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Synthesis of novel monomeric cyanine dyes containing 2-hydroxypropyl and 3-chloro-2-hydroxypropyl substituents – Noncovalent labels for nucleic acids

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Abstract

Several 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 2-hydroxypropyl and 3-chloro-2-hydroxypropyl substituents. All derivatives absorb in the region 476–519 nm and have a molar absorptivity of 49 000–106 000 L mol^{-1} cm^{-1}. The reaction products were characterized by ^{1}H NMR spectroscopy and elemental analysis.

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

1. Introduction

A growing scientific and commercial interest in the synthesis and application of cyanine dyes that are suitable as noncovalent nucleic acid labels [1–7] and our earlier 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 a higher fluorescence quantum yield upon binding to nucleic acids 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.

Recently the preparation of dye TOTO-1-6C bearing six positive charges in the molecule was also described [14]. This dye does not fluoresce in TE buffer and bidistilled water, but becomes strongly fluorescent upon binding to dsDNA. In another article we described the synthesis of several novel di- and tricationic monomeric and tetracationic homodimeric monomethine cyanine dyes based on oxazolo [4,5-*b*] pyridinium and quinolinium end groups, which exhibit a strong fluorescence enhancement in the presence of nucleic acids, very similar to SYBR Green I [15,16]. These interesting results, important for practical applications, provoked us to search for derivatives with new substituents and an increased number of positive charges in the molecule.

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, propylene oxide, epichlorohydrin, 1,3-dibromopropane, 1,3-diiodopropane, *N*-ethyldiisopropylamine, triethylamine,

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2-methylbenzothiazole and 1,4-diazabicyclo[2.2.2]octane (DABCO) are commercial products.

2.1. Synthesis of dyes **5a–5i** and **8**

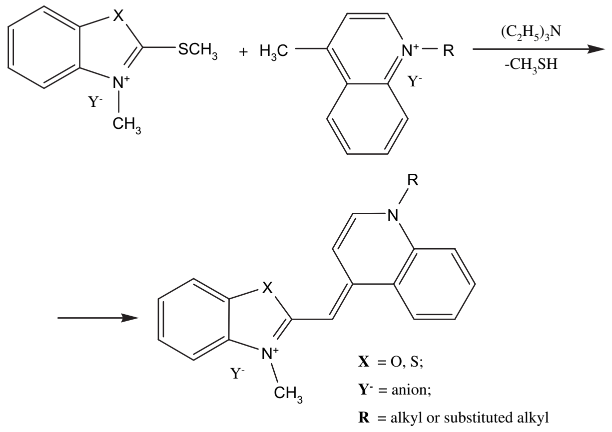
Corresponding amounts of **3a** or **3b** (Table 1) and of appropriate intermediates **4a–4g** or **7** were finely powdered together and suspended in 7–15 mL methanol in a reaction vessel equipped with electromagnetic stirrer. A twofold excess of *N*-ethyldiisopropylamine was added dropwise for about 1 min. Then the reaction mixture was stirred at room temperature for 2–7 h. The precipitate formed was suction-filtered, washed with methanol and air dried. Dyes **5a–5i** and **8** were recrystallized several times from methanol acidified with 1–2 drops of concentrated hydrochloric acid.

2.2. Synthesis of dyes **10a** and **10b**

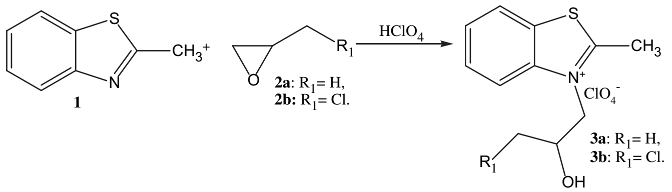
Equimolar amounts of **5h** or **5i** and **9** were finely powdered together. The mixture was suspended in the correspondent amount of methoxyethanol (Table 1) in a reaction vessel equipped with reflux condenser and mechanical stirrer. The reaction mixture was vigorously stirred and refluxed for 4 (**5i**) or 7 (**5h**) hours (TLC monitoring). After cooling to room temperature 40 mL diethyl ether was added and the precipitated dye was suction-filtered and air dried. Dyes **10a** and **10b** were recrystallized several times from methanol acidified with 1–2 drops of concentrated hydrochloric acid.

3. Results and discussion

During the last decades, most new asymmetric monomethine cyanine dyes have been prepared by the conventional method involving the reaction of 2-methylmercaptobenzoxazolium (Scheme 1) or 2-methylmercaptobenzothiazolium salts with 1-alkyl-4-methylquinolinium salts [17,18]. This method has a substantial drawback related to the evolution of methylmercaptan – a pollutant with very unpleasant odour. Another drawback is that the alkyl groups at sulphur and nitrogen in the quaternized 2-alkylmercapto starting materials may exchange their position, thus leading to unexpected reaction products [19,20].



Scheme 1. Synthesis of monomethine cyanine dyes by the conventional method.

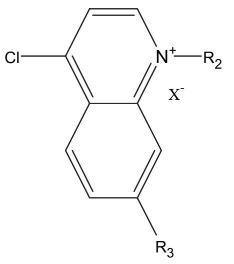


Scheme 2. Synthesis of intermediates **3a** and **3b**.

To avoid the aforementioned disadvantages we used an improved procedure. The original method has been patented [21] 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. On the other hand, in previous work we found that the fluorescence quantum yield increases with increasing number of positive charges in the dye molecule [14]. Therefore, we now synthesized several monomethine cyanine dyes bearing one, two or three positive charges in the molecule. The attached 2-hydroxypropyl and 3-chloro-2-hydroxypropyl

Table 1
Reaction conditions, melting points and yields of dyes **5a–5i**, **8**, **10a**, and **10b**

Product number	Starting compounds (mmol/number)	Solvent (mL)/net (iPr)$_2$ (mmol)	Reaction time (h) (at r.t.)	Yield (%)	M.p. (°C)
5a	3.25/**3a** and 3.89/**4a**	15 MeOH/6.5	2	82	263–264
5b	2.92/**3b** and 3.2/**4a**	15 MeOH/5.84	2	70	244–245
5c	3.25/**3a** and 3.89/**4b**	15 MeOH/6.5	2	73	273–275
5d	3.25/**3a** and 3.5/**4c**	10 MeOH/6.5	2	72	>300
5e	2.92/**3b** and 3.5/**4c**	10 MeOH/6.1	2	77	259–260
5f	6.81/**3a** and 8.52/**4d**	15 MeOH/13.63	2	97	243–245
5g	5.01/**3a** and 6.3/**4e**	10 MeOH/10	4	60	247–249
5h	2.27/**3a** and 2.73/**4f**	10 MeOH/4.54	2	50	233–236
5i	3.5/**3a** and 4.2/**4g**	10 MeOH/7	2	68	225–227
8	3.2/**3a** and 4.2/**7**	7 MeOH/6.4	6	58	268–270
10a	1.8/**5h** and 1.8/**9**	11 $CH_3O(CH_2)_2OH$	7 (reflux)	89	217–219
10b	1.66/**5i** and 1.66/**9**	15 $CH_3O(CH_2)_2OH$	4 (reflux)	84	216–218



No	R_2	R_3	X^-
4a	CH_3	H	$CH_3SO_4^-$
4b	CH_3	Cl	$CH_3SO_4^-$
4c	$CH_2CH(OH)CH_2Cl$	Cl	ClO_4^-
4d	CH_2Ph	H	Br^-
4e	$CH_2CH_2CONH_2$	H	ClO_4^-
4f	$(CH_2)_3Br$	H	Br^-
4g	$(CH_2)_3I$	H	I^-

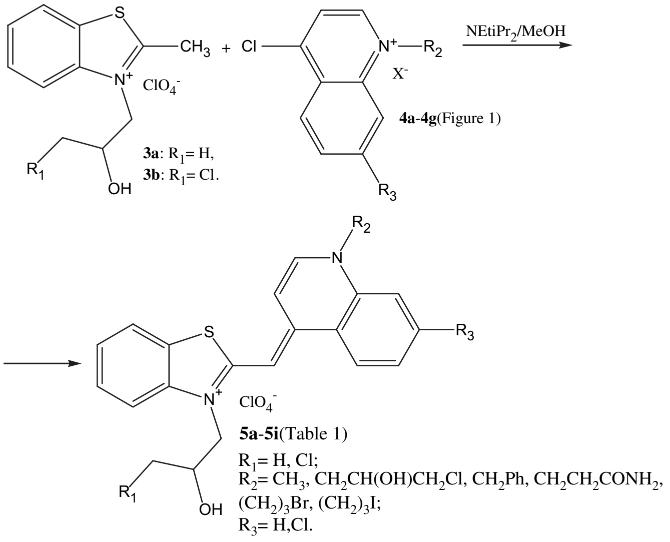
Fig. 1. Intermediates **4a–4g**.

substituents could be converted to epoxy substituents. This makes possible further functionalization of the derived dyes [22]. Additionally the synthesized stains bearing 2-hydroxypropyl substituents exhibit good solubility in water, methanol, ethanol and dimethyl sulfoxide, which is another important advantage.

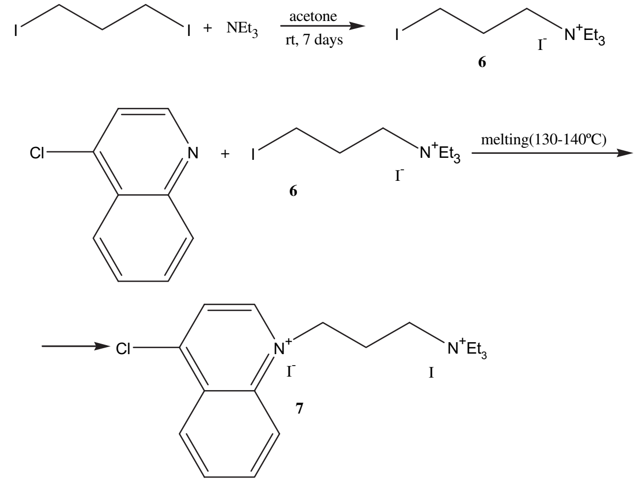
Apparently the reaction method with 4-chloroquinolinium salts has been scarcely used [2,18]. The method does not involve the evolution of methylmercaptane and offers more synthetic possibilities. The relatively easy preparation of the intermediate 4-chloroquinoline by a published method was used [23].

The intermediates **3a**, **3b** (Scheme 2) and **4c** (Fig. 1) by quaternization of 2-methylbenzothiazole or 4,7-dichloroquinoline with propylene oxide or epichlorohydrin were synthesized according to our procedure described recently [22].

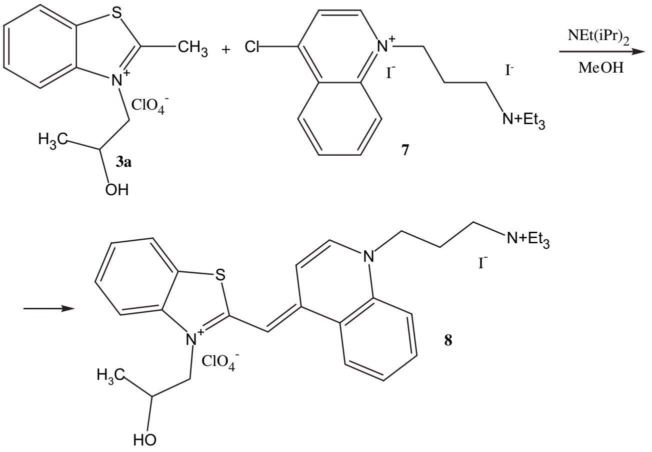
The starting compounds **4a** and **4b** (Fig. 1) were prepared by quaternization with dimethylsulphate [24]. The intermediate **4d** is new and was prepared by melting equimolar amounts of 4-chloroquinoline and benzylbromide and subsequent crystallization of the product in acetone. Intermediate **4e** is a product of the reaction between 4-chloroquinolinium perchlorate and acrylamide in acetic acid solution [25]. The starting compounds **4f** and **4g** were prepared by refluxing 4-chloroquinoline in a large excess of 1,3-diiodopropane or 1,3-dibromopropane [2]. Since the intermediates **4a–4g** are highly hygroscopic and unstable on silica gel as well as at increased temperatures, recrystallization and complete purification are not possible. Their structures were verified by determination of the structures of the derived dyes.



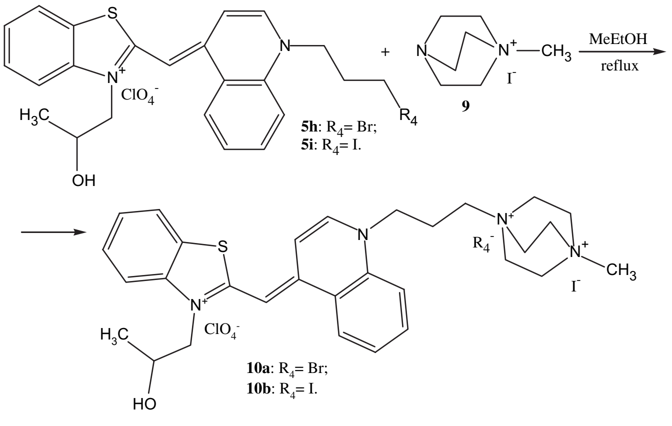
Scheme 3. Synthesis of monocationic monomethine cyanine dyes **5a–5i**.



Scheme 4. Synthesis of intermediates **6** and **7**.



Scheme 5. Synthesis of dicationic dye **8**.



Scheme 6. Synthesis of tricationic dyes **10a** and **10b**.

Table 2
Structures, names and ^{1}H NMR spectra of dyes **5a–5i**, **8a**, **10a**, and **10b**

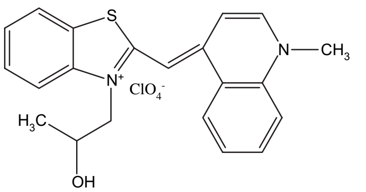
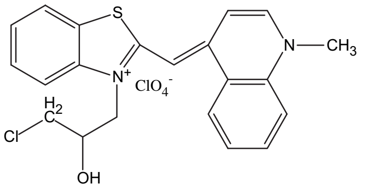
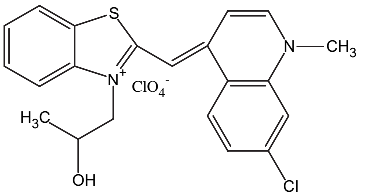
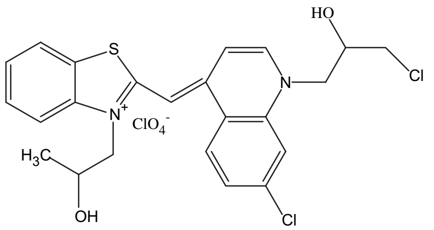
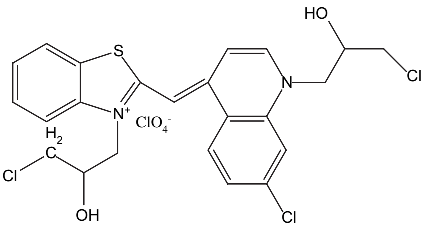
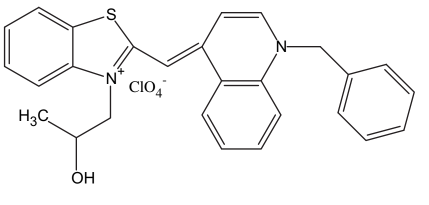
Product number	Structure/name	^{1}H NMR (d_6-DMSO, δ (ppm))
5a	 1-Methyl-4-[(3-(2-hydroxypropyl)-3*H*-benzothiazol-2-ylidene) methyl]-quinolinium perchlorate	1.32 d (3H, CH_3), 4.11 s (3H, N^+CH_3), 4.17–4.22 m (1H, *CH*OH), 4.83 d (1H, $N^+\mathit{CH_2}CH$), 4.85 d (1H, $N^+\mathit{CH_2}CH$), 7.03 s (1H, CH), 7.29–8.62 m (10H, Ar)
5b	 1-Methyl-4-[(3-(3-chloro-2-hydroxypropyl)-3*H*-benzothiazol-2-ylidene)methyl]-quinolinium perchlorate	3.84–3.98 m (2H, CH_2Cl), 4.14 s (3H, N^+CH_3), 4.24–4.27 m (1H, *CH*OH), 5.21 d (1H, $N^+\mathit{CH_2}CH$), 5.23 d (1H, $N^+\mathit{CH_2}CH$), 7.00 s (1H, CH), 7.32–8.60 m (10H, Ar)
5c	 1-Methyl-4-[(3-(2-hydroxypropyl)-3*H*-benzothiazol-2-ylidene) methyl]-7-chloroquinolinium perchlorate	1.33 d (3H, CH_3), 4.10 s (3H, N^+CH_3), 4.17–4.22 m (1H, *CH*OH), 4.86 d (1H, $N^+\mathit{CH_2}CH$), 4.88 d (1H, $N^+\mathit{CH_2}CH$), 7.03 s (1H, CH), 7.27–8.65 m (9H, Ar)
5d	 1-(3-Chloro-2-hydroxypropyl)-4-[(3-(2-hydroxypropyl)-3*H*-benzothiazol-2-ylidene)methyl]quinolinium perchlorate	1.33 d (3H, CH_3), 3.76–3.88 m (2H, CH_2Cl), 3.90–4.15 m (1H, *CH*OH), 4.18–4.25 m (1H, *CH*OH), 4.69 d (1H, $N^+\mathit{CH_2}CH$), 4.73 d (1H, $N^+\mathit{CH_2}CH$), 5.17–5.77 m (2H, N^+CH_2), 7.07 s (1H, CH), 7.40–8.68 m (9H, Ar)
5e	 1-(3-Chloro-2-hydroxypropyl)-4-[(3-(3-chloro-2-hydroxypropyl)-3*H*-benzothiazol-2-ylidene)methyl]-7-chloroquinolinium perchlorate	3.81–3.95 m (4H, 2 × CH_2Cl), 4.20–4.27 m (1H, *CH*OH), 4.30–4.68 m (1H, *CH*OH), 4.71–4.75 m (2H, N^+CH_2), 5.75–5.84 m (2H, N^+CH_2), 7.05 s (1H, CH), 7.37–8.65 m (9H, Ar)
5f	 1-Benzyl-4-[(3-(2-hydroxypropyl)-3*H*-benzothiazol-2-ylidene)methyl]quinolinium perchlorate	1.34 d (3H, CH_3), 4.20–4.25 m (1H, *CH*OH), 4.88 d (1H, $N^+CH_2\mathit{CH}$), 4.91 d (1H, $N^+CH_2\mathit{CH}$), 5.86 s (2H, N^+CH_2Ph), 7.13 s (1H, CH), 7.28–8.77 m (15H, Ar)

Table 2 (*continued*)

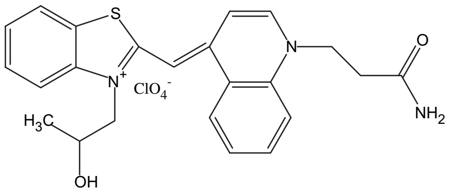
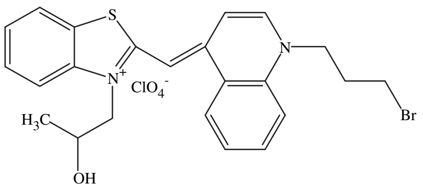
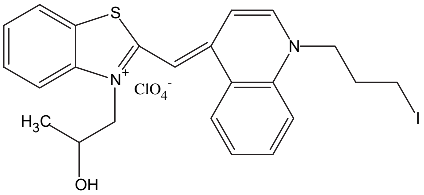
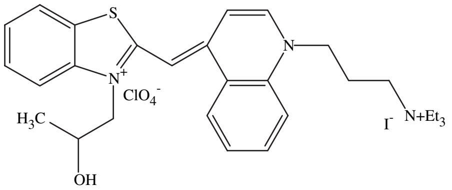
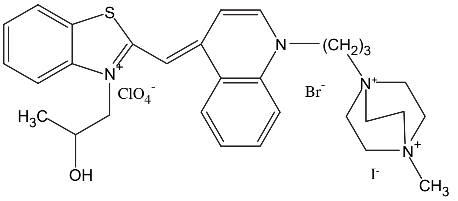
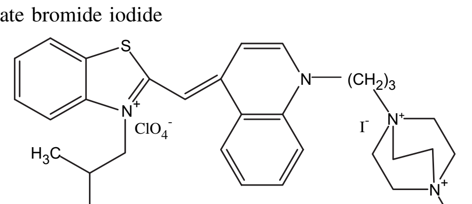
Product number	Structure/name	^{1}H NMR (d_6-DMSO, δ (ppm))
5g	 1-(2-Carbamoyl-ethyl)-4-[(3-(2-hydroxypropyl)-3*H*-benzothiazol-2-ylidene)methyl]quinolinium perchlorate	1.33 d (3H, CH$_3$), 2.73 t (2H, CH$_2$CO), 3.84 d (1H, N$^+$*CH*$_2$CH), 3.86 d (1H, N$^+$*CH*$_2$CH), 4.18–4.53 m (1H, *CH*OH), 4.77 t (2H, N$^+$CH$_2$), 5.22 br s (1H, OH), 7.06 s (1H, CH), 7.23 d (2H, NH$_2$), 7.10–8.64 m (10H, Ar)
5h	 1-(3-Bromopropyl)-4-[(3-(2-hydroxypropyl)-3*H*-benzothiazol-2-ylidene)methyl]quinolinium perchlorate	1.32 d (3H, CH$_3$), 2.44–2.48 m (2H, CH$_2$), 3.36 br s (2H, CH$_2$Br), 4.17–4.20 m (1H, *CH*OH), 4.79 t (2H, N$^+$CH$_2$), 4.84 d (1H, N$^+$*CH*$_2$CH), 4.86 (1H, N$^+$*CH*$_2$CH), 7.05 s (1H, CH), 7.31–8.62 m (10H, Ar)
5i	 1-(3-Iodopropyl)-4-[(3-(2-hydroxypropyl)-3*H*-benzothiazol-2-ylidene)methyl]quinolinium perchlorate	1.32 d (3H, CH$_3$), 2.33 d (2H, CH$_2$), 3.30 d (2H, CH$_2$I), 4.16–4.23 m (1H, *CH*OH), 4.38–4.65 m (4H, 2 × N$^+$*CH*$_2$CH + N$^+$CH$_2$), 7.03 s (1H, CH), 7.33–8.61 m (10H, Ar)
8a	 1-[3-(*N*-triethylammonio)propyl]-4-[(3-(2-hydroxypropyl)-3*H*-benzothiazol-2-ylidene)methyl]quinolinium perchlorate iodide	1.20 t (9H, *CH*$_3$CH$_2$), 1.34 d (3H, *CH*$_3$CH), 2.20–2.28 m (2H, CH$_2$), 3.22–3.30 q (6H, N$^+$*CH*$_2$CH$_3$), 4.19–4.24 m (1H, CHOH), 4.60 t (2H, N$^+$CH$_2$), 4.88 d (1H, N$^+$*CH*$_2$CH), 4.91 d (1H, N$^+$*CH*$_2$CH), 7.12 s (1H, CH), 7.40–8.66 m (10H, Ar)
10a	 1-[3-(*N*-(1-methyl-1,4-diazoniabicyclo[2.2.2]octane))propyl]-4-[(3-(2-hydroxypropyl)-3*H*-benzothiazol-2-ylidene)methyl]quinolinium perchlorate bromide iodide	1.34 d (3H, CH*CH*$_3$), 2.33 br s (2H, CH$_2$), 3.27 s (3H, N$^+$CH$_3$), 3.68 t (2H, N$^+$CH$_2$), 3.85 s (12H, N$^+$CH$_2$– Dabco), 4.16–4.28 m (1H, *CH*OH), 4.64 t (2H, N$^+$CH$_2$), 4.89 d (1H, N$^+$*CH*$_2$CH), 4.91 d (1H, N$^+$*CH*$_2$CH), 7.14 s (1H, CH), 7.41–8.72 m (10H, Ar)
10b	 1-[3-(*N*-(1-methyl-1,4-diazoniabicyclo[2.2.2]octane))propyl]-4-[(3-(2-hydroxypropyl)-3*H*-benzothiazol-2-ylidene)methyl]quinolinium perchlorate diiodide	1.34 d (3H, CH*CH*$_3$), 2.35 br s (2H, CH$_2$), 3.28 s (3H, N$^+$CH$_3$), 3.72 br s (2H, N$^+$CH$_2$), 3.88 s (12H, N$^+$CH$_2$– Dabco), 4.19–4.25 m (1H, *CH*OH), 4.61 t (2H, N$^+$CH$_2$), 4.88 d (1H, N$^+$*CH*$_2$CH), 4.90 d (1H, N$^+$*CH*$_2$CH), 7.13 s (1H, CH), 7.40–8.71 m (10H, Ar)

Table 3
Spectral characteristics and elemental analysis of dyes **5a–5i**, **8**, **10a**, and **10b**

Dye number	λ_{max} (nm) (ϵ L mol^{-1} cm^{-1})	Molecular formulae (M_m)	Analysis	Calculated Found	
			C (%)	H (%)	N (%)
5a	505 (86 000)	$C_{21}H_{21}ClN_2O_5S$ (483.36)	56.19	4.72	6.24
			56.55	4.69	6.80
5b	503 (76 000)	$C_{21}H_{20}Cl_2N_2O_5S$ (483.36)	52.18	4.17	5.80
			52.87	4.52	6.17
5c	514 (78 000)	$C_{21}H_{20}Cl_2N_2O_5S$ (483.36)	52.18	4.17	5.80
			52.64	4.49	–
5d	515 (83 000)	$C_{23}H_{23}Cl_3N_2O_6S$ (561.86)	49.17	4.13	4.99
			–	–	5.19
5e	514 (85 000)	$C_{23}H_{22}Cl_4N_2O_6S$ (596.31)	46.33	3.72	4.70
			46.70	3.79	5.12
5f	510 (105 000)	$C_{27}H_{25}ClN_2O_5S$ (525.03)	61.77	4.80	5.34
			61.58	4.87	5.51
5g	508 (72 000)	$C_{23}H_{24}ClN_3O_6S$ (505.97)	54.60	4.78	8.30
			54.85	4.97	–
5h[a]	476 (66 000)	$C_{23}H_{24}BrClN_2O_5S$ (555.87)	49.70	4.35	5.04
	519 (49 000)		–	–	–
5i[a]	510 (79 000)	$C_{23}H_{24}ClIN_2O_5S$ (555.87)	45.82	4.01	4.65
			–	–	–
8	511 (85 000)	$C_{29}H_{39}ClIN_3O_5S$ (704.06)	49.47	5.58	5.97
			50.16	5.80	–
10a	512 (73 000)	$C_{30}H_{39}ClBrIN_4O_5S$ (809.98)	44.49	4.85	6.92
			44.44	5.05	–
10b	511 (65 000)	$C_{30}H_{39}ClI_2N_4O_5S$ (856.99)	42.05	4.59	6.54
			41.81	5.78	–

[a] Since dyes **5h** and **5i** are intermediates in the preparation of dyes **10a** and **10b**, their structures are proved only with ^{1}H NMR (Table 2).

The synthesis of monocationic monomethine cyanine dyes **5a–5i** was performed by condensation of the quaternized 2-methylbenzothiazolum salts **3a** and **3b**, and 4-chloroquinolinium salts **4a–4g** (Scheme 3, Table 1) in methanol in the presence of *N*-ethyldiisopropylamine.

1-(3-(*N*-triethylammonium)propyl)-4-chloroquinolinium diiodide **7** is a new intermediate and was prepared by the quaternization of 4-chloroquinoline and triethyl-(3-iodopropyl)-ammonium iodide **6** (Scheme 4). Compound **6** [26] and a small excess of 4-chloroquinoline were melted together for 30 s. After cooling to room temperature the product crystallized from acetone. The intermediate **7** is highly hygroscopic and unstable, too. Its structure was confirmed via determination of the structure of dye **8**.

Dicationic dye **8** was synthesized from intermediates **3a** and **7** in methanol with a twofold excess of *N*-ethyldiisopropylamine (Scheme 5 and Table 1).

Tricationic dyes **10a** and **10b** were prepared as outlined in Scheme 6 by refluxing of **5h** or **5i** in methoxyethanol with 1-methyl-4-aza-1-azonia-bicyclo[2.2.2]octane iodide **9**. The chemical structures of the new dyes were proven by ^{1}H NMR spectroscopy (Table 2) and elemental analysis (Table 3).

The longest wavelength absorption maxima of the studied dyes are in the region 476–519 nm. The corresponding molar absorptivities are high with values between 49 000 and 105 000 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 **5a–5i**, **8**, **10a** and **10b** in the presence of nucleic acids are in progress.

4. Conclusions

Twelve novel mono-, di-, and tricationic monomeric monomethine cyanine dyes, containing 2-hydroxypropyl and 3-chloro-2-hydroxypropyl substituents, based on the Thiazole Orange chromophore, were synthesized in good to excellent yields using an improved synthetic procedure.

The dyes with 2-hydroxypropyl substituents show good solubility in water, methanol, ethanol and dimethyl sulfoxide. This is an advantage, very useful for their application as nucleic acid stains.

The conversion of the 3-chloro-2-hydroxypropyl substituent into an epoxy group offers the opportunity for additional functionalization of the dyes opening up new synthetic possibilities.

All dyes have high molar absorptivity between 49 000 and 105 000 L mol^{-1} cm^{-1}. The dyes are practically non-fluorescent, but become strongly fluorescent after binding to dsDNA. This fact makes the new dyes suitable for application in nucleic acid analysis.

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