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Preparation of Monomethine Cyanine Dyes for Nucleic Acid Detection

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ABSTRACT

By condensation of quaternary heterocyclic compounds having an active methyl group with 4-chloroquinolinium salts in the presence of basic agents, such as triethylamine, 10 monomethine cyanine dyes with one or two positive charges have been synthesized. Most of the dyes absorb in the region 505–525 nm, two of them in the region around 600 nm, and all dyes have a high molar absorptivity of 50 000–80 000 l mol^{-1} cm^{-1}. The dyes do not have fluorescent properties in aqueous solution, but in the presence of ds DNA, in most cases a large fluorescence enhancement is observed. © 1998 Elsevier Science Ltd

Keywords: monomethine cyanine dyes, nucleic acid detection, fluorescence enhancement.

INTRODUCTION

Recently we published a novel method for the preparation of asymmetric monomethine cyanine dyes by condensation of 2-imino-3-methylbenzothiazolines with 1-alkyl-4-methylquinolinium salts [1]. This type of dye has some valuable properties [2] such as high molar absorptivity, with extinction coefficient usually more than 50 000 l mol^{-1} cm^{-1} at visible wavelengths; very low

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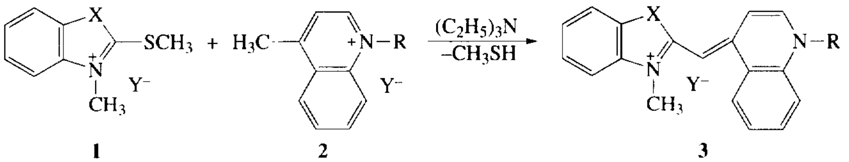
intrinsic fluorescence, with quantum yield usually less than 0.01 when not bound to nucleic acids; large fluorescence enhancement (often over 1000-fold) on binding to nucleic acids with an increase in quantum yield up to 0.9 [2], moderate to very high affinity (depending on the number of the positive charges in the dye molecule) to nucleic acids, with little or no staining of other biopolymers. Furthermore, representatives of this new class of nucleic acid stains have fluorescence excitations and emissions that cover the visible spectrum from blue to near infrared and with additional absorption peaks in the UV region, making them applicable in many different types of instrumentation. Also, we have observed that the change of even one substituent in the dye molecule can lead to novel and sometimes better properties by nucleic acid detection [3]. Novel representatives with improved properties have been patented [4, 5].

In this study, we present the preparation of 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 in the presence of basic agents.

RESULTS AND DISCUSSION

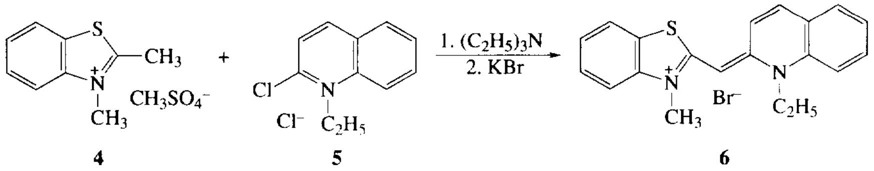
During the last few years, numerous new representatives have been synthesized [1, 4, 5]. Most of these novel asymmetric monomethine cyanine dyes have been prepared by the conventional method involving the reaction of 2-methylthiobenzoxazolium or 2-methylthiobenzothiazolium salts with 1-alkyl-4-methylquinolinium salts (Scheme 1) [6]. This method has a substantial drawback, related to the evolution of methylthiol—a strong pollutant with very unpleasant odour. Another drawback is that interchange is possible of the alkyl groups at the sulphur and nitrogen atoms in the quaternized 2-alkylthio starting materials, thus leading to unexpected reaction products [7, 8]. A novel method [1] developed by the authors avoids these disadvantages and is environmentally more friendly. A method has been patented [9] for the synthesis of asymmetric and symmetric monomethine



1 2 3

X = O,S; Y is anion; R is alkyl or substituted alkyl

Scheme 1

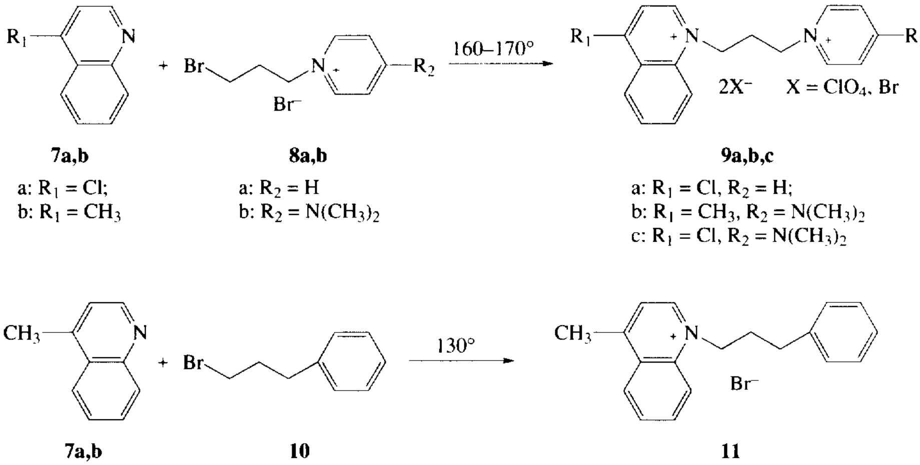


Scheme 2

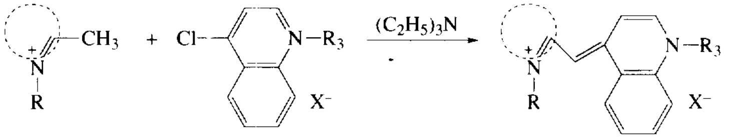
cyanine dyes by condensation of quaternized 2-chloro-heterocycles with quaternized 2- or 4-methyl-heterocyclic compounds in the presence of a basic agent such as triethylamine (Scheme 2).

To the best of our knowledge, this reaction method is used less frequently [6]. The method does not involve the evolution of methylthiol and offers more synthetic possibilities. The relatively easy preparation of the intermediate 4-chloroquinoline was used by the authors [10]. From this starting material, we prepared 1-methyl-4-chloroquinolinium methosulphate by quaternization with dimethylsulphate [11] and 1-[3-(pyridinio)propyl]-4-chloroquinolinium dibromide **9a** by quatenization with 3-bromopropylpyridinium bromide **8a**. We also synthesized 1-[3-(4-*N*,*N*-dimethylamino)pyridinio]-4-methylquinolinium diperchlorate **9b** by quaternization of lepidine **7b** with 3-bromopropyl(4-*N*,*N*-dimethylamino)pyridinium bromide **8b** and 1-(3-phenylpropyl)-4-chloroquinolinium bromide **11**. These are new intermediates (Scheme 3).

The dyes are easily prepared by the reaction of 4-chloroquinolinium salts with (quaternary salts of heterocyclic 2- or 4-methyl compounds in the presence of a basic reagent such as triethylamine. The reaction takes place at room temperature as well as at reflux in methanol or ethanol solutions (Scheme 4). The molecular structures of the intermediates **12** and of the dyes



Scheme 3



12 **9a,c; 13** **14a–j**

13: $R_3 = CH_3$, $X = CH_3SO_4$

X is an anion

Scheme 4

14a–14j are given in Table 1; dyes **14f** [12] and **14h** [13] are known, the other dyes **14a–14e** and **14g**, **14i**, **14j** being new compounds. Some physical characteristics of dyes **14a–14j** are shown in Table 2.

The dyes do not fluoresce in aqueous solutions. Preliminary investigations have shown that the addition of ds DNA in an amount of 4 μg ml^{-1} to the aqueous dye solutions leads to a slight batochromic shift—about 5 nm of the visible maximum, and to a two-fold decrease of the molar absorptivity. On excitation at 480 nm in the presence of ds DNA, a strong fluorescence appears in the region 530–550 nm for dyes **14a–14e**. In the presence of ds DNA and on excitation at around 580 nm dye **14i** has a strong fluorescence maximum at around 610 nm. A more detailed study of these dyes as nucleic acid non-covalent labels will be reported later.

EXPERIMENTAL

Melting points were determined on a Kofler apparatus and are uncorrected. The absorption spectra were recorded on a Carl Zeiss Jena Specord UV-VIS spectrophotometer (2×10^{-5} M l^{-1} in methanol).

Preparation of quinolinium intermediates

Preparation of 1-(3-pyridiniopropyl)-4-chloroquinolinium dibromide 9a.
1.63 g (0.01 mol) 4-chloroquinoline and 2.81 g (0.01 mol) 3-bromopropylpyridinium bromide were melted together at 180°C for 30 min. After cooling to room temperature, the melt was dissolved in 10 ml methanol and the solution used in the subsequent syntheses.

*Preparation of 1-[3-(4-*N,N*-dimethylamino)pyridiniopropyl]-4-methylquinolinium diperchlorate 9b*
6.48 g (0.02 mol) 3-bromopropyl(4-*N*,*N*-dimethylaminopyridinium) bromide and 2.86 g (0.02 mol) lepidine were melted together at 160–170°C for 15–20 min. After cooling, the glassy reaction mixture was dissolved in water and

TABLE 1
Structures of Intermediates **12**, and Dyes **14a–14j**

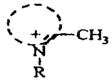
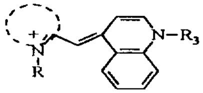
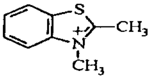
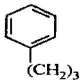
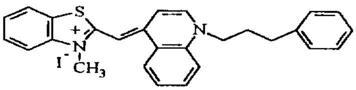
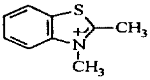
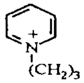
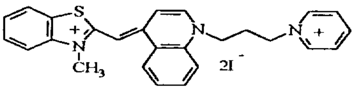
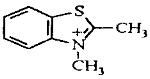
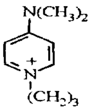
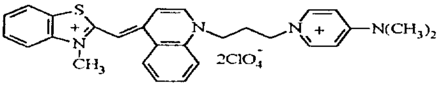
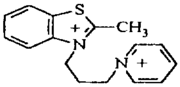
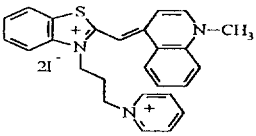
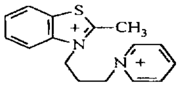
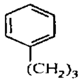
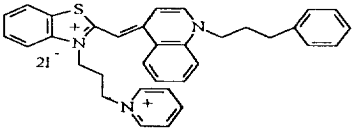
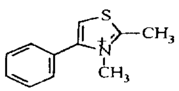
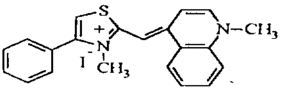
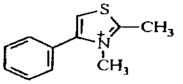
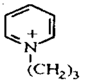
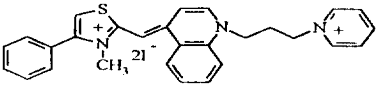
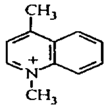
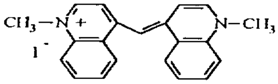
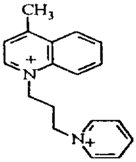
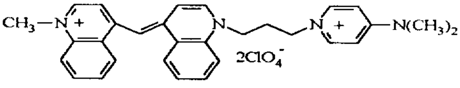
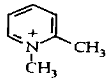
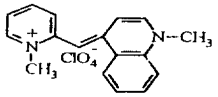
	*R_3*	*Dye*	
		**14a**	
		**14b**	
		**14c**	
	CH_3	**14d**	
		**14e**	
	CH_3	**14f**	
		**14g**	
	CH_3	**14h**	
	CH_3	**14i**	
	CH_3	**14j**	

TABLE 2
Characterisation Data for Dyes **14a–14j**

Dye	*m.p. (°C)*	*Yield (%)*	λ_{max} nm (ε l mol^{-1} cm^{-1})	*Molecular formulae*	*Analysis (%) found/calc.*		
					C	*H*	*N*
14a	249–250	50	505 (51000)	$C_{27}H_{25}1N_2S.0.5H_2O$	59.4 59.1	4.8 4.4	5.1 5.4
14b	265-266	60	515 (73900)	$C_{26}H_{25}I_2N_3S$	46.9 47.1	3.8 4.1	6.3 5.1
14c	225-226	50	515 (80900)	$C_{28}H_{30}Cl_2N_4O_8S.H_2O$	50.1 49.6	4.8 4.6	8.3 8.4
14d	265-266	50	505 (70800)	$C_{26}H_{25}I_2N_3S$	46.9 47.8	3.8 4.4	6.3 6.2
14e	269-270	55	510 (77500)	$C_{34}H_{33}I_2N_3S$	53.1 53.4	4.3 4.1	5.5 5.1
14f	283-284	60	515 (66300)	$C_{21}H_{19}1N_2S$	—	—	6.1 5.7
14g	275-276	69	520 (67600)	$C_{28}H_{27}I_2N_3S.H_2O$	47.4 47.7	4.1 4.1	5.9 5.7
14h	319-320	60	600 (83500)	$C_{21}H_{19}IN_2$	59.2 59.1	4.5 4.6	6.6 6.5
14i	179–180	52	600 (57300)	$C_{30}H_{32}Cl_2N_4O_8$	—	—	6.3 5.1
14j	216-217	60	525 (62500)	$4C_{17}H_{17}ClN_2O_4.0.5H_2O$	58.5 58.5	4.9 4.9	8.0 8.0

precipitated with sodium perchlorate The oily mass solidified after several days and was recrystallized from water; the pure product had m.p. 255–257°C.

Analysis for $C_{20}H_{26}C1_2N_3O_8$: calc. C%47.3 H%5.2 N%8.3 found 47.3 5.0 8.0

Preparation of 1-(3-phenylpropyl)-4-chloroquinolinium bromide 11

1.63 g (0.01 mol) 4-chloroquinoline and 1.99 g (0.01 mol) 1-bromo-3-phenylpropane were dissolved in 50 ml chlorobenzene. The reaction mixture was refluxed for 5 h and the solvent distilled off. The oily product (2.72 g, 75% yield) was used without further purification in further syntheses.

General method for preparation of dyes 14a–14j

0.006 mol of the appropriate quaternary salt (2,3-dimethylbenzothiazolium iodide, 2-methyl-3-(3-pyridiniopropyl)benzothiazolium dibromide, 2,3-dimethyl-4-phenylthiazolim iodide, 1,4-dimethylquinolinium methosulphate, 1-[3-(4-*N*,*N*-dimethylamino)pyridinio-propyl]-4-methylquinolinium diperchlorate, or 1,2-dimethylpyridinium iodide) and 0.006 mol of the corresponding quaternary salt of 4-chloroquinoline (1-methyl-4-chloroquinolinium methosulphate, 1-(3-pyridiniopropyl)-4-chloroquinolinium dibromide, or 1-(3-phenylpropyl)-4-chloroquinolinium, bromide) were dissolved in 20 ml methanol and 1.2 g (0.012 mol) triethylamine was added to the solution. The reaction mixtures was stirred at room temperature or refluxed for 30 min. After cooling, 1.0 g (0.006 mol) potassium iodide or 0.74 g (0.006 mol) sodium perchlorate was added to the dye solution. The precipitated dyes were filtered, washed with diethyl ether and air dried. All dyes were crystallized from methanol or ethanol.

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