Preparation of monomethine cyanine dyes as noncovalent labels for nucleic acids

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Abstract

By condensation of quaternary benzothiazolium, quinolinium and acridinium salts having an active methyl group with 1-(3-bromopropyl)-4-chloroquinolinium or 1-(3-bromo-2-hydroxypropyl)-4-chloroquinolinium salts in the presence of a basic agent such as triethylamine, 8 asymmetric and symmetric monomethine cyanine dyes bearing ω-bromopropyl substituent with one or two positive charges, were synthesized. Additionally, two of the dyes were quaternized with pyridine, and monomethine cyanines with two and three positive charges are prepared. Most of the dyes showed high molar absorptivity (70 000–100 000 l mol$^{-1}$ cm$^{-1}$). The acridinium dyes showed broad peaks with lower intensity of 30 000 l mol$^{-1}$ cm$^{-1}$. In the presence of nucleic acid in aqueous solutions, a strong enhancement of the fluorescence of these new dyes was observed. © 1998 Elsevier Science Ltd. All rights reserved.

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1. Introduction

In recent years, there has been increased interest in the field of the synthesis and application of cyanine (polymethine) dyes absorbing in different visible spectral regions, suitable as nucleic acid labels. The number of both patents [1–5] and scientific publications [6–9] is an evidence for the commercial [10], scientific [11–17] and practical importance of these probes in nucleic acid research, clinical and environmental analysis. Representatives of this class of nucleic acid stains have fluorescence excitations and emissions that cover the visible spectrum from blue to near infrared, with additional absorption peaks in the UV region [10], making them applicable in many different types of instrumentation with different light sources. The strong interest for the replacement of the radioactive probes with the more environmentally friendly and safe fluorescent probes, and the need for miniaturization and automatization of the nucleic acid analysis, ensures a positive future for such non-covalently binding labels. Replacement of even one substituent in the dye molecule can lead to novel and sometimes better properties by nucleic acid detection [11]. We believe that such an important research area needs many more new representatives to be investigated in order to establish their new properties by interaction with nucleic acids.

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In this study, we describe the preparation of some 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 (quaternized with 3-bromo- or 3-bromo-2-hydroxypropyl substituent) in the presence of basic agents.

2. Results and discussion

Such novel dyes have been synthesized by condensation of quaternized 2-, or 4-chloroheterocycles with quaternized 2- or 4-methyl heterocyclic salts in the presence of basic agents [7,18]. Although this preparation is less frequently used, it does not have the main disadvantage of the so called alkylthio method [19], where a strong pollutant is evolved, method involving the reaction of 2- or 4-methylthio quaternized heterocycles with 2- or 4-methyl quaternized heterocyclic salts in the presence of basic agents [19]. The dye synthesis requires some novel intermediates to be prepared. 2-Methylbenzothiazole 1 was quaternized with 1-(3-bromopropyl)pyridinium bromide 2a or (3-bromopropyl)-N,N-dimethylaminopyridinium bromide 2b by melting together the components at around 160°C for 30 min (Scheme 1), thus giving products 3a and 3b.

4-Chloroquinoline [20] 4 was quaternized with 1,3-dibromopropane 5a or 1,3-dibromo-3-hydroxypropane 5b in trichloroethylene (Scheme 2) for the preparation of compounds 6a and 6b.

Dyes 7a–f were prepared by condensation of the quaternized 2-methylbenzothiazolium salts 3a–c with the 4-chloroquinolinium salts 6a and 6b in the presence of triethylamine (Scheme 3 and Table 1).

Dyes with quinolinium 9a and acridinium 9b end groups are synthesized by the same method with 1,4-dimethylquinolinium methosulfate 8 and 9,10-dimethylacridinium iodide [21,22] 9 (Scheme 4 and Table 1).

Dyes 7a–f, 9a and 9b can be used as intermediates for the preparation of monomeric nucleic acid dyes bearing more than one positive charge, thus having higher binding affinity to nucleic acids. Dyes 11 and 12 with two and three positive
charges, respectively are synthesized by additional quaternization of 9b and 7c with pyridine 10 (Scheme 5 and Table 1).

The monomethine cyanine benzothiazolium dyes 7a-f and 12 have high molar absorptivity ($\varepsilon = 70,000-90,000$) at around 504–507 nm. This holds also for the quinolinium monomethine dye 9a ($\varepsilon = 100,300$) at 590 nm. The monomethine cyanines with acridinium and quinolinium end groups 9b and 11 have broad peaks with lower intensity ($\varepsilon \sim 30,000$), absorbing at 605–620 nm (Table 2).

The newly synthesized monomethine cyanines 7a-f and 9a and 9b with $\omega$-bromopropyl substituent can also be used as building blocks for the synthesis of homo- [21] and heterodimeric [23–25] nucleic acid dyes. In the presence of nucleic acids in aqueous solutions a strong enhancement of the fluorescence of the novel dyes is observed.

### 3. Experimental

Melting points were determined on a Kofler apparatus and are uncorrected. The absorption

### Table 1—contd

<table>
<thead>
<tr>
<th>Dye</th>
<th>Formula</th>
<th>Starting compounds</th>
<th>Yield %</th>
</tr>
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<tbody>
<tr>
<td>9b</td>
<td><img src="image1" alt="Structure" /></td>
<td>8b, 6a</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td><img src="image2" alt="Structure" /></td>
<td>9b, 10</td>
<td>63</td>
</tr>
<tr>
<td>11</td>
<td><img src="image3" alt="Structure" /></td>
<td>7c, 10</td>
<td>61</td>
</tr>
</tbody>
</table>

(continued)
spectra were recorded on a Perkin–Elmer Lambda 17 UV/VIS spectrophotometer (2×10⁻⁵ M/litre in methanol).

3.1. Preparation of 2-methyl-3-[(3-pyridinio)-propyl]benzothiazolium dibromide 3a and 2-methyl-3-[(3-(4-N,N-dimethylamino)pyridinio)-propyl]-benzothiazolium dibromide 3b

2-Methylbenzothiazole 1 (0.1 m) and 3-bromopyridinium bromide or 1-(3-bromopropyl)-4-N,N-dimethylaminopyridinium bromide (0.1 m) were melted together and the viscous mass was stirred and heated at 160°C for 30 min. The melt was cooled to 80°C and 30 ml acetone were added to the reaction mixture. The acetone layer above the solidified melt was decanted. The semisolid compound was transferred with heating to a storage vessel. The compounds were used without further purification in the next reaction steps.

3.2. Preparation of 1-(3-bromopropyl)-4-chloroquinolinium bromide 6a

Five grams (0.03 m) 4-Chloroquinoline were suspended in 7 ml chlorobenzene and 6.1 ml (12.1 g, 0.06 m) 1,3-dibromopropane were added. The reaction mixture was refluxed with stirring for 2 h. Additional 4.04 g (2 ml) 1,3-dibromopropane were added and refluxing continued for 2 further hours. The reaction mixture was cooled and the product then crystallized in a thick mass. The compound was filtered and dried under vacuum. Yield of the crude product 9.5 g (85%). The 1-(3-bromopropyl)-4-chloroquinolinium bromide was highly hygroscopic and needs to be stored in a
3.3. Preparation of 1-(3-bromo-2-hydroxypropyl)-4-chloroquinolinium bromide 6b

Five grams (0.03 m) 4-Chloroquinoline was suspended in 15 ml trichloroethylene and 6.1 ml (12.1 g, 0.06 m) 1,3-dibromo-2-hydroxy-propane added. The reaction mixture was refluxed with stirring for two hours. The solvent was distilled off/C128 (rotary evaporator) and a non-crystallizable oily product was obtained. Yield of crude product 7.2 g (78%). It was used in the next reaction steps without further purification.

3.4. Preparation of the dyes 7a-f, and 9a,b

Of the corresponding heterocyclic quaternary salt 0.0035 m (3a-c or 8a,b), 0.0035 m 1-(3-bromo-2-hydroxypropyl)-4-chloroquinolinium bromide 6a or 1-(3-bromo-2-hydroxypropyl)-4-chloroquinolinium bromide 6b and 0.97 ml (0.007 m) triethylamine were refluxed in 15 ml methanol for 30 min. A triple excess of saturated aqueous potassium iodide solution was added to the hot dye solution. After cooling the precipitated dye was filtered and dried. Some data are given in Tables 1 and 2.

3.5. Preparation of the dyes 11 and 12

Of the corresponding dye 0.00034 m 9b or 7c, 0.08 ml (0.001 m) pyridine in 3 ml 2-methoxy-ethanol were refluxed with stirring for 6 h. To the hot dye solution, a triple excess of saturated aqueous potassium iodide solution was added. After cooling the precipitated dye was filtered and dried. Some data are given in Tables 1 and 2.

References


Table 2
Characterization data for dyes 7a-f, 9a,b, 11 and 12

<table>
<thead>
<tr>
<th>Dye</th>
<th>m.p. °C</th>
<th>λ\text{max} nm (ε 1mol⁻¹ cm⁻¹)</th>
<th>Molecular formulae</th>
<th>Analysis (%) found/calc.</th>
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</thead>
<tbody>
<tr>
<td>7a</td>
<td>204–206</td>
<td>505 (87600)</td>
<td>C₂₁H₂₉BrIN₂S</td>
<td>–</td>
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<tr>
<td>7b</td>
<td>236–237</td>
<td>504 (72300)</td>
<td>C₂₁H₂₉BrIN₂OS.C₂H₅OH</td>
<td>–</td>
</tr>
<tr>
<td>7c</td>
<td>219–221</td>
<td>505 (77800)</td>
<td>C₂₆H₂₈Br₂I₂N₃S</td>
<td>46.6 (47.3) 4.5 (4.7)</td>
</tr>
<tr>
<td>7d</td>
<td>249–250</td>
<td>504 (89800)</td>
<td>C₂₆H₂₈Br₂I₂N₃OS</td>
<td>47.7 (45.2) 4.1 (4.8)</td>
</tr>
<tr>
<td>7e</td>
<td>236–239</td>
<td>478sh, 504 (52400, 70000)</td>
<td>C₃₀H₃₃Br₂I₂N₃S</td>
<td>46.4 (48.3) 4.9 (4.9)</td>
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<tr>
<td>7f</td>
<td>255–257</td>
<td>504 (90100)</td>
<td>C₃₀H₃₃Br₂I₂NaOS</td>
<td>–</td>
</tr>
<tr>
<td>9a</td>
<td>222–224</td>
<td>590 (103300)</td>
<td>C₂₃H₂₂BrIN₂O</td>
<td>–</td>
</tr>
<tr>
<td>9b</td>
<td>145–147</td>
<td>606 (28500)</td>
<td>C₂₃H₂₂BrIN₂</td>
<td>46.8 (43.3) 4.3 (4.4)</td>
</tr>
<tr>
<td>11</td>
<td>252–254</td>
<td>621 (29900)</td>
<td>C₂₃H₂₂I₂N₃</td>
<td>44.2 (41.4) 5.1 (5.6)</td>
</tr>
<tr>
<td>12</td>
<td>190–193</td>
<td>507 (78300)</td>
<td>C₁₃H₁₃I₃N₄S</td>
<td>44.0 (39.3) 5.9 (5.8)</td>
</tr>
</tbody>
</table>


