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A novel general method for preparation of neutral monomethine cyanine dyes

Todor Deligeorgiev*, Stefka Kaloyanova, Aleksey Vasilev

University of Sofia, Faculty of Chemistry, 1, James Bourchier Avenue, 1164 Sofia, Bulgaria

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ABSTRACT

Two novel general experimental procedures for preparation of neutral monomethine cyanine dyes without using a basic agent have been developed. The procedures allow synthesis of cyanine dyes by reaction of substituted methylthio aromatic heterocycles with different quaternized nitrogen containing heterocycles by simple melting or by their reaction in acetic anhydride for short reaction times. The proposed method is very versatile as a variety of different starting materials can be used.

1. Introduction

Recently, cyanine dyes have found applications in numerous diverse areas – biological, medical and drug development, imaging of bio-targets – molecules, cells and organelles and conformational studies by fluorescence energy transfer [1–4]. Their excellent staining properties make them useful fluorescent probes in such methods as flow cytometry, for the detection of nucleic acids in solution, gel electrophoresis and in fluorescence microscopy [5]. The wide spectrum of their biological applications (one of the "high-tech" fields) prompted researchers to investigate new methods for the synthesis.

Monomethine cyanine dyes are among the most important organic functional dyes. They are generally synthesized by the reaction of two heterocyclic quaternary salts, one bearing a reactive methyl group and the other a quaternized heterocycle containing a thioalkyl which is a good leaving group, in the presence of base [6]. In our earlier investigations we presented environmentally friendly novel procedures to synthesize monomethine cyanine dyes, avoiding some of the drawbacks of the alkylthio-method [7,8]. The continued interest in the development of the novel and the improvement of known synthetic procedures with reduced environmental impact has influenced our research program. In this study we present a novel method to prepare neutral monomethine cyanine dyes.

2. Experimental

^{1}H NMR spectra were recorded on a Bruker Avance DRX 250 MHz and AV 600 MHz instruments in DMSO-d_6. Elemental analyses were performed on a Vario 3 instrument. Absorption spectra were scanned on a Cecil Aurius CE 3021 UV–VIS spectrophotometer (1.10^{-5} mol/l in CH_3OH). Melting points were determined on a Kofler apparatus and are uncorrected. The progress of the reactions was monitored by TLC (Merck F 254 silica gel; CH_2Cl_2:CH_3OH:CH_3COOH 86:13:1). All starting materials and solvents were commercial products from Sigma–Aldrich, except for compounds **2b**, **8**, which were synthesized by known procedures [9,10].

2.1. Method A

Quaternary heterocyclic 2-methylthio **1**, **5a** – **5d** (1 mmol) and the heterocyclic compounds bearing a reactive methyl group **2a** – **2e** (1.1 mmol) or the quaternary heterocyclic 2-methyl salt **7a** – **7c** (1 mmol) and 4-methylthio heterocyclic compound **8** (1 mmol) were melted together and the reaction mixture was heated and stirred at the appropriate temperature and time (Table 1). The melt was diluted with 10 ml ethanol and cooled. The dyes, which precipitate in the alcoholic solution, were directly isolated by filtration. Where the products precipitated as oils, the isolation was carried out by adding a saturated aqueous solution of potassium iodide or sodium perchlorate (anion exchange). The precipitated dye was filtered and air dried.

* Corresponding author. Tel.: +359 2 8161 269; fax: +359 2 8625438.
E-mail address: toddel@chem.uni-sofia.bg (T. Deligeorgiev).

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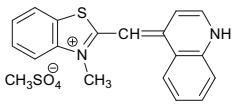
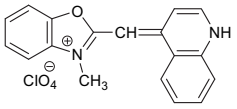
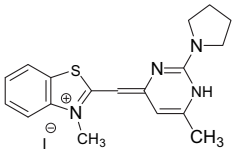
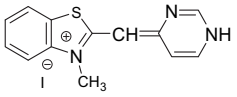
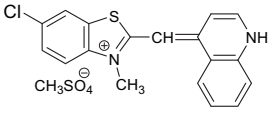
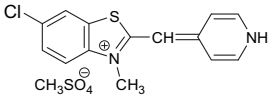
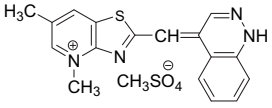
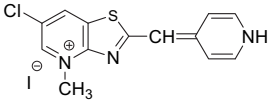
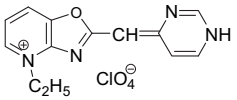
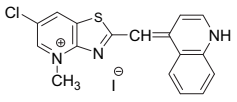
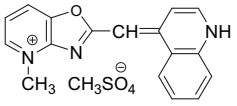
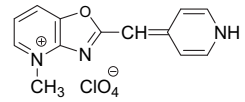
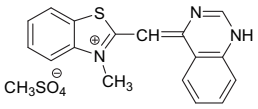
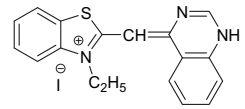
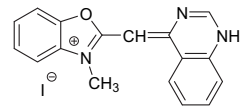
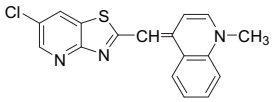
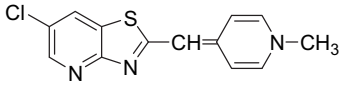
Table 1
Structures of dyes **3a** – **3d**, **6a**–**6f**, **9a**–**9c**, **12** and **13** reaction times and isolated yields by **Method A** and **Method B**.

Dye	Structure	Method	Time [min]	Temperature [°C]	Yield [%]
3a		A	10	100	91
		B	20	90	79
3b		A	40	55–60	63
		B	20	60	79
3c		A	10	100	57
		B	25	70	45
3d		B	25	80	41
3e		A	no reaction		
		B	15	70	95
3f		A	no reaction		
		B	20	90	93
6a		A	10	100	52
6b		B	25	70	21
6c		A	no reaction		
		B	60	110	43
6d		A	no reaction		
		B	20	90	49
6e		B	60	90	37

(*continued on next page*)

Table 1 (*continued*)

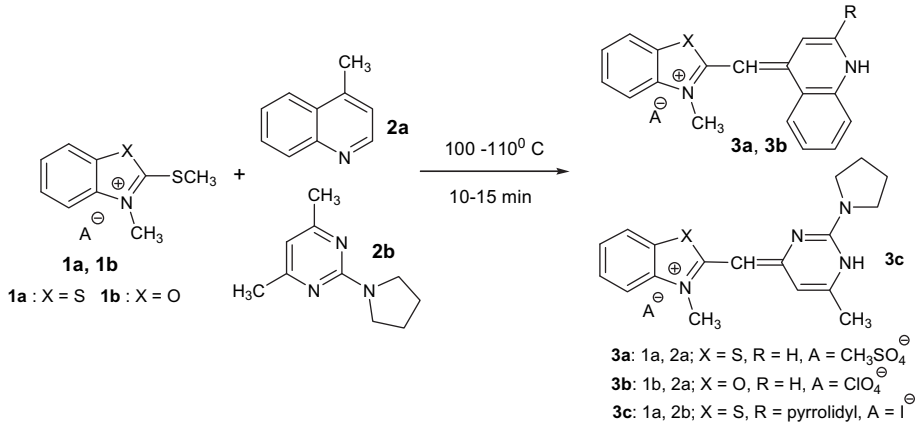
Dye	Structure	Method	Time [min]	Temperature [°C]	Yield [%]
6f		B	60	80	33
9a		A B	5 30	100 80	59 53
9b		A B	10 30	110 100	62 50
9c		A B	15 25	100 70	45 35
12		A	25	120	64
13		A	15	90	57

2.2. Method B

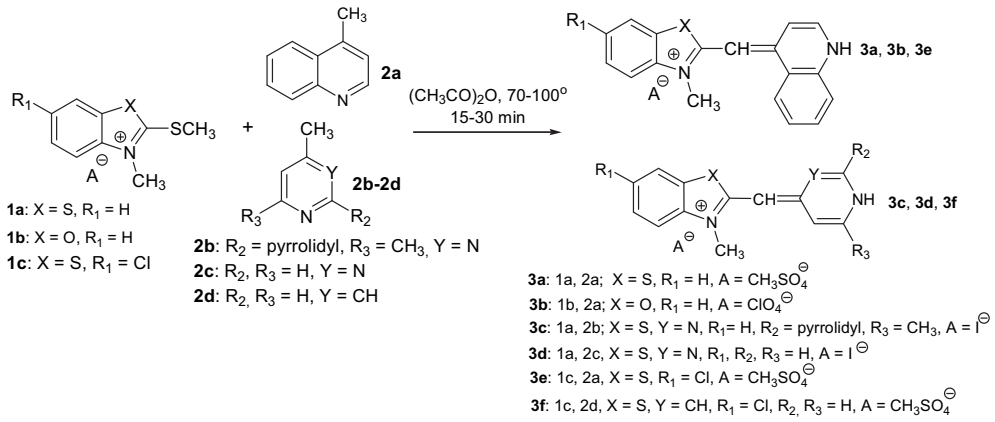
Quaternary heterocyclic 2-methylthio **1**, **5a** − **5d** (1 mmol) and the heterocyclic compound bearing a reactive methyl group **2a** − **2e** (1.1 mmol) or the quaternary heterocyclic 2-methyl salt **7a** − **7c** (1 mmol) and 4-methylthio heterocyclic compounds **8** (1 mmol) were stirred and heated together in 2–4 ml acetic anhydride at the appropriate temperature and time (Table 1). The reaction mixture was cooled, diluted with 15 ml diethyl ether and the precipitate was filtered and air dried. Products, which precipitate as oils were dissolved in 10–15 ml ethanol and were worked-up as in *Method A* by anion exchange.

2.3. Preparation of the neutral forms of the synthesized dyes

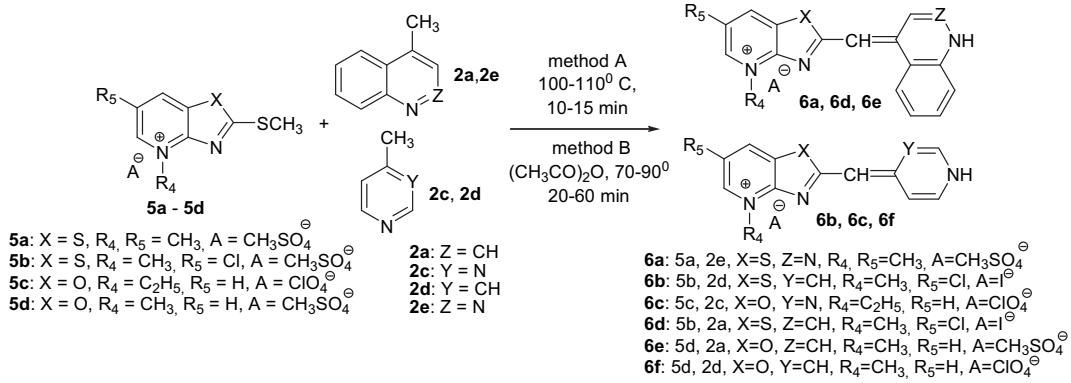
The neutral forms of the dyes in *Method A* and *Method B* were obtained before the anion exchange procedure, by dissolving the precipitate or the oil in water and adding NH_3. The unprotonated dyes were filtered and air dried.



Scheme 1. Synthesis of monomethine cyanine dyes in solvent-free conditions without using basic agent (Method A).



Scheme 2. Synthesis of monomethine cyanine dyes in acetic anhydride without using of a basic agent (Method B).



Scheme 3. Synthesis of dyes **6a**–**6f** by **Method A** or **Method B**.

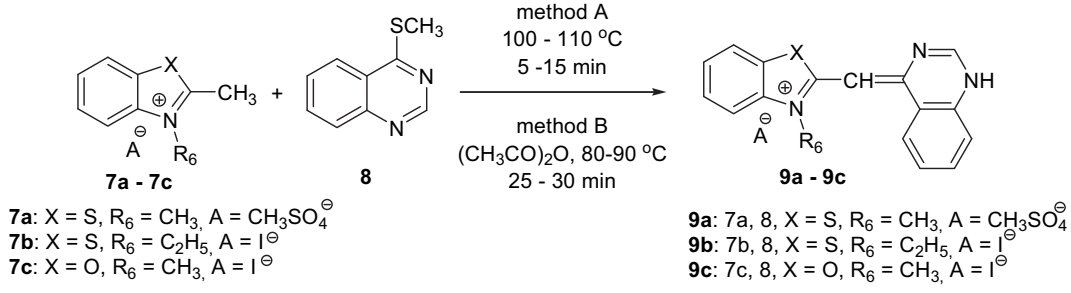
Analytical samples of the dyes were obtained by recrystallization from ethanol.

3. Results and discussion

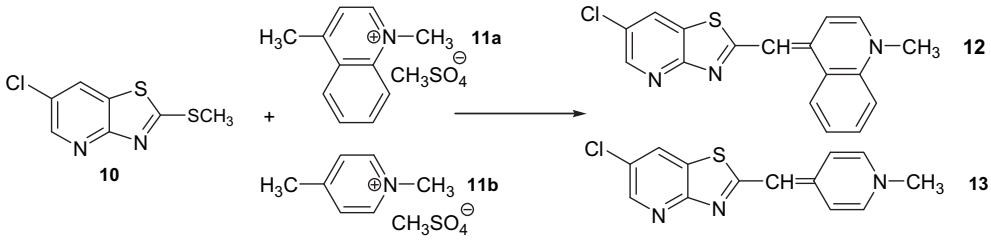
The synthesis of photostable dyes is very important from practical points of view – the analytical applications of the some cyanine dyes requires long fluorescence lifetimes and good fluorescence quantum yields. These features could be achieved by alteration of the molecular structure of the dyes; therefore diverse and improved synthetic approaches are of significant importance to progress this area.

As a part of our investigations into monomethine cyanine dyes, we developed syntheses for dye structures using a mixture of a quaternary heterocyclic methylthio salt (**1**) and a heterocyclic compound bearing a reactive methyl group (**2**) in solvent-free conditions and without the presence of a basic agent, which is obligatory in all other reported methods thus far [11]. We aim to simplify the known alkylthio-method by avoiding the use of two quaternary compounds, which will reduce the synthetic steps by obtaining derivatives as starting materials for the monomethine cyanine dyes synthesis (Scheme 1).

Direct heating of the starting materials allows shorter reaction times and, furthermore, avoids one of the main drawbacks of the alkylthio-method– the possibility of interchange of the alkyl groups at the sulphur and nitrogen atoms in the quaternized starting materials in the presence of a strong base, which gives unexpected reaction products [12–14]. The products were only isolated upon dilution with small amounts of ethanol followed by filtration.



Scheme 4. Synthesis of dyes **9a**–**9c**.



Scheme 5. Synthesis of neutral dyes **12** and **13**.

A detailed study on these syntheses showed that this approach works well only if one of the starting materials is a liquid, or if both have similar melting points. If the melting points of the starting materials vary widely, the method either does not proceed at all, or proceeds only partially, since decomposition of the starting derivative with the lower melting point is faster than dye formation. For this reason, we performed the same reaction using acetic anhydride as solvent in the temperature range 70–100 °C (Scheme 2). In all cases the reaction progress was monitored by TLC.

Supplementary experiments were carried out to find the optimal reaction temperature in acetic anhydride (Table 1). An exact temperature value, valid for all of the synthesized structures, cannot be specified, but best yields under these conditions were obtained in the temperature range 70–100 °C. Heating some of the dyes to higher temperatures, or boiling them in acetic anhydride, produced undesired side products – we believe this results from an autocondensation process, which was monitored by TLC.

Table 2
Melting points, elemental analysis and ^{1}H NMR data of the synthesized dyes.

Dye	M.p. [°C]	Elemental analyses				^{1}H NMR (250, 600 MHz, DMSO-d_6) δ(ppm)
		C % calc. found	H % calc. found	N % calc. found	S % calc. found	
3a	140–145	56.70 / 56.23	4.51 / 4.12	6.96 / 7.08	15.93 / 15.57	3.65 s (3H, –NCH_3); 6.46 s (1H, –CH=); 7.07 t (1H, ArH); 7.27–7.35 m (2H, ArH); 7.41 d (1H, ArH); 7.53–7.75 m (3H, ArH); 7.95 d (1H, ArH); 8.42 d (1H, ArH); 8.74 d (1H, ArH); 14.01 brs (1H, NH);
3b	128–132	56.69 / 56.32	4.03 / 4.28	7.47 / 7.09	–	3.91 s (3H, –NCH_3); 6.29 s (1H, –CH=); 7.28–7.36 m (3H, ArH); 7.40 d (2H, ArH); 8.21–8.33 m (2H, ArH); 8.46–8.55 m (2H, ArH); 8.76 d (1H, ArH); 13.70 brs (1H, NH);
3c	192–196	47.49 / 47.13	4.68 / 4.09	12.39 / 11.98	7.09 / 6.77	2.02 s (3H, –CH_3)2.17 t (4H, CH_2NCH_2); 2.19–2.33 m (4H, CH_2CH_2); 4.12 s (3H, –NCH_3); 6.71 s (1H, –CH=); 7.32 s (1H, ArH); 7.71–7.73 m (2H, ArH); 7.79–7.66 m (2H, ArH); 13.91 brs (1H, NH);
3d	210 (decomposed)	42.29 / 41.93	3.28 / 3.19	12.38 / 11.98	8.68 / 8.77	3.96 s (3H, –NCH_3); 6.51 s (1H, –CH=); 6.64 d (1H, ArH); 6.71 d (1H, ArH); 7.52–7.71 m (3H, ArH); 7.85 d (1H, ArH); 8.61 s (1H, ArH)
3e	270–272	52.23 / 51.85	3.92 / 3.72	6.41 / 6.07	14.68 / 14.27	3.97 s (3H, –NCH_3); 6.87 s (1H, –CH=); 7.38 d (1H, ArH); 7.63 dd (1H, ArH); 7.73–7.75 m (2H, ArH); 7.89–7.96 m (2H, ArH); 8.17 d (1H, ArH); 8.63 d (1H, ArH); 8.73 d (1H, ArH); 14.01 brs (1H, NH);
3f	160–165	46.57 / 46.15	3.91 / 3.77	7.24 / 6.87	16.58 / 16.37	3.95 t (3H, –NCH_3); 6.85 s (1H, –CH=); 7.21 d (2H, ArH); 7.30 d (1H, ArH); 7.60 dd (1H, ArH); 7.70 d (1H, ArH); 8.00–8.06 m (2H, ArH); 14.05 brs (1H, NH);
6a	>300	51.66 / 51.15	4.34 / 2.92	13.39 / 12.97	15.32 / 14.86	3.50 s (3H, –CH_3); 3.73 s (3H, N^+–CH_3); 6.85 s (1H, –CH=); 7.12 s (1H, ArH); 7.64 t (1H, ArH); 7.86–7.95 m (2H, ArH); 8.28 d (1H, ArH); 8.73 d (1H, ArH); 8.86 s (1H, ArH); 14.01 brs (1H, NH);
6b	142–146	38.68 / 38.25	2.75 / 2.37	10.41 / 11.99	7.94 / 7.56	3.72 s (3H, –NCH_3); 6.34 s (1H,=CH–); 7.52 d (1H, ArH), 7.57 d (1H, ArH), 8.36 d (1H, ArH), 8. 91 m (2H, ArH), 9.48 d (1H, ArH); 13.97 brs (1H, NH);
6c	194–198	45.83 / 45.35	3.85 / 3.28	16.44 / 16.07	–	1.47 t (3H, –NCH_2–CH_3); 4.80 q (2H, –NCH_2–CH_3); 6.61 s (1H, –CH=); 6.64 d (1H, ArH); 6.71 d (1H, ArH); 7.52 s (1H, ArH); 7.83–7.95 m (3H, ArH); 13.92 brs (1H, NH);
6d	210–214	45.00 / 44.75	2.89 / 2.47	9.26 / 9.19	7.07 / 6.86	4.13 s (3H, –NCH_3); 6.91 s (1H, –CH=); 7.55 d (1H, ArH); 7.67–7.75 m (2H, ArH); 8.25 d (1H, ArH); 8.40–8.43 m (2H, ArH); 8.51–8.60 m (2H, ArH); 13.31 brs (1H, NH);
6e	140–144	55.80 / 55.22	4.42 / 4.12	10.85 / 10.39	8.28 / 7.94	4.16 s (3H, –NCH_3); 6.95 s (1H, –CH=); 7.57 d (1H, ArH); 7.70–7.77 m (2H, ArH); 8.29 d (1H, ArH); 8.42–8.44 m (2H, ArH); 8.54–8.62 m (3H, ArH); 13.35 brs (1H, NH);
6f	192–198	47.94 / 47.42	3.71 / 3.18	12.90 / 12.43	–	3.71 s (3H, –NCH_3); 6.37 s (1H,=CH–); 7.54 d (1H, ArH), 7.59d (1H, ArH), 8.38 d (1H, ArH), 8.45–8.57 m (3H, ArH), 8.78 d (1H, ArH); 13.99 brs (1H, NH);
9a	272	46.69 / 46.92	3.69 / 3.58	9.61 / 9.20	7.33 / 6.96	3.86 s (3H, –NCH_3), 6.57 s (1H, –CH=), 7.52 t (1H, ArH), 7.59–7.64 m (2H, ArH), 7.69 t (1H, ArH), 7.75 d (1H, ArH), 7.81 d (1H, ArH), 7.91 t (1H, ArH), 8.60 s (1H, ArH), 8.65 d (1H, ArH);
9b	>300	48.88 / 48.53	3.87 / 3.85	9.50 / 9.37	7.25 / 6.90	1.42 t (3H, –NCH_2–CH_3); 4.84 q (2H, –NCH_2–CH_3); 7.33 t (1H, –CH=); 7.54 t (1H, ArH); 7.66–7.73 m (3H, ArH); 7.92–7.96 m (2H, ArH); 8.16 d (1H, ArH), 8.74 d (1H, ArH); 8.88 s (1H, ArH);
9c	280–282	48.47 / 48.24	3.83 / 3.50	9.98 / 9.60	–	3.96 s (3H, –NCH_3); 6.51 s (1H, –CH=); 7.53 t (1H, ArH); 7.59 t (1H, ArH); 7.72 t (2H, ArH); 7.80 d (1H, ArH); 7.86 d (1H, ArH); 7.96 t (1H, ArH); 8.63 s (1H, ArH); 8.66 d (1H, ArH);
12	126–128	62.67 / 62.23	3.71 / 3.25	12.90 / 12.67	9.84 / 9.52	2.85 s (3H, –CH_3); 6.81 s (1H, –CH=); 7.15 d (1H, ArH); 7.62 t (1H, ArH); 7.76–7.85 m (2H, ArH); 8.29–8.34 m (2H, ArH); 8.75 d (1H, ArH); 8.89 s (1H, ArH);
13	122–126	56.62 / 56.29	3.60 / 3.25	15.24 / 15.17	11.63 / 11.12	2.85 s (3H, –CH_3); 6.89 s (1H, –CH=); 6.92 d (2H, ArH); 7.05 d (2H, ArH); 7.92 d (1H, ArH); 8.74 d (1H, ArH);

The underlined protons from the NMR spectra data give the protons, which are assigned as triplet or quartet.

Table 3
Absorption maxima and molar absorptivities of the protonated and neutral forms of dyes **3a** – **13**.

Dye (protonated form)[a]	λ_{max} [nm] (protonated form)	ε [l mol^{-1} cm^{-1}] (protonated form)	λ_{max} [nm] (neutral form)[b]	ε [l mol^{-1} cm^{-1}] (pH = 8, neutral form)
3a	492	58 100	427	33 500
3b	466	17 200	408	9500
3c	420	33 500	397	30 200
3d	436	20 900	398	15 800
3e	492	20 000	423	9800
3f	498	28 800	419	19 900
6a	556	79 900	548	58 400
6b	502	23 000	484	21 200
6c	455	22 500	decomposition	
6d	548	96 800	decomposition	
6e	461	27 600	decomposition	
6f	465	21 300	decomposition	
9a	443	49 800	442	59 000
	471	45 300		
9b	446	64 600	443	71 100
	471	61 800		
9c	413	43 500	423	52 900
	436	42 900		
12	543	10 500	548	73 500
13	498	12 000	505	37 900

[a] 1 drop HCl was added to the dye solution.
[b] 1 drop NH_3 was added to the dye solution.

The use of acetic anhydride as solvent did not significantly change the yields of the dyes obtained by either method. In all cases, the heating times for Method B were greater than, for Method A.

The structures **3a**–**3f** obtained by this procedure could be used as starting materials for further quaternization reactions with alkyl, substituted alkyl, aralkyl, substituted aralkyl halides or with other quaternization agents. They could also be isolated as neutral forms by dissolving in water/NH_3 solution followed by filtration of the deprotonated compounds.

A literature survey has revealed only two patented synthetic procedures for obtaining of neutral monomethine cyanine dyes using quaternary 3-methyl-2-methylthiobenzoxa(thia)zolium salts and heterocyclic compounds with a reactive methyl group (2- or 4-methylquinoline and its derivatives or 4-methylpyridine) as starting materials [15,16]. In one of the procedures described [15] acetic anhydride is used as solvent and the reaction mixture is heated for 4 h in the presence of DMF. Larive and co-workers described in a patent [16] method for synthesizing of neutral monomethine cyanine dyes, which requires use of sulfones as starting materials, as well as the presence of triethylamine as base. All other reported procedures to obtain asymmetric monomethine cyanine dyes, including thia(oxa)zolopyridinum and quinazolinium moieties, are based on the alkylthio-method [12,17–22]. The method described in a patent [21] requires an additional step of dequaternization to prepare the neutral cyanine dyes. We successfully synthesized dyes by a simpler method, reacting 4-methyl-2-(methylthio)thiazolopyridinium and 4-methyl-2-(methylthio)oxazolopyridinium moieties by the new Methods A or B (Scheme 3).

These initial results prompted us to carry out additional experiments. We found that the monomethine cyanine dye structure can also be obtained by melting together 2-methyl-3-alkylbenzothia(oxa)zolium salt (**7a**–**7c**) and 4-methylthio heterocyclic compound (**8**) without using a basic agent (Scheme 4).

The neutral forms of the dyes **9a**–**9c** could be obtained by dissolving them in water/NH_3 and filtration of the deprotonated compounds.

By melting together the 2-methylthiothiazolopyridinium compound with the quaternary 4-methylthio heterocyclic compound without the use of solvent, a neutral monomethine cyanine dye was obtained (Scheme 5).

The reaction times and yields of dyes **3a**–**3f**, **6a**–**6f**, **9a**–**9c**, **12** and **13** are summarized in Table 1.

The synthesized monomethine cyanine dyes **3a** – **3f**, **6a** – **6f**, **9a** – **9c** could be used in their neutral forms after workup in H_2O/NH_3 or directly as derivatives to synthesize novel dyes. The structures **3a** and **3b** were previously reported as fluorescent stains for nucleic acids or oligonucleotides in cells, gels and solutions [15,23]. The quaternized analogs of **9a**–**9c** were recently patented as dyes more sensitive to dsDNA and useful for real time PCR [24].

The analytical samples of the synthesized dyes were obtained by recrystallization in ethanol and their structures were evaluated by ^{1}H NMR spectra and elemental analysis (Table 2).

The spectral characteristics of the synthesized dyes – protonated and neutral forms, were investigated in CH_3OH at room temperature (Table 3).

In summary, we report two simple novel experimental procedures to prepare neutral monomethine cyanine dyes without using a basic agent. These approaches require shorter reaction times, have an easy workup procedure and reduce the synthetic steps to obtain neutral monomethine cyanine dyes. The method gives products with good to high yields and with a high purity. This novel general method, according to us, is superior in comparison with the known procedures and methods by simplicity and versatility. Moreover the novel method provides numerous possibilities to prepare classical cyanine dyes by quaternization with different alkylation agents.

References

[1] Hilal H, Taylor JA. Cyanine dyes for the detection of double stranded DNA. J Biochem Biophys Methods 2008;70:1104–8.
[2] Deligeorgiev T, Gadjev N, Vasilev A, Drexhage K-H, Yarmoluk SM. Synthesis of novel monomeric and homodimeric cyanine dyes with thioacetyl substituents for nucleic acid detection. Dyes Pigm 2007;72:28–32.
[3] Yarmoluk SM. Symmetric cyanine dyes for detecting nucleic acids. Biotech Histochem 2008;83:1–15.
[4] El-Shishtawy RM, Santos CR, Goncalves I, Marcelino H, Almeida P. New amino and acetamido monomethine cyanine dyes for the detection of DNA in agarose gels. Bioorg Med Chem 2007;15:5537–42.
[5] Haughland RP. Molecular probes. Handbook of fluorescent probes and research chemicals. Eugene: Molecular Probes; 2002. p. 679.
[6] Brooker LGS, Keyes GH, Williams WW. Colour and Constitution. V[1]. The absorption of unsymmetrical cyanines. Resonance as a basis for a classification of dyes. J Am Chem Soc 1942;64:199–210.

[7] Deligeorgiev T, Zaneva DA, Katerinopoulos HE, Kolev VN. A novel method for the preparation of monomethine cyanine dyes. Dyes Pigm 1999;41:49–54.
[8] Deligeorgiev T, Gadjev N, Drexhage K-H. Preparation of intercalating dye thiazole orange and derivatives. Dyes Pigm 1995;29:315–22.
[9] Pinner A. Über pyrimidine. Ber Dtsch Chem Ges 1889;22:1612–35.
[10] Kreutzberger A, Tesch UH. 2-Methoxy- and 2-(methylthio)pyrimidines. Chem Ber 1976;109:3255–61.
[11] Mishra A, Behera RK, Behera PK, Mishra BK, Behera GB. Cyanines during the 1990s: a review. Chem Rev 2000;100:1973–2011.
[12] Beilenson B, Hamer FM. Preparation of simple cyanines. J Chem Soc; 1939:143–51.
[13] Sexton WA. Reactions of benzthiazole derivatives. Part I. The reactivity of the methylthiol group in quaternary salts of 1-methylthiobenzthiazole. J Chem Soc; 1939:470–3.
[14] Hamer FM. The cyanine dyes and related compounds. New York: Interscience; 1964. pp. 58–59.
[15] Yue ST. Neutral unsymmetrical cyanine dyes. US Patent 5656449; 1997 (Molecular Probes, Inc.).
[16] Larive H, Collet PD. Method for preparing cyanine bases. US Patent 2 798 066; 1957 (Eastman Codac Company, Rochester).
[17] Lee LG, Mize PD. Novel fluorescent dye. US Patent 4 937 198; 1990 (Becton, Dickinson and Company).
[18] Josel H-P, Heindl D, Irlinger B, Weilke C. New ds DNA binding fluorescent dyes. WO 2008052742; 2008 (Roche Diagnostics GMBH).
[19] Gromov SP, Razinkin MA. Reaction of quinazoline derivatives with quaternary salts of cheterocyclc bases. Khim Geterotsikl Soedin 1992;5:662–70.
[20] Bethge L, Venkatrao Jarikote D, Seitz O. New cyanine dyes as base surrogates in PNA: forced intercalation probes (FIT-probes) for homogeneous SNP detection. Bioorg Med Chem 2008;16:114–23.
[21] Nakamura K, Takeuchi K. Method for optical measurement of multi-stranded nucleic acid. European Patent EP 1889880 A2; 2008 (FUJIFILM Corporation).
[22] Yue ST, Haugland RP. Dimers of unsymmetrical cyanine dyes containing pyridinium moieties, US Patent 5410030; 1995 (Molecular probes Inc.).
[23] Socher E, Jarikote DV, Knoll A, Röglin L, Burmeister J, Seitz O. FIT probes: peptide nucleic acid probes with a fluorescent base surrogate enable real-time DNA quantification and single nucleotide polymorphism discovery. Anal Biochem 2008;375:318–30.
[24] Weidong CAO, Rockville MD. Unsymmetrical cyanine dyes for high resolution nucleic acid melting analysis. US Patent 0167279; 2010 (CANON U.S. Life Sciences Inc.).