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A novel method for the preparation of monomethine cyanine dyes

Todor G. Deligeorgiev [a,*], Daphinka A. Zaneva [a],
Haralambos E. Katerinopoulos [b], Vihren N. Kolev [a]

[a] *Faculty of Chemistry, University of Sofia, 1 J. Bourchier Avenue, 1126 Sofia, Bulgaria*
[b] *Department of Chemistry, University of Crete, PO Box 1470, Iraklion, 71 409 Crete, Greece*

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Abstract

A novel method for the preparation of symmetric and asymmetric monomethine cyanine dyes was developed. Quaternary salts of a heterocyclic 2- or 4-methyl compound and *N*-heterocyclic 2- or 4-sulfobetainic compounds were reacted by simple melting or by boiling in different solvents in the absence of a basic compound. The preparation is fast and the yields are good to high, exceeding in some cases those by other methods. The novel method is environmentally more friendly. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Novel synthetic method; Monomethine cyanine dyes; Short reaction time

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1. Introduction

In the last several years there has been extensive growth [1–3] in the syntheses and applications of polymethine dyes as non-covalent labels to nucleic acid detection. Such dyes cover the visible and near infrared spectral region [3]. There are mono-, tri- and pentamethine cyanines, which are used as non-covalent nucleic acid labels. Most numerous are the asymmetric monomethine cyanine dyes used as nucleic acid stains. Among cyanines, they are the best non-covalently binding nucleic acid labels, with respect to their most important property—high fluorescence signal. With increasing length of the polymethine chain, the fluorescence quantum yield decreases upon binding with nucleic acids [2].

Since the discovery by Lee et al. [4] that the monomethine asymmetric dye 1-methyl-4-[(3-methylbenzothiazoline-2-ylidene)methyl]quinolinium tosylate (Thiazole Orange) has excellent properties as a biological non-covalent DNA or RNA label, many new useful representatives have been synthesized [5–7] and patented [8–11]. Most of the preparations of these dyes are based on the method in which quaternary salts of heterocyclic 2- or 4-alkylthiocompounds are reacted by heating in the presence of a basic reagent with a quaternary salt having a reactive methyl group [7–12]. This method has substantial drawbacks:

- evolution of methyl thiol—a very strong pollutant with a long-lasting unpleasant odour;

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* Corresponding author. Fax: +359-2-962-5438; *E-mail address*: toddel@chem.uni-sofia.bg

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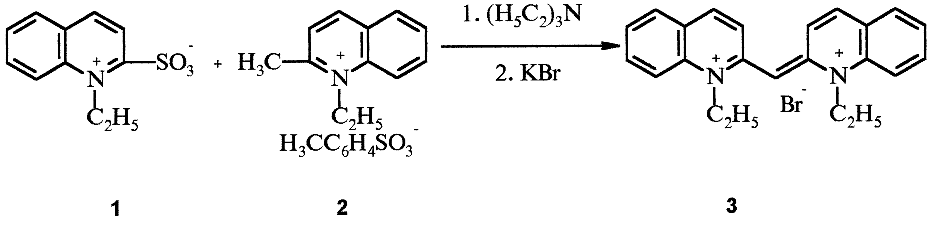
- in some cases an interchange between *S*-alkyl and *N*-alkyl substituents in the starting quaternized 2-alkylthioheterocycles is possible, leading to unexpected products [12];
- in the case of 2-alkylthiobenzoxazoles, the modification called 'fusion process' gives lower yields, because of the instability of such heterocycles in alkaline media.

In our search of more environmentally friendly methods, we have presented procedures [5,6] avoiding some of the drawbacks of the above mentioned alkylthio method. In this study we describe a novel method for the preparation of symmetric and asymmetric monomethine cyanine dyes.

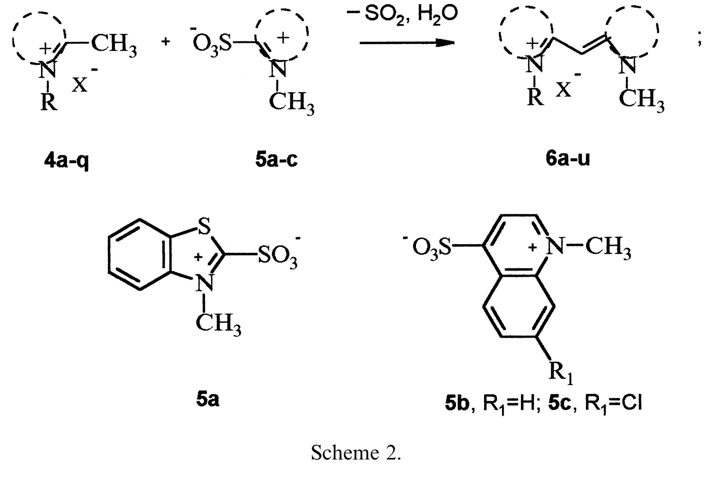
2. Results and discussion

It is known [13,14] that sulfobetaines from *N*-alkylheterocyclic compounds can react in the presence of a basic reagent with quaternary salts of heterocyclic 2- or 4-methylcompounds, thus giving monomethine cyanine dyes (Scheme 1).

We found that the preparation of monomethine cyanine dyes can be carried out by heating together a sulfobetaine from an *N*-alkylheterocyclic compound and the quaternary salt of a heterocyclic 2- or 4-methylcompound (Scheme 2) in the absence of a basic agent. The syntheses can be performed by simple melting of the starting compounds, or by boiling in common solvents or solvent mixtures. In both cases there is a strong evolution of an acid gas, which we believe to be SO_2. In the preparation by melting, the process is very fast—from 1 to several minutes. The applicability of this modification depends on the melting points of the compounds and their relative thermostability. If they are not sufficiently thermostable, the decomposition of the compounds is faster than the formation of the dye. Usually, the temperature range from 150–250°C is suitable for the preparation of monomethine dyes by the melting procedure. For less thermostable intermediates, preparation in boiling polar solvent or solvent mixtures is more suitable. Solvents or solvent mixtures, boiling in the temperature range 100–200°C can be utilised. The process duration in



Scheme 1.



Scheme 2.

boiling solvents is from 5 to 120 min, most often 15–20 min. In both modifications the yields are from good to excellent and sometimes reach 95–100%. Because of the absence of basic agent, monomethine cyanines derived from quaternized 2-methylbenzoxazolium salts can be also obtained (Tables 1 and 2). It is known that such heterocycles are easily hydrolyzed to 2-*N*-alkylacetaminophenols in 90% ethanol, alkali media or even in pure aqueous solutions [15,16]. In methods involving basic agents, such preparations are impossible, or in the best case (fusion method), are with very low yields [17]. By the above procedure we were able to prepare asymmetric monomethine cyanines from quaternized 3-methylbenzisoxazole, not synthesized so far.

Table 1
Molecular structures of intermediates **4a–4q** and dyes **6a–6u**

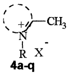
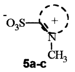
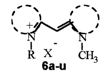
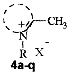
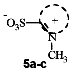
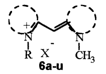
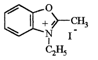
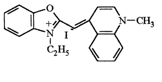
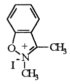
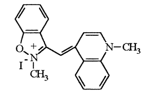
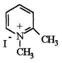
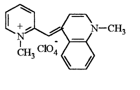
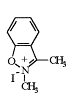
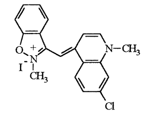
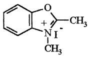
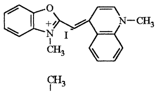
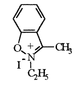
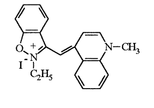
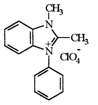
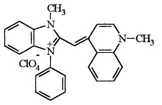
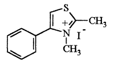
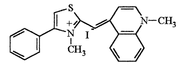
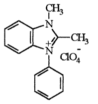
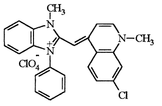
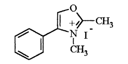
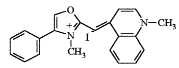
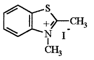
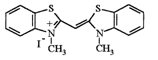
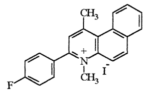
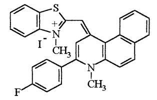
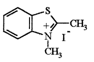
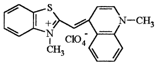
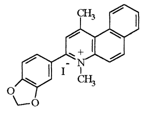
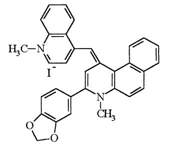
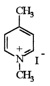
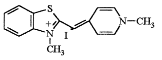
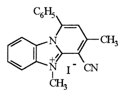
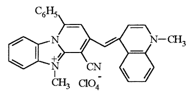
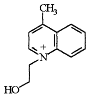
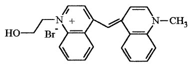
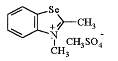
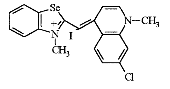
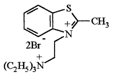
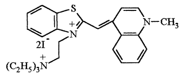
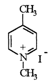
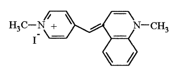
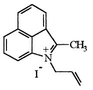
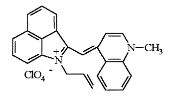
 4a-q	 5a-c	 6a-u	DYE	 4a-q	 5a-c	 6a-u	DYE
4a 	**5b**		**6a**	**4j** 	**5b**		**6l**
4b 	**5b**		**6b**	**4j** 	**5c**		**6m**
4c 	**5b**		**6c**	**4k** 	**5b**		**6n**
4d 	**5b**		**6d**	**4l** 	**5b**		**6o**
4d 	**5c**		**6e**	**4m** 	**5b**		**6p**
4e 	**5a**		**6f**	**4n** 	**5a**		**6q**
4e 	**5b**		**6g**	**4o** 	**5b**		**6r**
4f 	**5a**		**6h**	**4p** 	**5b**		**6s**
4g 	**5b**		**6i**	**4q** 	**5c**		**6t**
4h 	**5b**		**6j**	**4f** 	**5b**		**6u**
4i 	**5b**		**6k**				

Table 2
Characterization data for dyes **6a**–**6u**

Dye	Method (reaction time) Yield (%)	m.p. (°C)	λ_{max} nm ϵ ($L\,mol^{-1}\,cm^{-1}$)	Molecular formula	Analysis (%) Found/calc.		
					C	H	N
6a	A(5)	310–311	480	$C_{20}H_{19}IN_2O$	55.5	4.5	6.6
	65		(64300)		55.8	4.5	6.5
6b	B_1(30)	216–217	525	$C_{17}H_{17}ClN_2O_4$	59.2	4.7	7.6
	84		(62500)		58.6	4.9	7.0
6c	B_1(30)	299–300	478	$C_{19}H_{17}IN_2O$	53.5	4.2	6.3
	70		(70300)		53.6	4.8	6.6
6d	B_1(5)	236–237	478	$C_{25}H_{22}ClN_3O_4$	64.5	5.1	9.5
	70		(39500)		64.7	4.8	9.1
6e	B_1(5)	268–269	480	$C_{25}H_{21}Cl_2N_3O_4$	60.2	4.1	8.2
	70		(33000)		60.2	4.2	8.4
6f	B_1(10)	308–309 [17]	425	$C_{17}H_{15}IN_2S_2$	–	–	–
	51	(294–296)	(72500)				
6g	B_1(10)	253–255	507	$C_{19}H_{17}ClN_2O_4S$	–	–	–
	92		(71000)				
6h	A(5)	277–278	450	$C_{15}H_{15}IN_2S$	–	–	7.8
	78		(80000)		–	–	7.3
6i	B_1(10)	242–243	513	$C_{22}H_{21}BrN_2.H_2O$	64.5	5.4	6.8
	90		(87600)		64.2	5.6	6.8
6j	B_1(10)	293–294	505	$C_{27}H_{35}I_2N_3O.H_2O$	45.8	5.2	5.9
	60		(67700)		45.9	5.4	6.1
6k	B_1(105)	233–234	595	$C_{25}H_{21}ClN_2O_4$	67.1	4.9	5.9
	84		(32200)		66.9	4.7	6.2
6l	A(5)	294–295	480	$C_{19}H_{17}IN_2O.0.5H_2O$	53.4	4.0	6.3
	72		(60500)		53.7	4.3	6.6
6m	A(5)	301–302	480	$C_{19}H_{16}ClIN_2O$	50.3	3.6	6.1
	67		(61400)		50.6	3.6	6.2
6n	A(5)	310–311	480	$C_{20}H_{19}IN_2O$	55.7	4.7	6.5
	55		(64400)		55.8	4.5	6.5
6o	B_1(90)	284–285	515	$C_{21}H_{19}IN_2S$	55.6	4.6	5.7
	82		(66300)		55.0	4.2	6.1
6p	B_2(120)	267–268	480, 460sh	$C_{21}H_{19}IN_2O$	57.0	4.3	6.1
	65		(47000)		57.2	4.1	6.3
6q	B_2(40)	305–306	523	$C_{29}H_{22}FIN_2S$	60.6	4.0	4.8
	69		(66100)		60.4	3.8	4.8
6r	B_2(60)	231–232	619	$C_{32}H_{25}IN_2O_2$	64.2	4.0	4.3
	90		(66300)		64.4	4.2	4.2
6s	B_2(10)	310–311	586	$C_{30}H_{23}ClN_4O_4.0.5H_2O$	65.5	4.5	10.0
	79		(92800)		65.7	4.4	10.2
6t	B_1(15)	287–288	518	$C_{19}H_{16}ClIN_2Se$	44.2	3.4	5.2
	95		(47500)		44.4	3.1	5.2
6u	A(5)	194–195	535	$C_{17}H_{17}IN_2$	54.0	4.9	7.1
	95		(66400)		54.3	4.6	7.4

B_1, solvent methoxyethanol; B_2 solvent methoxyethanol:1-methyl-2-pyrrolidinone 1:1. The dyes were recrystallized from methanol or ethanol.

We believe that improvements in respect to yields and reaction times can be further made.

3. Experimental

Melting points were determined on a Kofler apparatus and are uncorrected. The absorption spectra were recorded on a Perkin–Elmer Lambda 17 UV/VIS spectrophotometer (2×10^{-5} L mol^{-1} cm^{-1} in methanol).

3.1. Preparation of the dyes ***6a–6t***

3.1.1. Method A

A quaternary heterocyclic compound **4a–q** (0.01 mole) and a sulfobetainic compound **5a–c** (0.012 mole), finely ground and mixed, were melted together with stirring for 2–3 min on an oil bath. Evolution of a gas and foaming were observed. The usually solidified melt was cooled to 70–80°C and 50–100 ml methanol were added. The reaction mixture was refluxed with stirring until dissolution of the dye. The solution was filtered hot and evaporated to one third of the initial volume. In some cases the anion could be exchanged by adding a saturated aqueous solution of potassium iodide or sodium perchlorate. The precipitate was filtered and air dried.

3.1.2. Method B

A quaternary heterocyclic compound **4a–q** (0.01 mole) and a sulfobetainic compound **5a–c** (0.012 mole) were suspended in 15–20 ml methoxyethanol, 1-methyl-2-pyrrolidinone-2, or mixture thereof, and refluxed for 5–120 min (see Table 2). The reaction mixture was cooled and ether was added to the dye solution. The dye was precipitated as an oil and the ether solution was decanted. The oil was dissolved in 50–100 ml methanol and worked up as in Method A.

Reaction times, melting points, λ_{max}, molar absorptivities and analysis for dyes **6a–6u** are given in Table 2.

4. Conclusions

The new method for the preparation of asymmetric and symmetric monomethine cyanine dyes by heating together, with or without solvent, a sulfobetaine from an *N*-alkylheterocyclic compound and a quaternary salt of a heterocyclic 2- or 4-methylcompound in the absence of a basic agent is a simple and reliable method and has the following advantages:

- the method is general and different types of monomethine symmetric or asymmetric cyanine dyes can be prepared;
- the reaction is more environmentally friendly, because the evolved acid gas can be readily neutralized and is not such a strong pollutant as methyl thiol;
- yields are from good to excellent and in many cases are exceeding those of known dyes prepared by other methods.

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