ELSEVIER

Dyes and Pigments 40 (1999) 181–186

DYES AND PIGMENTS

Preparation of monomethine cyanine dyes as noncovalent labels for nucleic acids

Nikolai I. Gadjev [a], Todor G. Deligeorgiev [a, *], Seok Hong Kim [b]

[a] *University of Sofia, Faculty of Chemistry, 1 James Bourchier Avenue, 1126 Sofia, Bulgaria*
[b] *Research Centre, Taekwang Industrial Co., Ltd., 462-3, Jeonmin-dong, Yusung-ku, Daejeon, South Korea*

Received 17 April 1998; accepted 22 May 1998

Abstract

By condensation of quaternary benzothiazolium, quinolinium and acridinium salts having an active methyl group with 1-(3-bromopropyl)-4-chloroquinolinium or 1-(3-bromo-2-hydroxypropyl)-4-chloroquinolinium salts in the presence of a basic agent such as triethylamine, 8 asymmetric and symmetric monomethine cyanine dyes bearing ω-bromopropyl substituent with one or two positive charges, were synthesized. Additionally, two of the dyes were quaternized with pyridine, and monomethine cyanines with two and three positive charges are prepared. Most of the dyes showed high molar absorptivity (70 000–100 000 l mol^{-1} cm^{-1}). The acridinium dyes showed broad peaks with lower intensity of 30 000 l mol^{-1} cm^{-1}. In the presence of nucleic acid in aqueous solutions, a strong enhancement of the fluorescence of these new dyes was observed.

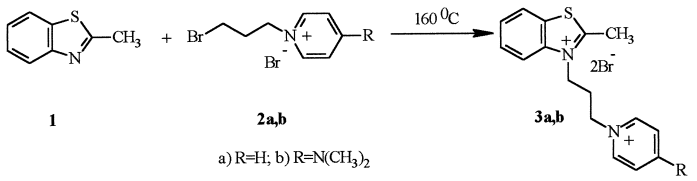
Keywords: Synthesis; Monomethines; Cyanine dyes; Nucleic acids; Fluorescence

1. Introduction

In recent years, there has been increased interest in the field of the synthesis and application of cyanine (polymethine) dyes absorbing in different visible spectral regions, suitable as nucleic acid labels. The number of both patents [1–5] and scientific publications [6–9] is an evidence for the commercial [10], scientific [11–17] and practical importance of these probes in nucleic acid research, clinical and environmental analysis. Representatives of this class of nucleic acid stains have fluorescence excitations and emissions that cover the visible spectrum from blue to near infrared, with additional absorption peaks in the UV region [10], making them applicable in many different types of instrumentation with different light sources. The strong interest for the replacement of the radioactive probes with the more environmentally friendly and safe fluorescent probes, and the need for miniaturization and automatization of the nucleic acid analysis, ensures a positive future for such non-covalently binding labels. Replacement of even one substituent in the dye molecule can lead to novel and sometimes better properties by nucleic acid detection [11]. We believe that such an important research area needs many more new representatives to be investigated in order to establish their new properties by interaction with nucleic acids.

* Corresponding author. Fax: +359-2-9625438; e-mail: toddel@chem.uni-sofia.bg

0143-7208/98/$—see front matter
PII: S0143-7208(98)00046-1



Scheme 1.

In this study, we describe the preparation of some asymmetric and symmetric monomethine cyanine dyes for nucleic acid detection by condensation of quaternary heterocyclic compounds, having an active 2- or 4-methyl group, with 4-chloroquinolinium salts (quaternized with 3-bromo- or 3-bromo-2-hydroxypropyl substituent) in the presence of basic agents.

2. Results and discussion

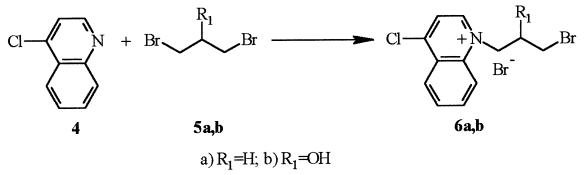
Such novel dyes have been synthesized by condensation of quaternized 2-, or 4-chloroheterocycles with quaternized 2- or 4-methyl heterocyclic salts in the presence of basic agents [7,18]. Although this preparation is less frequently used, it does not have the main disadvantage of the so called alkylthio method [19], where a strong pollutant is evolved, method involving the reaction of 2- or 4-methylthio quaternized heterocycles with 2- or 4-methyl quaternized heterocyclic salts in the presence of basic agents [19]. The dye synthesis requires some novel intermediates to be prepared. 2-Methylbenzothiazole **1** was quaternized with 1-(3-bromopropyl)pyridinium bromide **2a** or (3-bromopropyl)-4-*N*,*N*-dimethylaminopyridinium bromide **2b** by melting together the components at around 160°C for 30 min (Scheme 1), thus giving products **3a** and **3b**.

4-Chloroquinoline [20] **4** was quaternized with 1,3-dibromopropane **5a** or 1,3-dibromo-3-hydroxypropane **5b** in trichloroethylene (Scheme 2) for the preparation of compounds **6a** and **6b**.

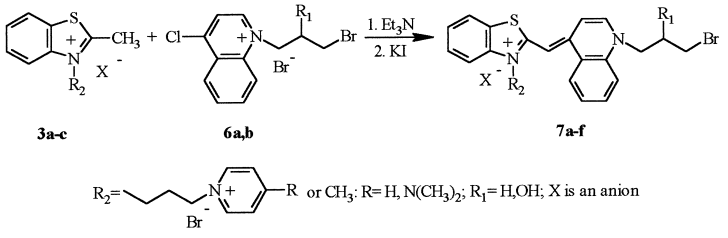
Dyes **7a–f** were prepared by condensation of the quaternized 2-methylbenzothiazolium salts **3a–c** with the 4-chloroquinolinium salts **6a** and **6b** in the presence of triethylamine (Scheme 3 and Table 1).

Dyes with quinolinium **9a** and acridinium **9b** end groups are synthesized by the same method with 1,4-dimethylquinolinium methosulfate **8** and 9,10-dimethylacridinium iodide [21,22] **9** (Scheme 4 and Table 1).

Dyes **7a–f**, **9a** and **9b** can be used as intermediates for the preparation of monomeric nucleic acid dyes bearing more than one positive charge, thus having higher binding affinity to nucleic acids. Dyes **11** and **12** with two and three positive

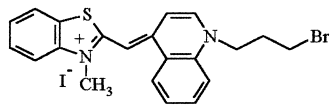
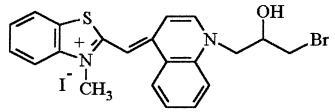
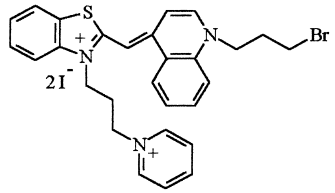
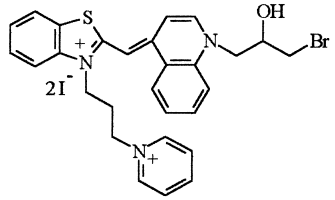
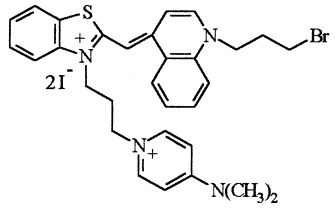
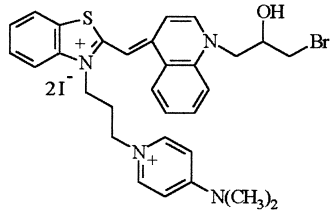
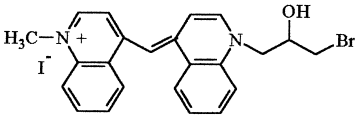


Scheme 2.



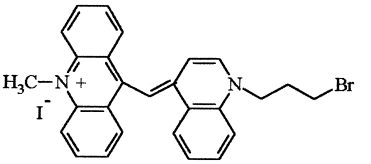
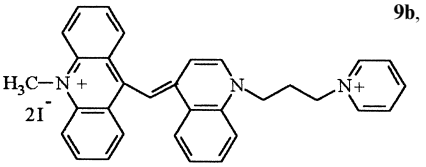
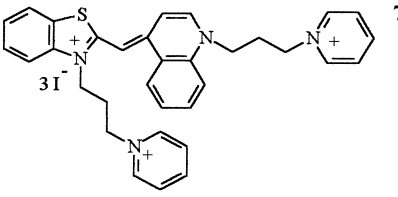
Scheme 3.

Table 1
Structure and yield for dyes **7a–f**, **9a,b**, **11** and **12**

Dye	Formula	Starting compounds	Yield %
7a		**3c**, **6a**	56
7b		**3c**, **6b**	64
7c		**3a**, **6a**	67
7d		**3a**, **6b**	71
7e		**3b**, **6a**	60
7f		**3b**, **6b**	64
9a		**8a**, **6b**	49

(continued)

Table 1—*contd*

9b		**8b**, **6a**	83
11		**9b**, **10**	63
12		**7c**, **10**	61

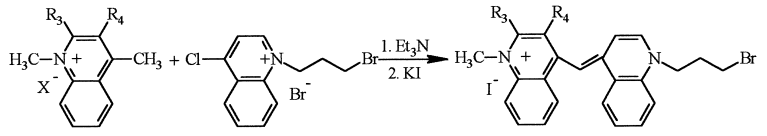
charges, respectively are synthesized by additional quaternization of **9b** and **7c** with pyridine **10** (Scheme 5 and Table 1).

The monomethine cyanine benzothiazolium dyes **7a–f** and **12** have high molar absorptivity ($\epsilon = 70\,000$–$90\,000$) at around 504–507 nm. This holds also for the quinolinium monomethine dye **9a** ($\epsilon = 100\,300$) at 590 nm. The monomethine cyanines with acridinium and quinolinium end groups **9b** and **11** have broad peaks with lower intensity ($\epsilon \sim 30\,000$), absorbing at 605–620 nm (Table 2).

The newly synthesized monomethine cyanines **7a–f** and **9a** and **9b** with ω-bromopropyl substituent can also be used as building blocks for the synthesis of homo- [21] and heterodimeric [23–25] nucleic acid dyes. In the presence of nucleic acids in aqueous solutions a strong enhancement of the fluorescence of the novel dyes is observed.

3. Experimental

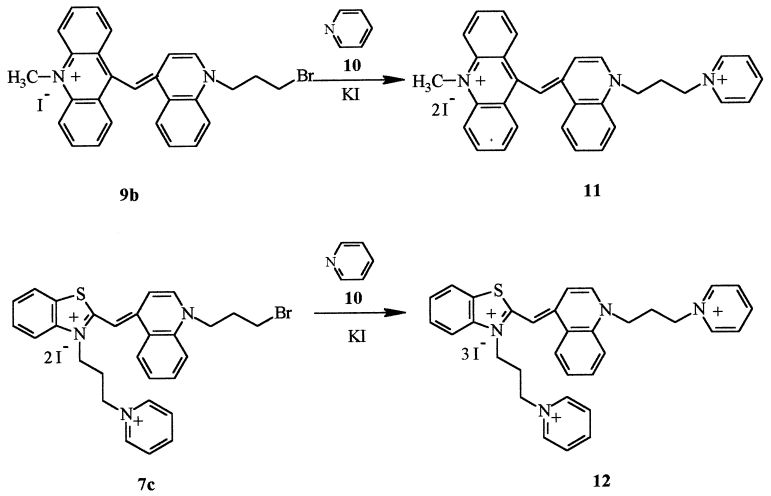
Melting points were determined on a Kofler apparatus and are uncorrected. The absorption



8a,b 9a,b

a) R_3=R_4=H; b) R_3,R_4= CH=CHCH=CH; X is an anion

Scheme 4.



9b 11 7c 12

Scheme 5.

spectra were recorded on a Perkin–Elmer Lambda 17 UV/VIS spectrophotometer (2×10^{-5} M/litre in methanol).

3.1. Preparation of 2-methyl-3-[(3-pyridinio)-propyl]benzothiazolium dibromide ***3a*** *and 2-methyl-3-{[3-(4-*N,N*dimethylamino)pyridinio]-propyl}-benzothiazolium dibromide* ***3b***

2-Methylbenzothiazole **1** (0.1 m) and 3-bromopyridinium bromide or 1-(3-bromopropyl)-4-*N*,*N*-dimethylaminopyridinium bromide (0.1 m) were melted together and the viscous mass was stirred and heated at 160°C for 30 min. The melt was cooled to 80°C and 30 ml acetone were added to the reaction mixture. The acetone layer above the solidified melt was decanted. The semisolid compound was transferred with heating to a storage vessel. The compounds were used without further purification in the next reaction steps.

3.2. Preparation of 1-(3-bromopropyl)-4-chloro-quinolinium bromide ***6a***

Five grams (0.03 m) 4-Chloroquinoline were suspended in 7 ml chlorobenzene and 6.1 ml (12.1 g, 0.06 m) 1,3-dibromopropane were added. The reaction mixture was refluxed with stirring for 2 h. Additional 4.04 g (2 ml) 1,3-dibromopropane were added and refluxing continued for 2 further hours. The reaction mixture was cooled and the product then crystallized in a thick mass. The compound was filtered and dried under vacuum. Yield of the crude product 9.5 g (85%). The 1-(3-bromopropyl)-4-chloroquinolinium bromide was highly hygroscopic and needs to be stored in a

Table 2
Characterization data for dyes **7a–f**, **9a,b**, **11** and **12**

Dye	m.p. °C	λ_{max} nm (ϵ l mol^{-1} cm^{-1})	Molecular formulae	Analysis (%) found/calc.		
				C	H	N
7a	204–206	505 (87600)	$C_{21}H_{20}BrIN_2S$	–	–	5.2/5.2
7b	236–237	504 (72300)	$C_{21}H_{20}BrIN_2OS.C_2H_5OH$	46.5/45.9	4.1/4.3	5.0/4.7
7c	219–221	505 (77800)	$C_{28}H_{28}Br_2I_2N_3S$	43.3/43.5	3.8/3.6	5.4/5.4
7d	249–250	504 (89800)	$C_{28}H_{28}BrI_2N_3OS$	42.7/42.6	3.6/3.6	5.0/5.3
7e	236–239	478sh, 504 (52400, 70000)	$C_{30}H_{33}BrI_2N_4S$	44.4/44.2	3.9/4.0	6.9/6.9
7f	255–257	504 (90100)	$C_{30}H_{33}BrI_2N_4OS$	–	–	6.7/6.7
9a	222–224	590 (103300)	$C_{23}H_{22}BrIN_2O$	–	–	5.4/5.1
9b	145–147	606 (28500)	$C_{27}H_{24}BrIN_2$	55.8/55.6	4.3/4.1	4.6/4.8
11	252–254	621 (29900)	$C_{32}H_{29}I_2N_3$	54.2/54.2	4.1/4.1	5.6/5.9
12	190–193	507 (78300)	$C_{33}H_{33}I_3N_4S$	44.0/44.1	3.9/3.7	5.8/6.2

desiccator. It was used in the next reaction steps without further purification.

3.3. Preparation of 1-(3-bromo-2-hydroxypropyl)-4-chloroquinolinium bromide *6b*

Five grams (0.03 m) 4-Chloroquinoline was suspended in 15 ml trichloroethylene and 6.1 ml (12.1 g, 0.06 m) 1,3-dibromo-2-hydroxy-propane added. The reaction mixture was refluxed with stirring for two hours. The solvent was distilled off (rotary evaporator) and a non-crystallizable oily product was obtained. Yield of crude product 7.2 g (78%). It was used in the next reaction steps without further purification.

3.4. Preparation of the dyes *7a–f*, and *9a,b*

Of the corresponding heterocyclic quaternary salt 0.0035 m (**3a–c** or **8a,b**), 0.0035 m 1-(3-bromopropyl)-4-chloroquinolinium bromide **6a** or 1-(3-bromo-2-hydroxypropyl)-4-chloroquinolinium bromide **6b** and 0.97 ml (0.007 m) triethylamine were refluxed in 15 ml methanol for 30 min. A triple excess of saturated aqueous potassium iodide solution was added to the hot dye solution. The solution was cooled and the precipitated dyes was filtered and dried. Some data for the dyes are given in Tables 1 and 2.

3.5. Preparation of the dyes *11* and *12*

Of the corresponding dye 0.00034 m **9b** or **7c**, 0.08 ml (0.001 m) pyridine in 3 ml 2-methoxyethanol were refluxed with stirring for 6 h. To the hot dye solution, a triple excess of saturated aqueous potassium iodide solution was added. After cooling the precipitated dye was filtered and dried. Some data are given in Tables 1 and 2.

References

[1] Roth B, Millard P, Yue S, Wells S, Haugaland RP. (Molecular Probes, Inc.). PCT Int Appl WO94 24 213; Chem Abstr 1995;122:163503t.
[2] Yue S, Singer VI, Roth B, Mozer T, Millard P, Jones L, Jin X, Haugland RP, Poot M. (Molecular Probes, Inc.). PCT Int Appl WO96 13 552; Chem Abstr 1996;125:81256n.
[3] Yue S, Johnson I, Huang Z, Haugaland RP. (Molecular Probes, Inc.). US Patent 5 321 130, 1994; Chem Abstr 1994;121:129393b.
[4] Yue S, Singer VI, Roth B, Mozer T, Millard P, Jones L, Jin X, Haugland RP, Poot M. (Molecular Probes, Inc.). US Patent 5 658 751, 1997; Chem Abstr 1997;127:231608m.
[5] Lee LG. (Biometric Imaging, Inc.). PCT Int Appl, WO97 17 471; Chem Abstr 1997;127:15178d.
[6] Deligeorgiev TG, Gadjev NI, Drexhage K-H, Sabnis RW. Dyes and Pigments 1995;29:315.
[7] Deligeorgiev TG, Zaneva DA, Kim SH, Sabnis RW. Dyes and Pigments, 1998;37:205–211.

[8] Yarmoluk I, Zhyvolup A, Koval'ska V, Klimenko Il, Kukharenko A, Kovtun Yu, Slominsky Yu. Biopolim kletka 1996;12:69.
[9] Moreda W, Forrester AR. Tetrahedron 1997;57:12595.
[10] Haugland RP. Handbook of fluorescent probes and research chemicals. 6th ed Eugene, OR: Molecular Probes, 1996.
[11] Timcheva Il, Maximova VA, Deligeorgiev TG, Gadjev NI, Sabnis RW, Ivanov IG. FEBS Letters 1997;407:141.
[12] Zeng Zh, Clark S, Mathies R, Glazer A. Anal Biochem 1997;252:110.
[13] Josef M, Taylor J, McGown L, Pitner JB, Linn CP. Biospectroscopy 1996;2:173.
[14] Millard P, Roth B, Thi H-Ph T, Yue S, Haugland RP. Appl Environ Microbiol 1997;63:2897.
[15] Roth B, Poot M, Yue S, Millard P. Appl Environ Microbiol 1997;63:2421.
[16] Gurrieri S, Wells S, Johnson I, Bustamante C. Anal Biochem 1997;249:44.
[17] Clark S, Mathies R. Anal Chem 1997;69:1335.
[18] Larive H, Dennilouler RJ. Eastman Kodak Co. US Patent 3 149 105, 1964.
[19] Hamer FM. The Cyanine dyes and related compounds. New York: Interscience Publisher, 1964:58–59.
[20] Lappin RG, Adelson H, Jackson I, Albisetti J, Dobson RM, Baker RH. Journal Amer Chem Soc 1946;68:1264.
[21] Tsuge Ot, Nishinokava M, Tashiro M. Bull Chem Soc Japan 1963;36:1447; Chem Abstr 60:5455.
[22] Zakhs E. Khim Get Soed 1973;4:539.
[23] Rye HS, Yue S, Wemmer DE, Quesada MA, Haugland RP, Maties RA, Glazer AN. Nucleic Acids Res 1992;20:2803.
[24] Benson SC, Mathies RA, Glazer AN. Nucleic Acids Res 1993;21:5720.
[25] Benson SC, Mathies RA, Glazer AN. Nucleic Acids Res 1993;21:5727.