2H, NH_2), 7.30–8.80 m (9H, Ar)
	1-(2-Carbamoylethyl)-4-[(3-(2-carbamoylethyl)-3*H*-benzothiazol-2-ylidene)methyl]-7-chloroquinolinium perchlorate	

Table 2 (*continued*)

Product no	Structure/name	^{1}H NMR (DMSO-d_6, δ (ppm))
8e		0.95–1.16 m (6H, CH_2), 1.51–1.60 m (4H, CH_2), 2.70 t (4H, 2 × CH_2CO), 4.84 t (4H, 2 × N^+CH_2), 6.99 s (1H, CH), 7.47–8.23 m (9H, Ar + NH)
	1-(2-Carbamoylethyl)-4-[(3-(2-cyclohexylcarbamoylethyl)-3*H*-benzothiazol-2-ylidene)methyl]pyridimium perchlorate	
8f		0.85–1.08 m (6H, CH_2), 1.44–1.48 m (4H, CH_2), 2.65 t (CH_2CO), 3.40–3.44 m (1H, *CH*NH), 4.10 s (3H, N^+CH_3), 4.79 t (2H, N^+CH_2), 6.95 s (1H, CH), 7.24–8.74 m (10H, Ar + NH)
	1-Methyl-4-[(3-(2-cyclohexylcarbamoylethyl)-3*H*-benzothiazol-2-ylidene)methyl]-7-chloroquinolinium perchlorate	
8g		0.89–1.07 m (6H, CH_2), 1.44–1.49 (4H, CH_2), 2.66 t (2H, CH_2CO), 2.74 t (2H, CH_2CO), 3.35–3.50 m (1H, *CH*NH), 4.80 t (4H, 2 × N^+CH_2), 6.99 s (1H, CH), 7.23 d (2H, NH_2), 7.31–8.74 m (11H, Ar + NH)
	1-(2-Carbamoylethyl)-4-[(3-(2-cyclohexylcarbamoylethyl)-3*H*-benzothiazol-2-ylidene)methyl]quinolinium perchlorate	
8h		1.10 s (6H, $C(CH_3)_2$), 1.77 s (3H, CH_3CO), 2.49 t (2H, *CH*$_2$CONH), 2.70 s (2H, $COCH_2$), 4.08 s (3H, N^+CH_3), 4.74 t (2H, N^+CH_2), 7.00 s (1H, CH), 7.20–8.75 m (10H, Ar + NH)
	1-Methyl-4-[(3-(2-(1,1-dimethyl-3-oxobutylcarbamoyl)ethyl)-3*H*-benzothiazol-2-ylidene)methyl]-7-chloroquinolinium perchlorate	

(*continued on next page*)

Table 2 (*continued*)

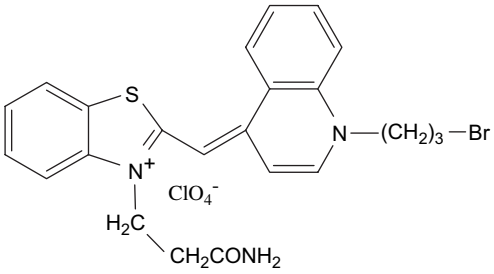
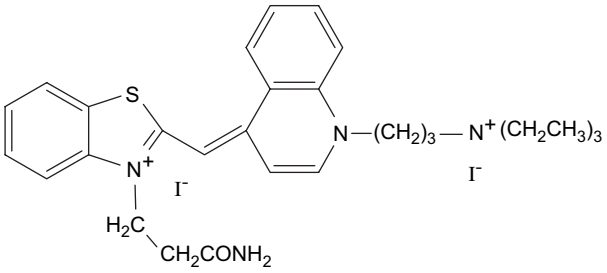
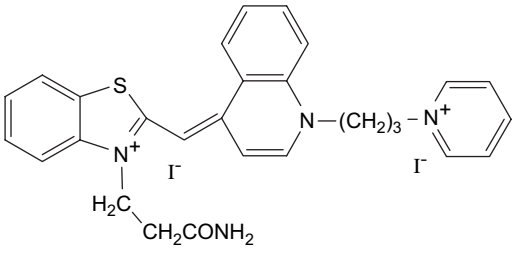
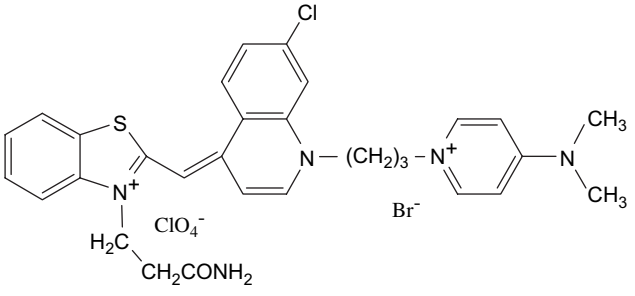
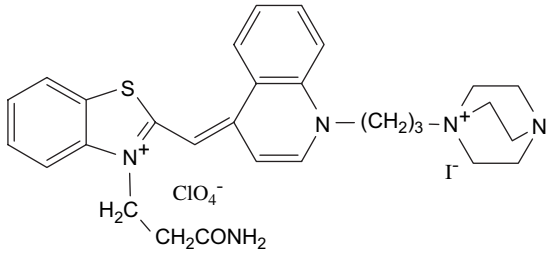
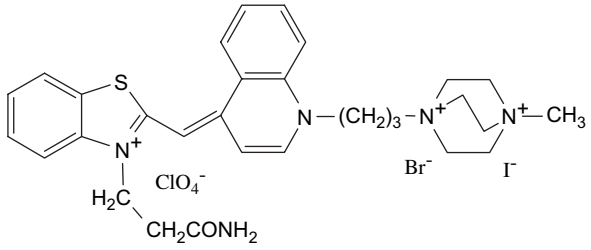
Product no	Structure/name	^{1}H NMR (DMSO-d_6, δ (ppm))
8i		2.42 t (2H, CH_2), 2.72 t (2H, CH_2Br), 3.65 t (2H, CH_2CO), 4.70 t (2H, N^+CH_2), 4.80 t (2H, N^+CH_2), 7.13 s (1H, NH), 7.14 s (1H, CH), 7.37–8.80 m (11H, Ar + NH)
	1-(3-Bromopropyl)-4-[(3-(2-carbamoylethyl)-3*H*-benzothiazol-2-ylidene)methyl]quinolinium perchlorate	
8j		1.20 t (9H, CH_3), 2.21 br s (2H, CH_2), 2.73 t (2H, CH_2CO), 3.25 q (6H, N^+CH_2), 3.35 t (2H, N^+CH_2), 4.68 t (2H, N^+CH_2), 4.80 t (2H, N^+CH_2), 7.18 s (1H, CH), 7.29 d (2H, NH_2), 7.40–8.80 m (10H, Ar)
	1-[3-(*N*-triethylammonio)propyl]-4-[(3-(2-carbamoylethyl)-3*H*-benzothiazol-2-ylidene)methyl]quinolinium diiodide	
8k		2.52 t (2H, CH_2), 2.58 t (2H, CH_2CO), 4.72 t (2H, N^+CH_2), 4.82 t (4H, 2 × N^+CH_2), 7.16 s (1H, CH), 7.36 d (2H, NH_2), 7.37–9.12 m (15H, Ar)
	1-(3-Pyridiniopropyl)-4-[(3-(2-carbamoylethyl)-3*H*-benzothiazol-2-ylidene)methyl]quinolinium diiodide	
8l		2.38 t (2H, CH_2), 2.72 t (2H, CH_2CO), 3.16 s (6H, $N(CH_3)_2$), 4.33 t (2H, N^+CH_2), 4.60 t (2H, N^+CH_2), 4.83 t (2H, N^+CH_2), 7.13 s (1H, CH), 7.34 d (2H, NH_2), 7.00–8.80 m (13H, Ar)
	1-[3-(4-Dimethylammonio)propyl]-4-[(3-(2-carbamoylethyl)-3*H*-benzothiazol-2-ylidene)methyl]-7-chloroquinolinium perchlorate bromide	

Table 2 (*continued*)

Product no	Structure/name	^{1}H NMR (DMSO-d_6, δ (ppm))
9a		2.32 br s (2H, CH_2), 2.73 br s (2H, CH_2CO), 3.02 br s (6H, NCH_2–**DABCO**), 3.30 br s (6H, N^+CH_2, **DABCO**), 3.42 (2H, N^+CH_2), 4.60 br s (2H, N^+CH_2), 4.77 br s (2H, N^+CH_2), 7.10 s (1H, CH), 7.35–8.75 m (12H, Ar + NH_2)
1-[3-(1-Azonia-1,4-diazabicyclo[2.2.2]octane)propyl]-4-[(3-(2-carbamoylethyl)-3*H*-benzothiazol-2-ylidene)methyl]quinolinium perchlorate iodide		
9b		2.31 br s (2H, CH_2), 2.67 t (2H, CH_2CO), 3.45–3.66 m (12H, N^+CH_2–**DABCO**), 3.89 s (3H, N^+CH_2), 4.66 t (2H, N^+CH_2), 4.80 t (2H, N^+CH_2), 7.15 s (1H, CH), 7.03–8.80 m (12H, Ar + NH_2)
1-[3-(*N*-(1-Methyl-1,4-diazoniabicyclo[2.2.2]octane))propyl]-4-[(3-(2-carbamoylethyl)-3*H*-benzothiazol-2-ylidene)methyl]quinolinium perchlorate bromide iodide		

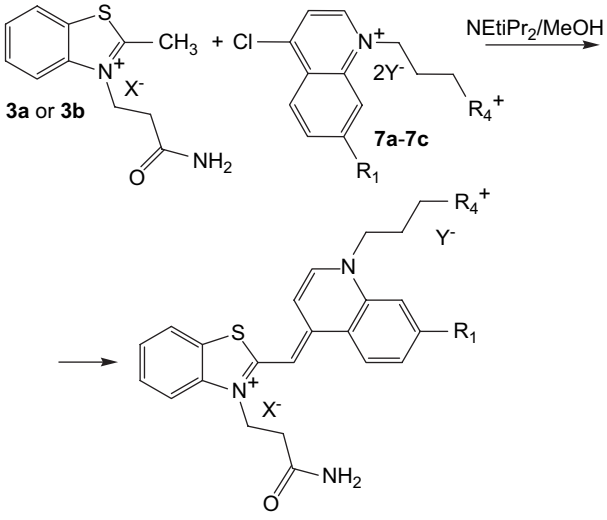
3. Results and discussion

Lee et al. [16,17] have shown that the monomethine cyanine dye, Thiazole Orange (TO) (Fig. 1), has excellent properties as a biological noncovalent DNA or RNA label.

This finding stimulates the research in the field of cyanine dyes as nucleic acid stains and many representatives of this new class of dyes, covering the spectrum from blue to the near infrared region, are developed.

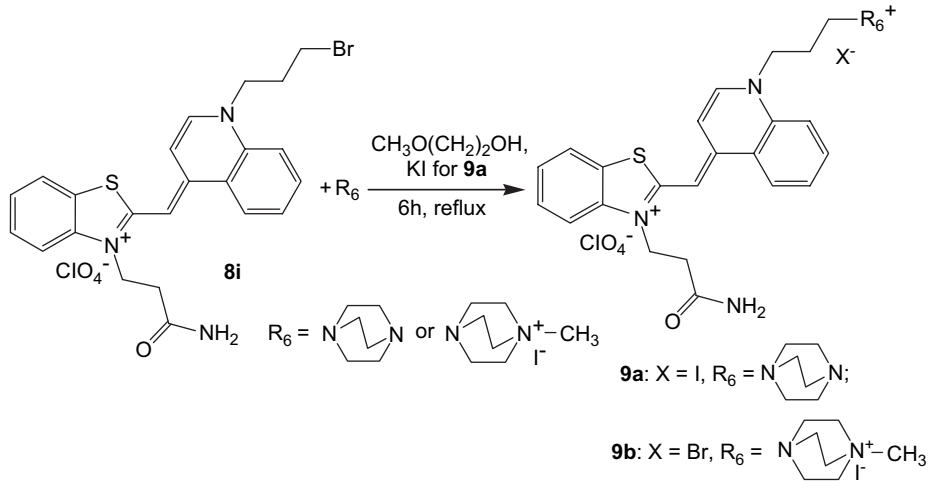
Thiazole Orange has a 14-fold higher absorption coefficient at about 509 nm than ethidium bromide and has a characteristic of monointercalator with strong enhancement of the fluorescence on binding to ds DNA. It has been shown that the fluorescence enhancement of TO on binding to ds DNA is over 1000-fold [16]. This dye has proven to have suitable physical properties for flow cytometric analysis of blood reticulocytes. The dye does not fluoresce until intercalated with DNA or RNA and after binding with DNA, it has a fluorescence quantum yield of 0.2. It is cell membrane permeable and absorbs at 509 nm, which allows its excitation with one of the lines of the argon laser. This fluorogenic dye is also useful in monitoring the growth and multiplication of malarial parasites in vitro [18]. Rye et al. [19] have proven that TO is 50-fold more sensitive than the most common ethidium bromide dye. These valuable properties have attracted our attention and provoked us to search for new representatives of TO-family.

TO and related dyes can be synthesized according to the method of Brooker et al. [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 sulphur and nitrogen atoms in the quaternized 2-alkylthio starting materials, which



8j: R_1 = H, R_4 = NEt_3, X = Y = I;
8k: R_1 = H, R_4 = pyridine, X = Y = I;
8l: R_1 = Cl, R_4 = 4-dimethylaminopyridine, X = ClO_4, Y = Br.

Scheme 6. Preparation of dyes **8j**–**8l**.



Scheme 7. Synthesis of dyes **9a** and **9b**.

lead to unexpected reaction products [21,22]. To avoid the aforementioned disadvantages we used an improved procedure. The original method has been patented [23] for the synthesis of asymmetric and symmetric monomethine cyanine dyes by the condensation of quaternized 2-chloro-heterocycles with quaternized 2- or 4-methyl-heterocyclic compounds in the presence of basic agents such as triethylamine. The method does not involve the evolution of methylthiol and offers more synthetic possibilities. The relatively easy preparation of the intermediate 4-chloroquinoline **4a** by the published method was used [24].

We prepared the starting compounds **3a**–**3d** (Scheme 1) by the quaternization of 2-methylbenzothiazolium hydrobromide **1a** or hydroperchlorate **1b** with acrylamides **2a**–**2c** in acetic

Table 3
Spectral characteristics and elemental analysis of dyes **8a**–**8l**, **9a** and **9b**

Dye no	λ_{max} nm (ε l mol^{-1} cm^{-1})	Molecular formulae (Mm)	Analysis		
			C%	H%	N%
			Calculated Found		
8a	503 (87 550)	$C_{21}H_{20}IN_3O_5S \cdot 0.5H_2O$ (498.38)	50.61 50.54	4.30 5.00	8.43 8.82
8b	507 (90 500)	$C_{27}H_{24}BrN_3OS$ (518.47)	62.55 62.46	4.67 5.14	8.10 8.03
8c	505 (81 800)	$C_{23}H_{23}ClN_4O_6S$ (518.97)	53.23 53.20	4.47 4.76	10.80 10.40
8d	512 (64 900)	$C_{24}H_{22}Cl_2N_4O_6S$ (553.41)	49.92 49.62	4.01 4.39	10.12 9.89
8e	428 (58 400)	$C_{25}H_{31}ClN_4O_6S$ (551.06)	54.49 54.65	5.67 5.83	10.17 –
8f	511 (57 800)	$C_{28}H_{30}C_{l2}N_3O_5S$ (578.51)	56.06 55.89	5.05 4.95	7.26 –
8g	505 (75 600)	$C_{29}H_{33}ClN_4O_6S$ (601.12)	57.95 57.77	5.53 5.88	9.32 –
8h	511 (82 400)	$C_{27}H_{29}Cl_2N_3O_6S$ (594.51)	54.55 54.50	4.92 4.79	7.07 –
8i	506 (74 900)	$C_{23}H_{23}BrClN_3O_5S$ (568.87)	48.56 –	4.08 –	7.39 –
8j	508 (87 500)	$C_{29}H_{38}I_2N_4OS$ (744.51)	46.78 46.42	5.14 5.42	7.53 7.95
8k	508 (70 800)	$C_{28}H_{28}I_2N_4OS$ (722.42)	46.55 46.62	3.91 3.75	7.76 7.24
8l	515 (72 400)	$C_{30}H_{32}BrCl_2N_5O_5S$ (725.48)	49.67 50.89	4.45 4.55	9.65 –
9a	509 (60 000)	$C_{29}H_{35}ClIN_5O_5S$ (728.11)	47.84 47.42	4.85 5.24	9.62 –
9b	509 (69 400)	$C_{30}H_{37}ClIN_5O_5S \cdot 2CH_3CH_2OH$ (915.12)	44.63 44.91	5.51 5.25	7.65 –

acid solution according to our recently published procedure [25].

The intermediates **5a** and **5b** (Scheme 2) were synthesized by the quaternization of **4a** or 4,7-dichloroquinoline **4b** with dimethylsulphate [26]. The intermediate **5c** is new and was prepared by melting together of equimolar amounts of **4a** and benzylbromide and subsequent crystallization of the product in acetone. The starting compound **5d** was prepared by refluxing **4a** and large excess of 1,3-dibromopropane [27].

Compounds **5e–5g** (Scheme 3) were synthesized by the reaction of **4a**, **4b** or 4-chloropyridinium hydrochloride **4c** in acetic acid solution according to Ref. [25]. The synthesis of intermediates **7a–7c** was performed according to Scheme 4 by melting together a small excess of **4a** or **4b** and compounds **6a–6c** [5,9] for 30 s and after cooling to room temperature the products were crystallized from acetone. All products **5a–5g** and **7a–7c** are highly hygroscopic and unstable on silicagel, and high temperatures in most solvents, which prevents their complete purification. Their structures were proved on the appropriate dyes.

The synthesis of monocationic monomethine cyanine dyes **8a–8i** was carried out by the condensation of the quaternized 2-methylbenzothiazolium salts **3a–3d**, and 4-chloroquinolinium salts **5a–5g** (Scheme 5, Tables 1 and 2) in methanol with the presence of *N*-ethyldiisopropylamine as a basic reagent.

Dicationic dyes **8j–8l** were synthesized from intermediates **3a** or **3b** and **7a–7c** in methanol and with double excess of *N*-ethyldiisopropylamine (Scheme 6, Tables 1 and 2).

Tricationic dyes **9a** and **9b** were prepared as outlined in Scheme 7 by refluxing **8i** in methoxyethanol/ethylene glycol – 10/8 ml with **DABCO** or 1-methyl-4-aza-1-azoniabicyclo[2.2.2]octane iodide. All dyes are new and their chemical structures were proven by ^{1}H NMR spectra (Table 2) and elemental analysis (Table 3).

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