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Dyes and Pigments 70 (2006) 185–191

DYES and PIGMENTS

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Synthesis of novel monomeric cyanine dyes containing mercapto and thioacetyl substituents for nucleic acid detection

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Received 9 February 2005; received in revised form 11 March 2005; accepted 25 May 2005
Available online 22 July 2005

Abstract

Fifteen novel mono-, di- and tricationic monomeric monomethine cyanine dyes, bearing mercapto and thioacetyl substituents, useful for nucleic acid detection are described. All derivatives absorb in the region 417–508 nm and have molar absorptivities of 61 000–101 300 $\text{l}\,\text{mol}^{-1}\,\text{cm}^{-1}$. The products were characterized by ^{1}H NMR, HPLC–MS and elemental analysis.

Keywords: Monomeric; Cationic monomethine cyanine dyes; Mercapto; Thioacetyl substituents; Nucleic acids

1. Introduction

Cyanine dyes have been known for a long time and are used in a variety of applications [1], such as photosensitizers for color photography [2], markers for flow cytometry [3] and as phototherapeutic agents [4]. In recent years there has been extensive growth [5–11] in the synthesis of cyanine dyes suitable as non-covalent labels for nucleic acid detection. As a part of our investigations on novel intermediates [12] as well as on monomeric and homodimeric cyanine dyes suitable as fluorescent probes for nucleic acid detection [13–19] we have synthesized 15 novel monomethine monointercalating cyanine dyes with one, two or three positive charges in the molecule.

2. Results and discussion

During the last decade of the 20th century most new asymmetric monomethine cyanine dyes have been prepared by the conventional method involving the reaction of 2-methylmercaptobenzoxazolium or 2-methylmercaptobenzothiazolium salts with 1-alkyl-4-methylquinolinium salts [20,21]. This method has disadvantages, relating to the evolution of methylmercaptan – a strong pollutant with very unpleasant odour. Another drawback is that the alkyl groups at sulfur and nitrogen in the quaternized 2-alkylmercapto starting materials may exchange their position, thus leading to unexpected reaction products [22,23]. The dye intermediates used by the authors avoid these problems and are environmentally friendlier. Another advantage of these intermediates is the possibility to generate mercapto end groups in the formed cyanine dyes, which can be additionally modified as described by Ishiguro et al. [24].

The preparation of the dye intermediates can be carried out by heating 2-methylmercaptobenzoxazole

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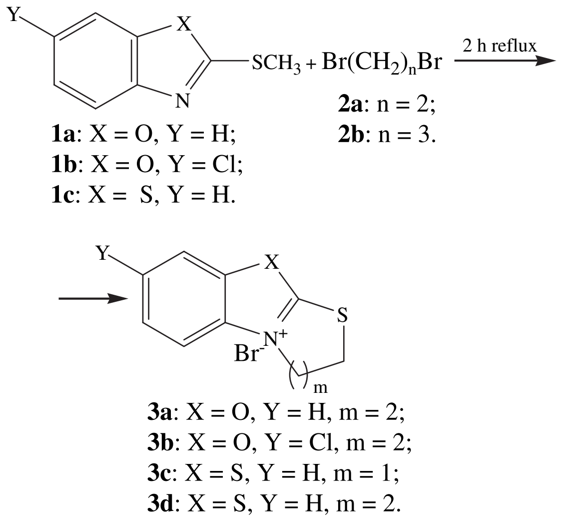
0143-7208/$ - see front matter
doi:10.1016/j.dyepig.2005.05.010

1a, 2-methylmercapto-6-chlorobenzoxazole **1b** or 2-methylmercaptobenzothiazole **1c** with equimolar amounts of 1,2-dibromoethane **2a** or 1,3-dibromopropane **2b** (Scheme 1) using procedures known in the literature [25,26]. Due to their reactive alkylmercapto group solutions of the heterocyclic compounds **3a**–**3d** are not stable when treated with silicagel and decompose on heating. Therefore the products were used in a further synthetic step without purification.

The intermediates **4a**–**4h** (Table 1) were synthesized by methods described in the literature [27–31] and were used without further purification.

Dyes **5a**–**5k** were prepared by condensation of the quaternary 1-alkyl-4-methylpyridinium or 1-alkyl-4-methylquinolinium compounds **4a**–**4f** with the aforementioned intermediates **3a**–**3d** in acetic anhydride (ethanol in case of dye **5e**) in the presence of triethylamine or *N*-ethyldiisopropylamine (Scheme 2 and Table 1). The reaction conditions are presented in Table 2. **5i**–**5k** are intermediates for dyes **6a**–**6d** and were used as crude reaction products. Their structures were proved with the confirmation of the structures of **6a**–**6d**. On the other hand dyes **6a**–**6d** were prepared by the quaternization of pyridine **4g** or 1-methyl-4-aza-1-azoniabicyclo[2.2.2]octane iodide **4h** with **5i**–**5k** in methoxyethanol. All dyes were characterized by ^{1}H NMR, HPLC–MS and elemental analysis.

The longest-wavelength absorption maxima of the dyes in methanolic solution are in the region 417–508 nm. The corresponding molar absorptivities are high between 61 000 and 101 000 l mol^{-1} cm^{-1} (Table 3). More detailed studies on the photo-physical properties of the described novel dyes **5a**–**5k** and **6a**–**6d** in the presence of nucleic acids are in progress.



Scheme 1. Synthesis of intermediates **3a**–**3d**.

3. 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). HPLC–MS spectra were obtained on an Agilent 1100 apparatus with a nucleosil RP 18 column and with solvent – 80% water, 19.95% acetonitrile and 0.05% trifluoroacetic acid. Lepidine, pyridine, 1,3-diiodopropane and 1,4-diazabicyclo[2.2.2]octane (DABCO) are commercial products.

*3.1. Preparation of heterocyclic quaternary ammonium salts **3a**, **3b**, **3c** and **3d** [25,26]*

Dyes **1a**, **1b** or **1c** of 0.03 mol and 0.03 mol **2a** or **2b** were refluxed in a reaction vessel equipped with mechanical stirrer and reflux condenser. After cooling to room temperature the formed precipitate was suction filtered, washed with ether and dried in a desiccator. The yields of crude products were quantitative and the purity as shown by ^{1}H NMR is more than 95%. These intermediates were used in the next stages without further purification because they are moisture sensitive.

3,4-Dihydro-2*H*-1,3-thiazino[2,3-*b*]benzoxazolium bromide **3a**: ^{1}H NMR (δ (ppm), DMSO-d_6) 2.45–2.56 m (2H, CH_2); 3.73 t (2H, SCH_2); 4.53 t (2H, N^+CH_2); 7.68–7.73 m (2H, Ar); 8.00–8.06 m (2H, Ar).

7-Chloro-3,4-dihydro-2*H*-1,3-thiazino[2,3-*b*]benzoxazolium bromide **3b**: ^{1}H NMR (δ (ppm), DMSO-d_6) 2.15–2.19 m (2H, CH_2); 3.17 t (2H, SCH_2); 3.9 t (2H, N^+CH_2); 6.83 d (1H, Ar); 6.95 s (1H, Ar); 7.12 d (1H, Ar).

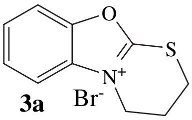
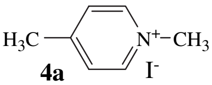
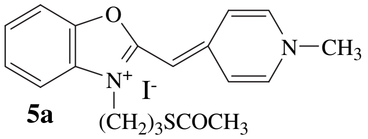
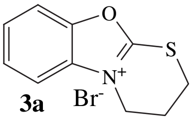
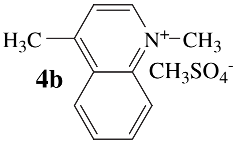
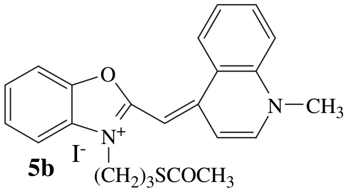
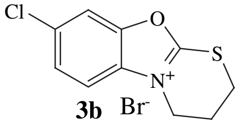
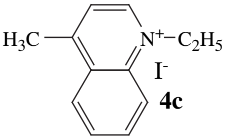
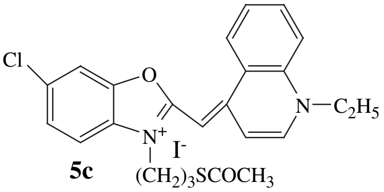
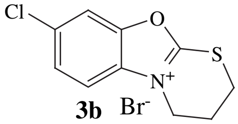
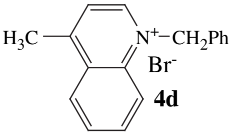
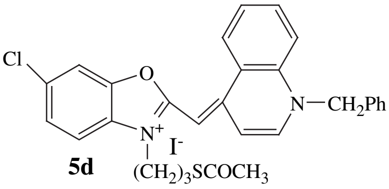
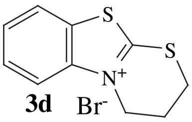
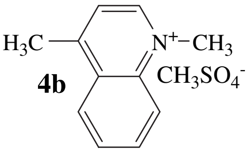
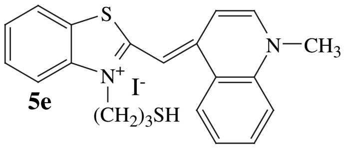
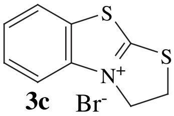
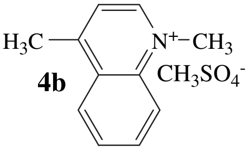
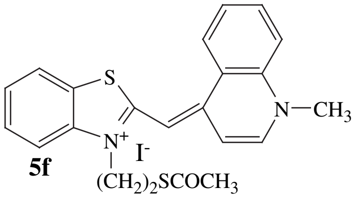
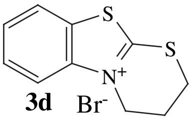
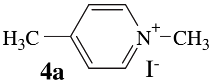
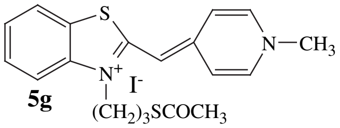
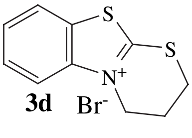
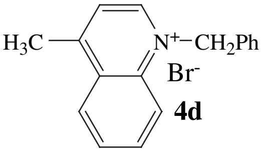
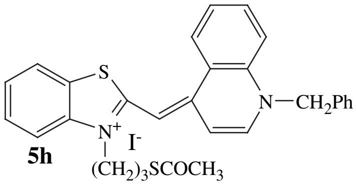
2,3-Dihydrothiazolo[2,3-*b*]benzothiazolium bromide **3c**: ^{1}H NMR (δ (ppm), DMSO-d_6) 3.76 t (2H, SCH_2); 4.97 t (2H, N^+CH_2); 7.63–7.66 m (1H, Ar); 7.72–7.99 m (1H, Ar); 7.97 d (1H, Ar); 8.29 d (1H, Ar).

3,4-Dihydro-2*H*-1,3-thiazino[2,3-*b*]benzothiazolium bromide **3d**: ^{1}H NMR (δ (ppm), DMSO-d_6) 2.50–2.57 m (2H, CH_2); 3.62 t (2H, SCH_2); 4.63 t (2H, N^+CH_2); 7.65–7.81 m (2H, Ar); 8.11 d (1H, Ar); 8.38 d (1H, Ar).

*3.2. Preparation of dyes **5a**–**5k***

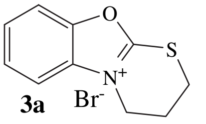
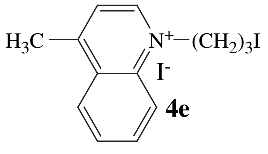
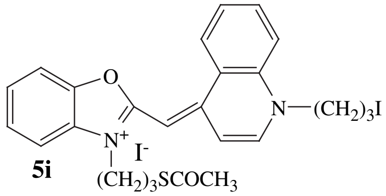
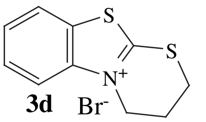
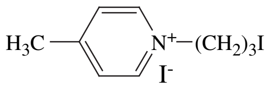
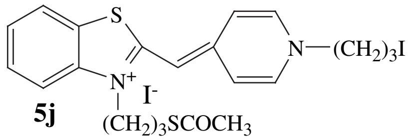
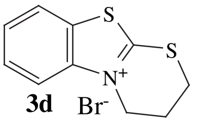
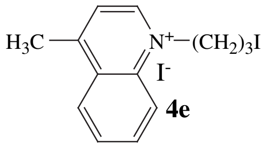
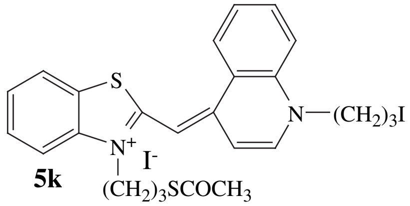
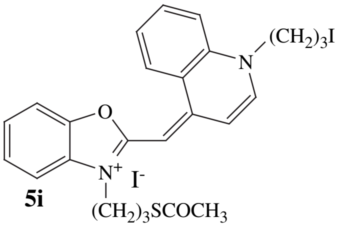
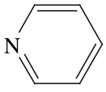
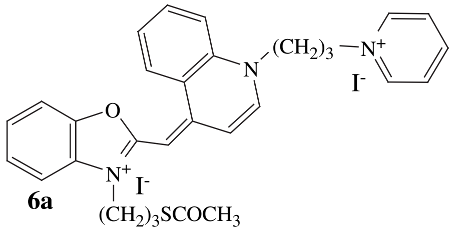
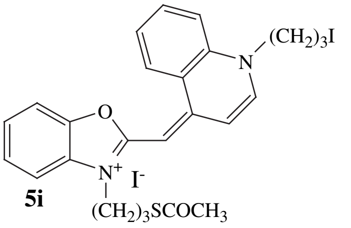
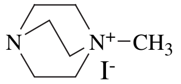
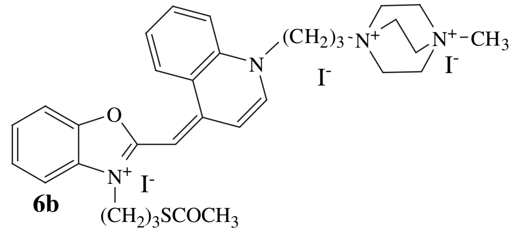
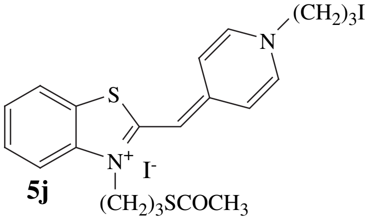
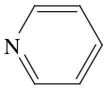
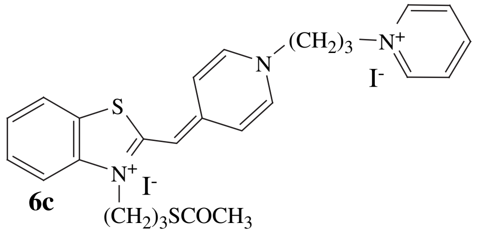
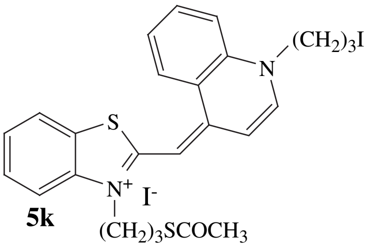
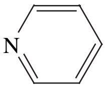
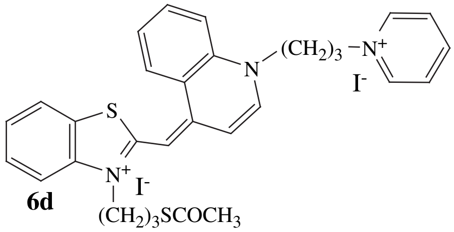
Corresponding amounts of the compounds **3a**–**3d** and of appropriate intermediates **4a**–**4f**, triple excess of triethylamine (or *N*-ethyldiisopropylamine) and 7–20 ml acetic anhydride or ethanol were vigorously stirred and heated for 2–10 min at appropriate temperature. The reaction mixture was cooled to room temperature and stirred from 1 to 7 h. The precipitated dye was suction filtered, washed with ether and air dried. The formed bromides of some of the dyes were dissolved in 15 ml methanol and 5 ml saturated water solution

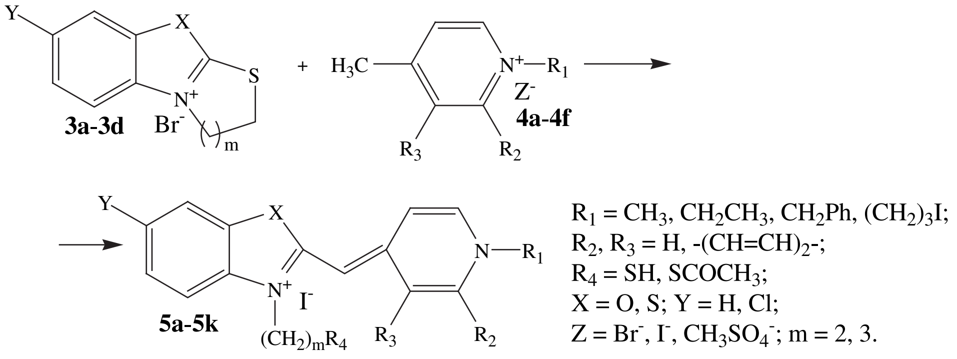
Table 1
Structures of starting compounds and dyes

Starting compounds (structure, No)	**Starting compounds (structure, No)**	**Dye (structure/No)**
 **3a**	 **4a**	 **5a**
 **3a**	 **4b**	 **5b**
 **3b**	 **4c**	 **5c**
 **3b**	 **4d**	 **5d**
 **3d**	 **4b**	 **5e**
 **3c**	 **4b**	 **5f**
 **3d**	 **4a**	 **5g**
 **3d**	 **4d**	 **5h**

(*continued on next page*)

Table 1 (*continued*)

Starting compounds (structure, No)	**Starting compounds (structure, No)**	**Dye (structure/No)**
 **3a**	 **4e**	 **5i**
 **3d**	 **4f**	 **5j**
 **3d**	 **4e**	 **5k**
 **5i**	 **4g**	 **6a**
 **5i**	 **4h**	 **6b**
 **5j**	 **4g**	 **6c**
 **5k**	 **4g**	 **6d**



Scheme 2. Synthesis of dyes **5a–5k**.

containing KI was added. The mixture was left at 5 °C in refrigerator for several days. The resulting precipitate was suction filtered and air dried. Dyes **5a–5k** were recrystallized from ethanol.

1-Methyl-4-[(3-(3-acetylsulfanylpropyl)-2(3*H*)-benzoxzol-2-ylidene)methyl]pyridinium iodide **5a**: ^{1}H NMR (δ (ppm), DMSO-d_6) 1.95–2.01 m (2H, CH_2); 2.30 s (3H, $COCH_3$); 2.94 t (2H, CH_2S); 3.97 s (3H, N^+CH_3); 4.12 t (2H, N^+CH_2); 5.59 s (1H, CH); 7.24–8.22 m (8H, Ar).

Table 2
Reaction conditions, amounts of the starting compounds, yields and melting points of dyes **5a–5k**

Dye	Starting compounds (mmol/number)	Ac_2O (ml)/NEt_3 (mmol)	Reaction time – heating/room temp.	Yield (%)	M.p. (°C)
5a	4.85/**3a** and 4.85/**4a**	15/9.7	10 min refl/7 h rt	98	174–176
5b	3.7/**3a** and 3.5/**4b**	20/7	4 h rt	80	239–241
5c	3.26/**3b** and 3.3/**4c**	8/6.6	4 h rt	90	201–203
5d	3.26/**3b** and 3.3/**4d**	7/6.6	4 h rt	97	247–249
5e	4.85/**3d** and 4.85/**4b**	10[a]/10	4 h rt	95	257–259
5f	3.8/**3c** and 3.72/**4b**	10/11	5 h rt	95	245–247
5g	5/**3d** and 5/**4a**	20/12	2 min 120 °C/3 h rt	97	218–220
5h	4.4/**3d** and 4.5/**4d**	8/9	2 min 120 °C/1.5 h rt	98	209–211
5i	2.8/**3a** and 2.73/**4e**	15/9[b]	5 h rt	71	196–198
5j	2.8/**3d** and 2.73/**4f**	10/9[b]	10 min refl/5 h rt	86	180–183
5k	3.5/**3d** and 3.4/**4e**	8/7[b]	2 min 90 °C/1 h rt	80	184–186

[a] Dye **5e** was prepared in ethanol instead of acetic anhydride as a solvent.

[b] *N*-ethyldiisopropylamine was used instead of triethylamine as a base.

1-Methyl-4-[(3-(3-acetylsulfanylpropyl)-2(3*H*)-benzoxazol-2-ylidene)methyl]quinolinium iodide **5b**: ^{1}H NMR (δ (ppm), DMSO-d_6) 2.05–2.11 m (2H, CH_2); 2.30 s (3H, $COCH_3$); 3.00 t (2H, CH_2S); 4.17 s (3H, N^+CH_3); 4.46 t (2H, N^+CH_2); 6.28 s (1H, CH); 7.38–8.04 m (8H, Ar); 8.49 d (1H, Ar); 8.77 d (1H, Ar).

1-Ethyl-4-[(3-(3-acetylsulfanylpropyl)-6-chloro-2(3*H*)-benzoxzol-2-ylidene)methyl]quinolinium iodide **5c**: ^{1}H NMR (δ (ppm), DMSO-d_6) 1.46 t (3H, CH_3); 1.99–2.06 m (2H, CH_2); 2.29 s (3H, $COCH_3$); 2.98 t (2H, CH_2S); 4.42 t (2H, $N^+CH_2CH_2$); 4.65 q (2H, $N^+CH_2CH_3$); 6.19 s (1H, CH); 7.43–8.14 m (7H, Ar); 8.58 d (1H, Ar); 8.73 d (1H, Ar).

1-Benzyl-4-[(3-(3-acetylsulfanylpropyl)-6-chloro-2(3*H*)-benzoxzol-2-ylidene)methyl]quinolinium iodide **5d**: ^{1}H NMR (δ (ppm), DMSO-d_6) 2.04–2.09 m (2H, CH_2); 2.30 s (3H, $COCH_3$); 2.99 t (2H, CH_2S); 4.49 t (2H, $N^+CH_2CH_2$); 5.91 s (2H, CH_2Ph); 6.34 s (1H, CH); 7.27–8.06 m (7H, Ar); 8.76 d (1H, Ar); 8.78 d (1H, Ar).

1-Methyl-4-[(3-(3-mercaptopropyl)-2(3*H*)-benzothiazol-2-ylidene)methyl]pyridinium iodide **5e**: ^{1}H NMR (δ (ppm), DMSO-d_6) 2.08–2.14 m (2H, CH_2); 3.00 t (2H, CH_2SH); 4.15 s (3H, N^+CH_3); 4.53 t (2H, N^+CH_2); 6.71 s (1H, CH); 7.08–8.45 m (8H, Ar).

1-Methyl-4-[(3-(2-acetylsulfanylethyl)-2(3*H*)-benzothiazol-2-ylidene)methyl]quinolinium iodide **5f**: ^{1}H NMR (δ (ppm), DMSO-d_6) 2.36 s (3H, $COCH_3$); 3.27 t (2H, CH_2S); 4.18 s (3H, N^+CH_3); 7.04 s (1H, CH); 7.34–8.04 m (8H, Ar); 8.63 d (1H, Ar); 8.86 d (1H, Ar).

1-Methyl-4-[(3-(3-acetylsulfanylpropyl)-2(3*H*)-benzothiazol-2-ylidene)methyl]pyridinium iodide **5g**: ^{1}H NMR (δ (ppm), DMSO-d_6) 1.92–1.98 m (2H, CH_2); 2.32 s (3H, $COCH_3$); 2.99 t (2H, CH_2S); 3.99 s (3H, N^+CH_3); 4.29 t (2H, N^+CH_2); 6.29 s (1H, CH); 7.24–7.56 m (5H, Ar); 7.88 d (1H, Ar); 8.29 d (2H, Ar).

1-Benzyl-4-[(3-(3-acetylsulfanylpropyl)-2(3*H*)-benzothiazol-2-ylidene)methyl]quinolinium iodide **5h**: ^{1}H NMR

Table 3
Spectral characteristics (methanol), elemental analysis and HPLS–MS of dyes **5a–5k** and **6a–6d**

Dye	λ_{max}, nm (ε, l/mol cm)	Molecular formulae	Analysis	Found		HPLS–MS (m/z) – Calc./found
			C	Calc.		
				H	N	
5a	417(95 300)	$C_{19}H_{21}\ IN_2O_2S$	48.72	4.49	5.99	483/483
			49.25	4.54	6.40	
5b	476(80 000)	$C_{23}H_{23}IN_2O_2S$	53.12	4.36	6.19	391/391
			53.28	4.44	5.41	
5c	477(94 000)	$C_{24}H_{24}ClIN_2O_2S$	50.74	4.39	4.99	439/439
			50.84	4.24	4.94	(405 < 1%)
5d	481(88 000)	$C_{29}H_{26}ClIN_2O_2S$	54.73	4.15	4.87	501/501
			54.59	4.24	4.39	
5e	476(51 600)	$C_{21}H_{21}IN_2S$	50.15	4.17	6.01	364/364
	500(40 250)		50.29	4.39	5.59	
5f	502(86 000)	$C_{22}H_{21}IN_2OS$	50.69	4.44	–	393/393
			50.77	4.04	–	
5g	446(78 000)	$C_{18}H_{19}IN_2OS_2$	46.85	4.25	6.24	357/357
			47.11	4.34	5.78	
5h	507(85 700)	$C_{28}H_{25}IN_2OS_2$	56.76	4.00	4.96	483/483
			57.05	4.43	4.59	
5i	450(99 000)	$C_{21}H_{24}I_2N_2OS_2$	44.66	3.90	4.17	–
			–	–	–	
5j	505(85 700)	$C_{25}H_{26}I_2N_2OS_2$	39.51	3.79	4.39	–
			–	–	–	
5k	480(91 000)	$C_{25}H_{26}I_2N_2O_2S$	43.62	3.81	4.07	–
			–	–	–	
6a	482(81 000)	$C_{29}H_{29}I_2N_3O_2S$	46.78	3.94	5.56	497/497
			46.81	4.29	5.46	
6b	483(91 000)	$C_{31}H_{39}I_3N_4O_2S$	41.61	4.46	6.42	–
			41.47	4.43	6.05	
6c	454(94 500)	$C_{25}H_{27}I_2N_3OS_2$	43.91	5.35	6.16	463/463
			43.51	4.54	5.86	
6d	508(73 200)	$C_{29}H_{29}I_2N_3OS_2$	46.87	3.88	5.75	513/513
			46.94	4.04	5.48	

(δ (ppm), DMSO-d_6) 2.04–2.09 m (2H, CH_2); 2.31 s (3H, $COCH_3$); 3.05 t (2H, CH_2S); 4.70 t (3H, N^+CH_3); 5.90 s (2H, CH_2Ph); 6.97 s (1H, CH); 7.30–8.85 m (15H, Ar).

3.3. Preparation of dyes *6a–6d*

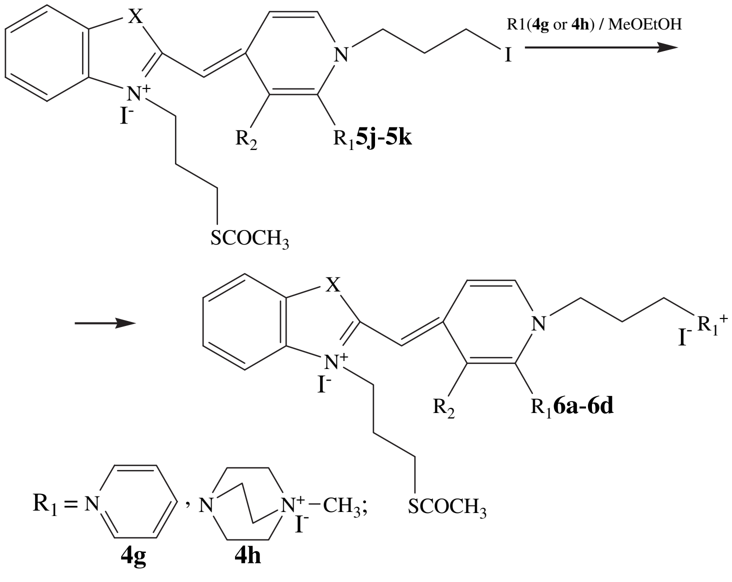
Dyes **5i**, **5j** or **5k** of 0.001 mol, 0.001 mol of pyridine **4g** or 1-methyl-4-aza-1-azoniabicyclo[2.2.2]octane iodide **4h** and 7 ml methoxyethanol were refluxed for 6 h in a 50 ml round bottomed flask, equipped with mechanical stirrer and reflux condenser. After cooling to room temperature the reaction mixture was left in a refrigerator for several days. The precipitated dyes were filtered off and dried in vacuum. They were recrystallized from ethanol (Scheme 3).

1-[3-(*N*-pyridinio)propyl]-4-[(3-(3-acetylsulfanylpropyl)-2(3*H*)-benzoxazol-2-ylidene)methyl]quinolinium diiodide **6a**: ^{1}H NMR (δ (ppm), DMSO-d_6) 2.04–2.10 m (2H, CH_2); 2.29 s (3H, $COCH_3$); 2.51–2.61 m (2H, CH_2); 3.00 t (2H, SCH_2); 4.50 t (2H, N^+CH_2); 4.75 t (2H, N^+CH_2); 4.86 (2H, N^+CH_2); 6.29 s (1H, CH); 7.39–9.16 m (15H, Ar).

1-[3-(*N*-(1-methyl-1,4-diazoniabicyclo[2.2.2]octane))-propyl]-4-[(3-(3-acetylsulfanylpropyl)-2(3*H*)-benzoxazol-2-ylidene)methyl]quinolinium triiodide **6b**: ^{1}H NMR (δ (ppm), DMSO-d_6) 2.05–2.11 m (2H, CH_2); 2.29 s (3H, $COCH_3$); 2.35 brs (2H, CH_2); 3.00 t (2H, SCH_2); 3.30 s (3H, N^+CH_3); 3.77 t (2H, N^+CH_2); 3.91 brs (12H, N^+CH_2–DABCO); 4.52 t (2H, N^+CH_2); 4.69 t (2H, N^+CH_2); 6.35 s (1H, CH); 7.41–8.84 m (10H, Ar).

1-[3-(*N*-pyridinio)propyl]-4-[(3-(3-acetylsulfanylpropyl)-2(3*H*)-benzothiazol-2-ylidene)methyl]pyridinium diiodide **6c**: ^{1}H NMR (δ (ppm), DMSO-d_6) 1.95–2.00 m (2H, CH_2); 2.33 s (3H, $COCH_3$); 2.48–2.52 m (2H, CH_2); 3.00 t (2H, SCH_2); 4.37 t (4H, $2 \times N^+CH_2$); 4.72 t (2H, N^+CH_2); 6.37 s (1H, CH); 7.30–9.12 m (13H, Ar).

1-[3-(*N*-pyridinio)propyl]-4-[(3-(3-acetylsulfanylpropyl)-2(3*H*)-benzothiazol-2-ylidene)methyl]quinolinium diiodide **6d**: ^{1}H NMR (δ (ppm), DMSO-d_6) 2.04–2.08 m (2H, CH_2); 2.31 s (3H, $COCH_3$); 2.56–2.62 m (2H, CH_2); 3.05 t (2H, SCH_2); 4.65–4.79 m (4H, $2 \times N^+CH_2$); 4.85 t (2H, N^+CH_2); 6.92 s (1H, CH); 7.37–9.15 m (15H, Ar).



R_1, R_2 = H, -$(CH{=}CH)_2$-; X = O, S.

Scheme 3. Synthesis of dyes **6a–6d**.

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