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# Synthesis of novel monomeric and homodimeric cyanine dyes with thioacetyl substituents for nucleic acid detection

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**Abstract**

Several novel di-, tri- and tetracationic monomeric and homodimeric monomethine cyanine dyes for nucleic acid detection were synthesized by condensation of 3,4-dihydro-(2*H*)-1,3-thiazino[2,3-*b*]benzooxazolium bromide and appropriate 1-(ω-bromoalkyl)-4-methylpyridinium or 1-(ω-bromoalkyl)-4-methylquinolinium bromide followed by quaternization with *N*,*N*,*N*′,*N*′-tetramethyl-1,3-propanediamine (TMPDA) or *N*,*N*,*N*,*N*′,*N*′-pentamethyl-1,3-propanediammonium iodide, or bisquaternization with TMPDA or *N*,*N*,*N*′,*N*′-tetramethyl-1,6-hexanediamine (TMHDA).


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

## 1. Introduction

It is well known that some non-fluorescent cyanine dyes become highly fluorescent in the presence of nucleic acids. Therefore they have replaced conventional dyes in some staining techniques because fluorescence methods provide far greater sensitivity due to enhanced signal-to-noise ratio and better color differentiation of stained cells [1]. For these reasons the application of new homodimeric and heterodimeric cyanine dyes as fluorescent stains is making a substantial impact on nucleic acid research [2–11]. As a part of our investigations [12,13] on novel and improved homodimers as fluorescent probes we have synthesized some new di- and tricationic monomeric as well as tetracationic homodimeric monomethine cyanine dyes.

## 2. Results and discussion

Thiazole orange and related dyes can be synthesized according to the method of Brooker et al. [14] 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 sulfur and nitrogen atoms in the quaternized 2-alkylthio starting materials, which leads to unexpected reaction products [15,16]. In our previous work [17] we described the synthesis of new cyanine dyes derived from 3,4-dihydro-(2*H*)-1,3-thiazino[2,3-*b*]benzothiazolium or benzooxazolium bromide and 4-methylpyridinium or 4-methylquinolinium salts, thus avoiding the

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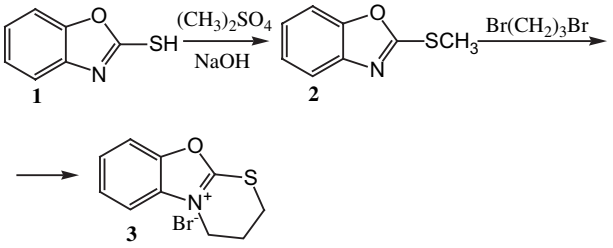



Scheme 1. Synthesis of intermediate **3**.

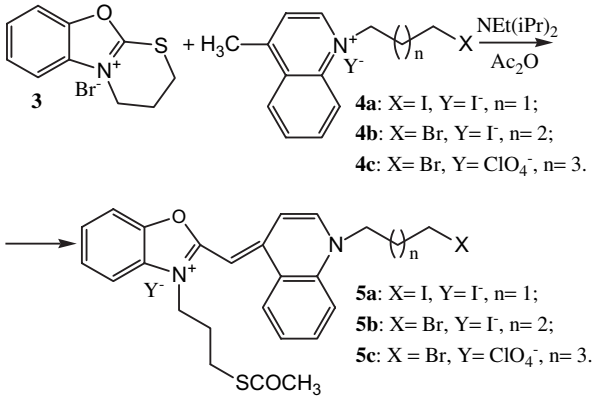
aforementioned disadvantages. Here we present an extension of our former work.

The starting compound 3,4-dihydro-(2*H*)-1,3-thiazino[2,3-*b*]benzooxazolium bromide **3** was prepared by the fusion of 2-methylthio-benzooxazole **2** (Scheme 1) with 1,3-dibromopropane [18,19].

The quaternized lepidines **4a**–**4c** were prepared by the reaction of lepidine with 1,3-diiodopropane or corresponding dibromoalkanes in the molar ratio 1:4, either neat or in a non-polar solvent at room temperature for 24–72 h [20].

Dyes **5a**–**5c** (Scheme 2) were prepared by condensation of the quaternized lepidines **4a**–**4c** with the heterocyclic intermediate **3** in acetic anhydride in the presence of the sterically hindered base *N*-ethyldiisopropyl amine.

The preparation of di- and tricationic monomeric dyes **6a**–**6c** was performed by heating **5a** or **5b** with *N*,*N*,*N*′,*N*′-tetramethyl-1,3-propandiamine **5d** or *N*,*N*-dimethyl-*N*′,*N*′,*N*′-trimethyl-1,3-propandiammonium iodide **5e** in methoxyethanol (Scheme 3). Similarly dyes

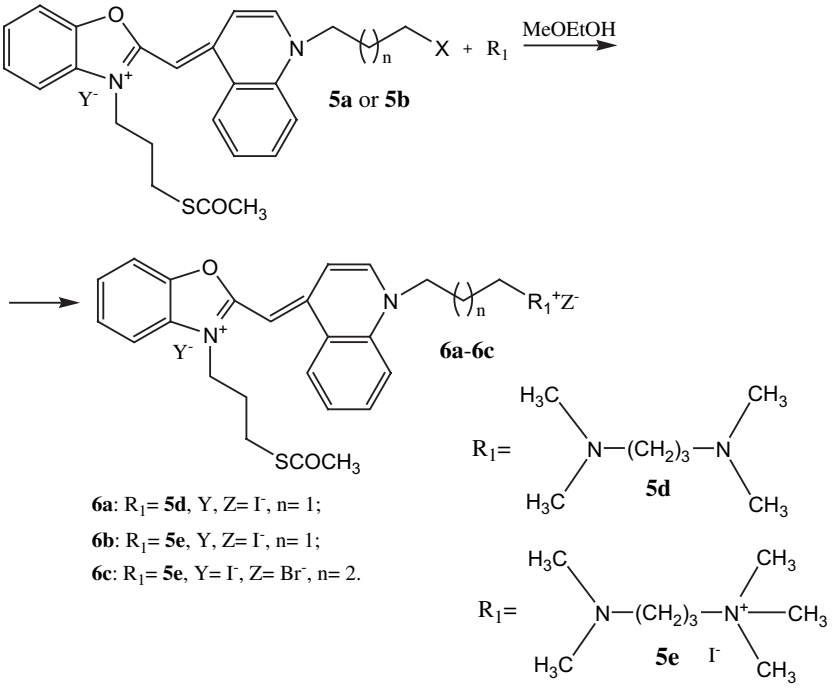



Scheme 2. Synthesis of monocationic monomeric monomethine cyanine dyes **5a**–**5d**.

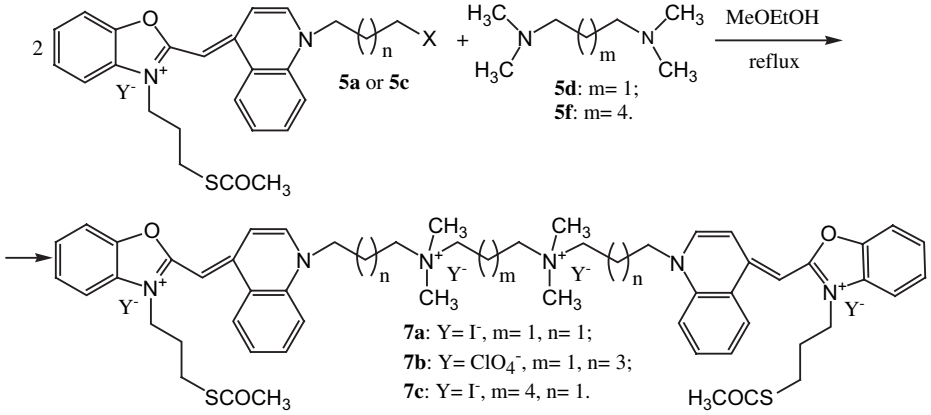
**7a**–**7c** were prepared by bisquaternization of dyes **5a** or **5c** with **5d** or *N*,*N*,*N*′,*N*′-tetramethyl-1,6-hexandiamine **5f** in methoxyethanol (Scheme 4).

The new dyes are practically non-fluorescent in solution (very low fluorescence quantum yield), but become strongly fluorescent after binding to dsDNA. For example the fluorescence quantum yield of the complex **7a**–dsDNA is 0.58 (fluorescence maximum at 512 nm), compared to 0.01 for the dye itself. More detailed studies on the photo-physical properties of the described novel dyes **6a**–**6c** and **7a**–**7c** in the presence of nucleic acids are in progress.

The following novel monomeric and homodimeric monomethine cyanine dyes with thioacetyl substituents were synthesized:

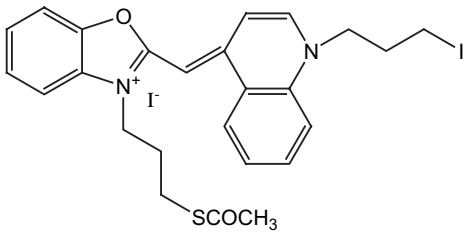



Scheme 3. Synthesis of di- and tricationic monomeric monomethine cyanine dyes **6a**–**6c**.

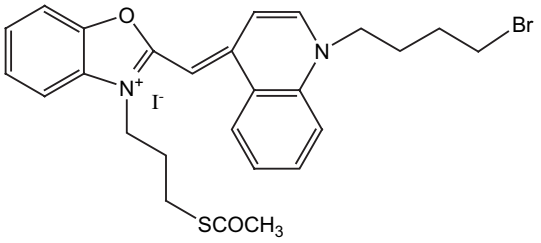



Scheme 4. Synthesis of tetracationic homodimeric monomethine cyanine dyes **7a**–**7c**.

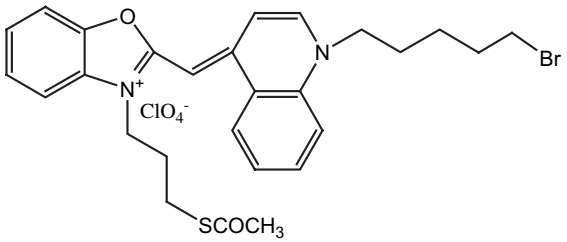
1-(3-iodopropyl)-4-[(3-(3-acetylsulfanylpropyl)-3*H*-benzooxazol-2-ylidene)methyl]quinolinium iodide **5a** [17]:



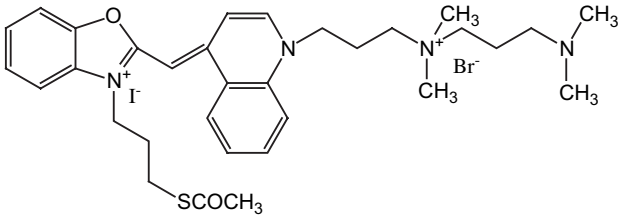
1-(4-bromobutyl)-4-[(3-(3-acetylsulfanylpropyl)-3*H*-benzooxazol-2-ylidene)methyl]quinolinium bromide **5b**:



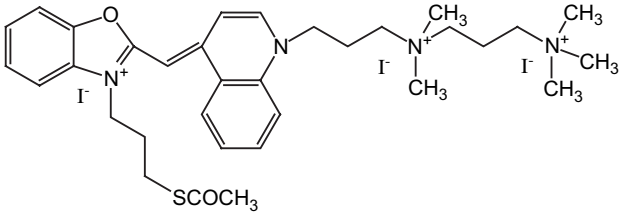
1-(5-bromopentyl)-4-[(3-(3-acetylsulfanylpropyl)-3*H*-benzooxazol-2-ylidene)methyl]quinolinium perchlorate **5c**:



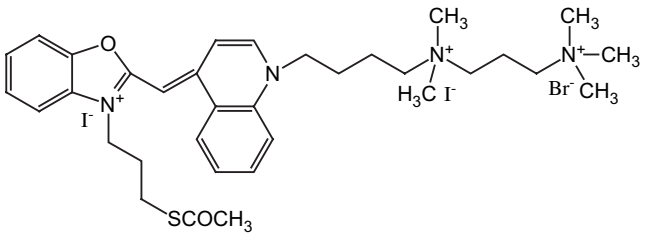
1-[((3-dimethylaminopropyl)-dimethylammonium)-propyl]-4-[(3-(3-acetylsulfanylpropyl)-3*H*-benzooxazol-2-ylidene)methyl]quinolinium bromide iodide **6a**:



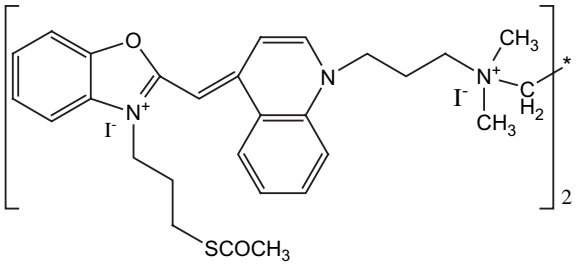
1-[3-(*N*,*N*,*N*′,*N*′,*N*′-pentamethyldiammonium-1,3-propyl)propyl]-4-[(3-(3-acetylsulfanylpropyl)-3*H*-benzooxazol-2-ylidene)methyl]quinolinium triiodide **6b**:



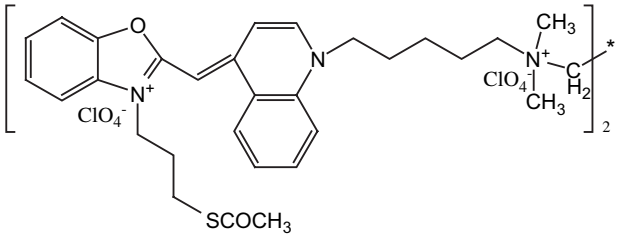
1-[4-(*N*,*N*,*N*′,*N*′,*N*′-pentamethyldiammonium-1,3-propyl)butyl]-4-[(3-(3-acetylsulfanylpropyl)-3*H*-benzooxazol-2-ylidene)methyl]quinolinium triiodide **6c**:



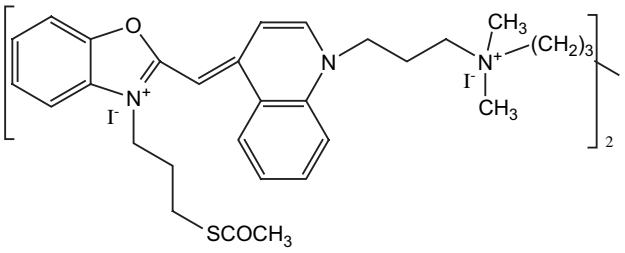
*N*,*N*,*N*′,*N*′-tetramethyl-*N*,*N*′-bis-{3-[4-[3-(3-acetylthiopropyl)-2(3*H*)-benzooxazol-2-ylidene]methyl]propylquinolinium-1-il}propandiammonium-1,3-tetraiodide **7a**:



*N*,*N*,*N*′,*N*′-tetramethyl-*N*,*N*′-bis-{5-[4-[3-(3-acetylthiopropyl)-2(3*H*)-benzooxazol-2-ylidene]methyl]pentylquinolinium-1-il}propandiammonium-1,3-tetraperchlorate **7b**:



*N*,*N*,*N*′,*N*′-tetramethyl-*N*,*N*′-bis-{3-[4-[3-(3-acetylthiopropyl)-2(3*H*)-benzooxazol-2-ylidene]methyl]propylquinolinium-1-il}hexandiammonium-1,6-tetraiodide **7c**:



All dyes are new and their chemical structures were proven by elemental analysis, UV–Vis spectra (Table 1) and $^1$H NMR spectra (Table 2).

## 3. Experimental part

Melting points were determined on a Kofler apparatus and are uncorrected. $^1H$ *NMR* spectra were obtained on a Bruker 250 MHz instrument in DMSO-$d_6$. Absorption spectra were scanned on a Specord M40 (Carl Zeiss, Jena) UV–Vis spectrophotometer ($1 \times 10^{-5}$ M in MeOH) and the corrected fluorescence spectra (excitation at 480 nm) on a Perkin Elmer MPF44 spectrofluorimeter. The emission spectra were corrected using a standard tungsten lamp, while the excitation spectra were corrected with Rhodamin B. The fluorescence

Table 1
Characterization data of dyes **5a–5c**, **6a–6c**, and **7a–7c**

| Dye | M.p. (°C) | Yield (%) | $\lambda_{max}$ nm ($\varepsilon \times 1\,mol^{-1}\,cm^{-1}$) | Molecular formulae (Mm) | Analysis | Found | |
|---|---|---|---|---|---|---|---|
| | | | | | | Calc. | |
| | | | | | C | H | N |
| **5a** | 196–198 | 67 | 480(91 000) | $C_{25}H_{26}I_2N_2O_2S$ (672.36) | 44.89 | 3.89 | 4.01 |
| | | | | | 44.66 | 3.90 | 4.17 |
| **5b** | 151–153 | 55 | 479(83 200) | $C_{26}H_{28}IN_2O_2SBr \cdot 2H_2O$ (675.42) | 46.53 | 4.71 | 4.38 |
| | | | | | 46.24 | 4.78 | 4.15 |
| **5c** | 113–115 | 76 | 478(87 900) | $C_{27}H_{30}BrClN_2O_6S$ (625.96) | 51.88 | 4.98 | 4.72 |
| | | | | | 51.81 | 4.83 | 4.48 |
| **6a** | 174–176 | 41.4 | 480(84 800) | $C_{32}H_{44}O_2N_4SIBr \cdot 1.5H_2O$ (782.62) | 49.06 | 5.46 | 7.38 |
| | | | | | 49.11 | 6.05 | 7.16 |
| **6b** | 168–170 | 56.7 | 482(78 600) | $C_{33}H_{47}O_2N_4SI_3 \cdot CH_3OH$ (976.58) | 41.65 | 5.19 | 5.69 |
| | | | | | 41.82 | 5.26 | 5.74 |
| **6c** | 152–155 | 63 | 481(73 000) | $C_{34}H_{49}O_2N_4SIBr \cdot 1.5H_2O$ (929.58) | 43.96 | 5.95 | 6.63 |
| | | | | | 43.51 | 5.58 | 5.97 |
| **7a** | 180–183 | 59.2 | 483(159 600) | $C_{57}H_{70}N_6O_4I_4S_2 \cdot CH_3OH$ (1508.01) | 45.94 | 5.14 | 6.27 |
| | | | | | 46.20 | 5.01 | 5.57 |
| **7b** | 150–152 | 61.6 | 479(162 000) | $C_{61}H_{78}Cl_4N_6O_{20}S_2$ (1421.25) | 51.70 | 5.81 | 6.51 |
| | | | | | 51.55 | 5.53 | 5.91 |
| **7c** | 198–201 | 66.7 | 482(114 000) | $C_{61}H_{78}I_4N_6O_4S_2$ (1517.04) | 47.96 | 4.78 | 5.92 |
| | | | | | 47.50 | 5.05 | 5.54 |

Table 2
$^1$H NMR-data of dyes **5a–5c**, **6a–6c**, and **7a–7c**

| Dye no | $^1$H NMR ($\delta$, ppm, DMSO-$d_6$) |
|---|---|
| **5a** | 2.08 m (2H, $CH_2$); 2.29 s (3H, $CH_3$); 2.37 m (2H, $CH_2$); 2.99 t (2H, $CH_2$); 3.34 t (2H, $SCH_2$); 4.49 t (2H, $NCH_2$); 4.62 t (2H, $N^+CH_2$); 6.32 s (H, CH); 7.4–8.8 m (10H, Ar). |
| **5b** | 1.89 m (4H, $CH_2$); 2.08 m (2H, $CH_2$); 2.29 s (3H, $CH_3$); 3.00 t (2H, $CH_2$); 3.34 t (2H, $SCH_2$); 4.49 t (2H, $NCH_2$); 4.62 t (2H, $N^+CH_2$); 6.32 s (H, CH); 7.4–8.8 m (10H, Ar). |
| **5c** | 1.38 m (2H, $CH_2$); 1.62 m (2H, $CH_2$); 1.86 m (2H, $CH_2$); 2.08 m (2H, $CH_2$); 2.29 s (3H, $CH_3$); 3.00 t (2H, $CH_2$); 3.98 t (2H, $SCH_2$); 4.49 t (2H, $NCH_2$); 4.62 t (2H, $N^+CH_2$); 6.32 s (H, CH); 7.4–8.8 m (10H, Ar). |
| **6a** | 1.97 m (2H, $CH_2$); 2.22 m (4H, $2\times CH_2$); 2.50 s (12H, $4\times CH_3$); 2.90 t (2H, $CH_2$); 3.03 s (3H, $CH_3$); 3.45 t (4H, $2\times N^+CH_2$); 4.08 t (2H, $SCH_2$); 4.46 t (2H, $NCH_2$); 4.66 t (2H, $N^+CH_2$); 6.22 s (H, CH); 7.4–8.8 m (10H, Ar). |
| **6b** | 2.08 m (2H, $CH_2$); 2.26 m (2H, $CH_2$); 2.29 s (3H, $CH_3$); 3.00 t (2H, $CH_2$); 3.11 s (6H, $CH_3$); 3.14 s (9H, $CH_3$); 3.31 t (6H, $3\times N^+CH_2$); 3.58 t (2H, $SCH_2$); 4.52 t (2H, $NCH_2$); 4.65 t (2H, $N^+CH_2$); 6.32 s (H, CH); 7.4–8.8 m (10H, Ar). |
| **6c** | 1.87 m (4H, $2\times CH_2$); 2.08 m (2H, $CH_2$); 2.20 m (2H, $CH_2$); 2.29 s (3H, $CH_3$); 3.00 t (2H, $CH_2$); 3.12 s (12H, $5\times CH_3$); 3.31 t (6H, $3\times N^+CH_2$); 4.50 t (2H, $NCH_2$); 4.67 t (2H, $N^+CH_2$); 6.33 s (H, CH); 7.4–8.8 m (10H, Ar). |
| **7a** | 2.08 m (4H, $CH_2$); 2.26 m (2H, $CH_2$); 2.29 s (6H, $2\times CH_3$); 2.29 t (4H, $2\times CH_2$); 3.14 s (12H, $4\times CH_3$); 3.31 t (8H, $4\times N^+CH_2$); 3.66 t (4H, $2\times SCH_2$); 4.49 t (4H, $2\times NCH_2$); 4.65 t (4H, $2\times N^+CH_2$); 6.3 s(2H, $2\times CH$); 7.4–8.8 m (20H, Ar). |
| **7b** | 1.38 m (4H, $2\times CH_2$); 1.62 m (6H, $3\times CH_2$); 1.86 m (4H, $2\times CH_2$); 1.97 s (6H, $2\times CH_3$); 2.20 t (8H, $4\times CH_2$); 2.50 s (12H, $4\times CH_3$); 2.87 m (4H, $2\times CH_2$); 3.98 t (4H, $2\times SCH_2$); 4.49 t (4H, $2\times NCH_2$); 4.62 t (4H, $2\times N^+CH_2$); 6.32 s (2H, $2\times CH$); 7.4–8.8 m (20H, Ar). |
| **7c** | 1.33 m (4H, $2\times CH_2$); 1.54 m (4H, $2\times CH_2$); 1.92 m (2H, $CH_2$); 2.18 m (2H, $CH_2$); 2.29 s (6H, $2\times CH_3$); 2.40 m (2H, $CH_2$); 2.79 m (2H, $CH_2$); 2.96 s (12H, $4\times CH_3$); 3.30 t (8H, $4\times N^+CH_2$); 3.53 t (4H, $2\times SCH_2$); 4.50 t (4H, $2\times NCH_2$); 4.64 t (4H, $2\times N^+CH_2$); 6.32 s (2H, $2\times CH$); 7.4–8.8 m (20H, Ar). |

quantum yield ($Q_f$) was determined relative to that of Rhodamin 6 G ($Q_f = 0.95$ in ethanol) [18].

### 3.1. Synthesis of monocationic dyes *5a–5c*

In a two-necked round-bottomed flask fitted with mechanical stirrer and reflux condenser 0.0074 mol of 3,4-dihydro-2*H*-thiazino[2,3-*b*]benzooxazolium bromide **3** and 0.0074 mol of the corresponding 1-($\omega$-iodo- or bromoalkyl)-4-methylquinolinium iodide **4a**, bromide **4b** or perchlorate **4c** were suspended in 28 ml acetic anhydride. The reaction mixture was stirred vigorously and heated to 45–50 °C for 3–5 min. After cooling to room temperature 0.0148 ml *N*-ethyldiisopropyl amine was added dropwise within 1–2 min. The reaction mixture was stirred at room temperature for 8 h. The formed precipitate was suction filtered, washed with diethyl ether and air-dried.

### 3.2. Synthesis of di- and tricationic monomeric cyanine dyes *6a–6c*

Dyes **5a–5c** of 0.0015 mol were dissolved under heating in 10 ml methoxyethanol. After the addition of 0.00074 mol **5d**, or 0.0015 mol **5e** the reaction mixture was refluxed and stirred vigorously for 9 h (TLC monitoring). After cooling to room temperature the precipitate of the formed dye was suction filtered and air-dried. The dyes were recrystallized from methanol.

### 3.3. Synthesis of tetracationic homodimeric monomethine cyanine dyes *7a–7c*

Dye **5a** or **5c** of 0.0015 mol was dissolved under heating in 10 ml methoxyethanol and 0.00074 mol *N*,*N*,*N*′,*N*′-tetramethylpropandiamine **5d** was added. The reaction mixture was refluxed and stirred vigorously for 9 h (TLC monitoring). After cooling to room temperature the precipitated dye was suction filtered and air-dried. Dyes **7a–7c** were recrystallized from methanol.

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