Synthesis of homodimeric monomethine cyanine dyes as noncovalent nucleic acid labels and their absorption and fluorescence spectral characteristics

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Dedicated to Professor Dr. Karl-Heinz Drexhage on the occasion of his 65th birthday

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Abstract

Several novel homodimeric asymmetric monomethine cyanine dyes based on the thiazole orange (TO) chromophore were synthesised via an improved synthetic procedure. The two TO chromophores \([1-(o\text{-bromoalkyl})-4-(3\text{-methyl-2-(3H)-benzothiazolilyden})\text{methyl}] \text{quinolinium iodide}\), with different chain lengths of the methylene linker between the quinolinium ring and the quaternary ammonium nitrogen, were connected by bisquaternization with \(N,N,N^0, N^0\)-tetramethyl-1,3-propanediamine, \(N,N,N^0, N^0\)-tetramethyl-1,6-hexanediamine, 1,4-diazabicyclo- [2,2,2]octane and 1,4'-bipyrididine. The homodimeric dyes have large molar absorptivity \((\epsilon \approx 130000–180000 \text{l mol}^{-1} \text{cm}^{-1})\) at 505–506 nm. In the presence of ds DNA, their fluorescence maxima were located at 530–534 nm and the fluorescence quantum yields were in the range 0.48–0.96. Fluorescence maxima between 560–650 nm and fluorescence quantum yields of 0.3–0.8 were observed in the presence of ss DNA. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Monomethine cyanine dyes; Homodimers; Synthetic method; Absorption; Fluorescence; Noncovalent nucleic acid labels

1. Introduction

In recent years there has been extensive research on the syntheses and applications of polymethine dyes as noncovalent labels to nucleic acid detection [1–3]. Penta-, tri- and monomethine cyanine dyes have been synthesised and used as nucleic acid stains, the latter ones being the most numerous and extensively studied. Among cyanines, they are the best noncovalently binding nucleic acid labels with respect to their most important property, viz. high fluorescence signal. As part of our search of novel and improved methods for the preparation...
of asymmetric monomethine cyanine dyes, we have synthesised a series of noncovalent nucleic acid labels [4–7] and studied their spectral profiles [8,9]. Nonradioactive labelled DNA stains, stable under electrophoretic conditions, are found among homodimeric monomethine cyanines with four positive charges. These dyes, known as TOTO-1 \([N,N,N',N'-tetramethyl-N,N'-bis-{3-[4-[(3-methyl-2(3H)benzothiazolilyden)methyl]quinolinium-1-yl]propyl}-1,3-propanediammonium tetraiodide}\) and YOYO-1 \([N,N,N',N'-tetramethyl-N,N'-bis-{3-[4-[3-(3-methyl-2(3H)benzoxazolilyden)methyl]quinolinium-1-yl]propyl}-1,3-propanediammonium tetraiodide]\), form highly fluorescent complexes with ds DNA [10]. Recently Staerk et al. [11] synthesised derivatives similar to TOTO-1, with extended methylene bridges between the quinolinium ring and the quaternary ammonium nitrogen. They observed that TOTO-1, and similar derivatives with extended linker, predominantly bisintercalate in the 5'-CTGAG-3' binding site of oligonucleotides [11,12].

In this study we present an improved synthetic method of novel homodimeric asymmetric monomethine cyanines similar to TOTO-1 and their absorption and fluorescence spectral characteristics.

2. Results and discussion

Rye et al. have synthesised TOTO-1 4a and YOYO-1 4b by reacting of 2-methylthio-3-methylbenzothiazolium 1a or 2-methylthio-3-methylbenzoxazolium 1b salts with 1-(3-iodopropyl)-4-methylquinolinium iodide 2 in the presence of a base. The obtained dyes 3a, 3b were bisquaternized with \(N,N,N',N'-tetramethyl-1,3-propanediamine\) (Scheme 1). Staerk et al. [11] have used the same preparation method to synthesise TOTO-1 derivatives by altering the length of the methylene linker between the quinolinium ring and the quaternary ammonium nitrogen (Scheme 1, X = S, \(n = 4,5\)).

The quaternized lepidines 7a, 7b were prepared by the reaction of lepidine 5 with dibromoalkanes 6a, 6b, in molar ratio 1:4, either neat or in a non-polar solvent and at room temperature for 24–72 h [13] (Scheme 2).

We have recently proposed a new synthetic method for the preparation of monomethine cyanines [7] in which quaternary salts of heterocyclic 2- or 4-methyl compounds and \(N\)-heterocyclic 2- or 4-sulfobetainic compounds were reacted by melting together or by refluxing in different solvents in the

![Scheme 1](image-url)
absence of a base. On this basis melting of anhydro-3-methyl-2-sulfobenzothiazolium hydroxide 8 with 1-(ω-brornoalkyl)-4-methylquinolinium bromides 7a, 7b yielded the starting monocationic ω-bromoalkyl monomethine cyanines 9a, 9b (Scheme 3).

The dyes 9a, 9b were bisquaternized with \( N,N,N',N' \)-tetramethyl-1,3-propanediamine, \( N,N,N',N' \)-tetramethyl-1,6-hexanediamine, 1,4-diazabicyclo[2.2.2] octane and 1,4'-bipyridine. The quaternization was carried out in 2-methoxyethanol for considerably shorter reaction time (15 min to 3 h) than previously described methods [10,11] (Scheme 4) giving the homodimeric dyes 10a–10f.

The following homodimeric monomethine cyanines were synthesised:

10a: \( N,N'-\text{bis-}\{3-[4\text{-}(3\text{-methyl-2}(3H)benzothiazolyliden) methyl]quinolinium-1-y]propyl\}-1,4\text{-diazabicyclo[2.2.2]octane tetraiodide} \)

10b: \( N,N'-\text{bis-}\{3-[4\text{-}(3\text{-methyl-2}(3H)benzothiazolyliden) methyl]quinolinium-1-y]butyl\}-1,4\text{-diazabicyclo[2.2.2]octane tetraiodide} \)

\[
\text{Scheme 2.}
\]

\[
\text{Scheme 3.}
\]

\[
\text{Scheme 4.}
\]
Some characterisation data for the homodimeric dyes 10a–f are given in Table 1. All dyes are new, except dye 10c [11].

The synthesised dyes did not exhibit any fluorescence in solution at room temperature, with the exception of 10c and 10f whose QF value was less than 0.001. They all became strongly fluorescent upon binding to DNA. Our investigations showed that both the position and the intensity of the fluorescence maxima depend strongly on the dye structure and the type of DNA used (Table 2).

All dyes showed similar fluorescence characteristics in TE buffer (pH 7.0) in the presence of ds DNA (5 µg/ml). Their fluorescence maxima were located at 530–534 nm and their quantum yields were in the range of 0.48–0.96. In the presence of ss DNA (5 µg/ml) the fluorescence maxima were between 560 and 650 nm and the fluorescence quantum yields were 0.3–0.8. The large difference (more than 40 nm) between the fluorescence maxima of compounds 10c and 10f in the presence of double- and single-stranded DNA (see Table 2) suggests the possible use of the two dyes for distinguishing between ds DNA and ss DNA in solution. The detection limit of ds DNA with compound 10f is less than 20 ng. Investigations on the fluorescence properties of these dyes, in the presence of different types of DNA and RNA, as well as studies on the mechanism of their interaction with nucleic acids, are in progress.

<table>
<thead>
<tr>
<th>Dye</th>
<th>Yield %</th>
<th>m.p. (°C)</th>
<th>λmax (nm)</th>
<th>ε (l mol⁻¹ cm⁻¹)</th>
<th>Molecular formula</th>
<th>Analysis (%) found/calc.</th>
<th>C H N</th>
</tr>
</thead>
<tbody>
<tr>
<td>10a</td>
<td>53 (3)</td>
<td>278–280</td>
<td>506 (129600)</td>
<td>–</td>
<td>C₄₈H₅₂I₄N₆S₂</td>
<td>– –</td>
<td>6.8</td>
</tr>
<tr>
<td>10b</td>
<td>93 (2)</td>
<td>220–222</td>
<td>506 (162300)</td>
<td>–</td>
<td>C₅₀H₅₆I₄N₆S₂</td>
<td>5.9 94.4</td>
<td>97.0</td>
</tr>
<tr>
<td>10c</td>
<td>54 (1.5)</td>
<td>204–206</td>
<td>505 (151100)</td>
<td>45.7</td>
<td>C₅₁H₆₂I₄N₆S₂</td>
<td>– 4.7</td>
<td>96.4</td>
</tr>
<tr>
<td>10d</td>
<td>54 (2)</td>
<td>215–217</td>
<td>506 (179400)</td>
<td>45.8</td>
<td>C₅₄H₅₂I₄N₆S₂·3 CH₃OH</td>
<td>46.7 4.3</td>
<td>6.5</td>
</tr>
<tr>
<td>10e</td>
<td>59 (1)</td>
<td>266–268</td>
<td>505 (170100)</td>
<td>46.0</td>
<td>C₅₂H₆₄I₄N₆S₂·CH₃OH</td>
<td>47.1 4.4</td>
<td>5.7</td>
</tr>
<tr>
<td>10f</td>
<td>60 (0.25)</td>
<td>222–223</td>
<td>505 (169000)</td>
<td>46.2</td>
<td>C₅₄H₅₈I₄N₆S₂·5 CH₃OH</td>
<td>46.2 5.6</td>
<td>5.8</td>
</tr>
</tbody>
</table>
3. Experimental

Melting points were determined on a Kofler apparatus and are uncorrected. The $^1$H NMR spectra of the dyes were obtained on a Bruker MSL 300 MHz in DMSO-$d_6$. The absorption spectra were recorded on a Perkin–Elmer Lambda 17 UV/ VIS spectrophotometer ($1 \times 10^{-5}$ mol $^{-1}$ cm$^{-1}$ in methanol). Fluorescence spectra (excitation at 480 nm) were scanned on a Perkin–Elmer MPF44 spectrofluorimeter. The fluorescence quantum yield ($Q_F$) was determined relative to that of dye TO ($Q_F = 0.2$) [2]. Stock solutions were prepared by dissolving 1 mg of the dye in 1 ml DMSO and subsequent dilution with TE buffer (10 mM Tris-HCL, 1 mM EDTA, pH 7.0) to a final concentration of $0.5 \times 10^{-5}$ mol/l. Salmon sperm DNA (native — ds, and denaturated — ss) was purchased from Sigma (USA).

3.1. Preparation of 1-(o-bromopropyl)-4-methylquinolinium bromide 7a and 1-(o-bromobutyl)-4-methylquinolinium bromide 7b [13]

Lepidine and 1,3-dibromopropane or 1,4-dibromobutane in molar ratio 1:4 were left for 24–72 h in the dark at room temperature. The resultant precipitate was filtered, washed with ether and air dried. 7a: 68%, yield, m.p. 136–140°C, literature m.p. 139–141 [13]; 7b: 50%, yield, m.p. 148–152°C, literature m.p. 150–152 [13].

3.2. Preparation of 1-(3-bromopropyl)-4-[(3-methyl-2(3H)benzothiazolilyden)methyl] quinolinium iodide 9a and 1-(3-bromobutyl)-4-[(3-methyl-2(3H)benzothiazolilyden)methyl] quinolinium iodide 9b

0.01 mol 7a or 7b and 0.01 mol anhydro 3-methyl-2-sulfobenzothiazolium hydroxide 8 were mixed and finely ground in a mortar. The reaction mixture was transferred into a flask and melted. The reaction mixture was then stirred for several minutes until evolution of sulphur dioxide ceased. The solidified melt was cooled to 70–80°C and sufficient methanol was added to dissolve the dye. The hot solution was filtered and the dye was precipitated by the addition of potassium iodide and cooling. The dyes were recrystallized from methanol. 9a: 56%, yield, m.p. 191–201°C, literature m.p. 204–206 [6]; 9b: 56%, yield, m.p. 230–235°C, literature m.p. 235–237 [6].

3.3. Preparation of homodimeric monomethine cyanine dyes 10a–f

0.002 mol 9a or 9b and 0.001 mol of N,N,N$^0$,N$^0$-tetramethyl-1,3-propanediamine, N,N,N$^0$,N$^0$-tetramethyl-1,6-hexanediamine, 1,4-diazabicyclo[2,2,2] octane or 1,4$^0$-bipyridine were refluxed in 5–9 ml 2-methoxyethanol for 15 min to 3 h. After cooling the precipitated dye was filtered and dried. The dye was dissolved in hot methanol and an excess of aqueous potassium iodide solution was added to the hot solution. The solution was cooled and the precipitated dye was filtered and dried. The dyes were recrystallized from methanol. Some data for dyes 10a–f are given in Table 1.

### Table 2

Fluorescence characteristics of the dyes in the presence of nucleic acids$^a$

<table>
<thead>
<tr>
<th>Dye</th>
<th>ds DNA</th>
<th>ss DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\lambda_F$ (nm)</td>
<td>$Q_F$</td>
</tr>
<tr>
<td>10b</td>
<td>529.2</td>
<td>0.69</td>
</tr>
<tr>
<td>10c</td>
<td>530.4</td>
<td>0.94</td>
</tr>
<tr>
<td>10d</td>
<td>531.2</td>
<td>–</td>
</tr>
<tr>
<td>10e</td>
<td>532.4</td>
<td>0.48</td>
</tr>
<tr>
<td>10f</td>
<td>529.0</td>
<td>0.94</td>
</tr>
</tbody>
</table>

$^a$ $\lambda_F$, position of the fluorescence maximum in nanometers; $Q_F$, fluorescence quantum yield; (–), no measurement; the numbers of the dyes correspond to those given in the text; the fluorescence characteristics of dye 10a were not measured.

3.3.1. NMR spectra of dyes 10b–f

3.3.1.1. Dye 10b. 7.39–8.84 (m, 20H, Ar); 6.96 (s, 2H, 2$\text{CH}$); 4.65 (br s, 4H, 2$\text{N–CH}_2$); 4.04 (s, 6H, 2$\text{N+–CH}_3$); 3.04–3.43 (m, 16H, CH$_2$N( CH$_2$CH$_2$)$_3$N+CH$_2$); 1.86 (br s, 8H, 2$\text{CH}_2$–CH$_2$).

3.3.1.2. Dye 10c. 7.28–8.78 (m, 20H, Ar); 6.88 (s, 2H, 2$\text{CH}$); 4.66 (br s, 4H, 2$\text{N+–CH}_3$); 3.04–3.43 (m, 16H, CH$_2$N( CH$_2$CH$_2$)$_3$N+CH$_2$); 1.86 (br s, 8H, 2$\text{CH}_2$–CH$_2$).

3.3.1.3. Dye 10d. 7.39–8.84 (m, 20H, Ar); 6.96 (s, 2H, 2$\text{CH}$); 4.65 (br s, 4H, 2$\text{N–CH}_2$); 4.04 (s, 6H, 2$\text{N+–CH}_3$); 3.04–3.43 (m, 16H, CH$_2$N( CH$_2$CH$_2$)$_3$N+CH$_2$); 1.86 (br s, 8H, 2$\text{CH}_2$–CH$_2$).

3.3.1.4. Dye 10e. 7.39–8.84 (m, 20H, Ar); 6.96 (s, 2H, 2$\text{CH}$); 4.65 (br s, 4H, 2$\text{N–CH}_2$); 4.04 (s, 6H, 2$\text{N+–CH}_3$); 3.04–3.43 (m, 16H, CH$_2$N( CH$_2$CH$_2$)$_3$N+CH$_2$); 1.86 (br s, 8H, 2$\text{CH}_2$–CH$_2$).

3.3.1.5. Dye 10f. 7.39–8.84 (m, 20H, Ar); 6.96 (s, 2H, 2$\text{CH}$); 4.65 (br s, 4H, 2$\text{N–CH}_2$); 4.04 (s, 6H, 2$\text{N+–CH}_3$); 3.04–3.43 (m, 16H, CH$_2$N( CH$_2$CH$_2$)$_3$N+CH$_2$); 1.86 (br s, 8H, 2$\text{CH}_2$–CH$_2$).
3.3.1.3. Dye 10d. 7.36–9.40 (m, 28H, Ar); 6.97 (s, 2H, 2CH); 4.76 (m, 4H, 2bipyN–CH2); 4.68 (m, 4H, 2N–CH2); 4.04 (s, 6H, 2N+–CH3); 2.14 (br s, 4H, 2CH2); 1.96 (br s, 4H, 2CH2).

3.3.1.4. Dye 10e. 7.37–8.84 (m, 20H, Ar); 6.96 (s, 2H, 2CH); 4.65 (br s, 4H, 2N–CH2); 4.05 (s, 6H, 2N+–CH3); 3.34–3.57 (m, 8H, N+(CH2)2); 3.12 (s, 12H, 2N+(CH3)2); 2.32 (br s, 4H, 2CH2); 1.75 (br s, 4H, 2CH2); 1.33 (br s, 4H, 2CH2).

3.3.1.5. Dye 10f. 7.26–8.77 (m, 20H, Ar); 6.86 (s, 2H, 2CH); 4.65 (br s, 4H, 2N–CH2); 3.99 (s, 6H, 2N+–CH3); 3.29–3.40 (m, 8H, N+(CH2)2); 3.07 (s, 12H, 2N+(CH3)2); 1.84 (br s, 8H, 2CH2–CH2); 1.77 (br s, 4H, 2CH2); 1.39 (br s, 4H, 2CH2).

References