Article

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Synthesis, Absorption and Fluorescence Spectral Characteristics of Trinucleus Dimethine Cyanine Dyes as Fluorescent Probes for DNA Detection

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Neste trabalho descreve-se a preparação de seis corantes cianina dimetina trinucleares com núcleos de piridina, obtidos a partir da condensação de iodeto de trimetilpiridínio com aldeídos aromáticos heterocíclicos. As propriedades de absorção e fluorescência dos corantes foram estudadas em solventes com polaridades diferentes. O deslocamento hipsocrômico dos máximos de absorção dos corantes foi observado com o aumento da polaridade do solvente. Foram estudadas as propriedades fluorescentes dos corantes em solução e na presença de DNA. Foi observado um aumento significativo do rendimento quântico de fluorescência na presença de DNA para quatro corantes.. Em particular, um dos corantes emite fluorescência fraca no tampão Tris-HCl, apresentando contudo fluorescência intensa na presença de DNA.

The preparation of six trinucleus dimethine cyanine dyes with pyridine nucleus obtained by the condensation of trimethylpyridinium iodides with heterocyclic aromatic aldehyde was described. The absorption and fluorescence properties of the dyes were studied in different polarity solvents. Blue shift of the maxima absorption of the dyes was observed with the increase of solvents polarity. The fluorescence properties of the dyes in solution and in presence of DNA were studied. Significant enhancement of the fluorescent quantum yield was observed in four dyes in the presence of DNA. Specially, one of six dyes emitted weak fluorescence in Tris-HCl buffer, but displayed bright fluorescence in the presence of DNA.

Keywords: trinucleus dimethine cyanine dyes, DNA, absorption properties, fluorescence properties, fluorescent dyes

Introduction

Methine cyanine dyes have been widely researched and explored as sensitizers in photography[1] and as optical recording materials in laser disk.[2] Recently, methine cyanine dyes have attracted attention owing to their excellent fluorescence properties[3-5] as well as their potential applications in probes for DNA in living cells.[6-10] From the applications of cyanine dyes it is known that the optical properties of cyanine dyes are often influenced by their conjugated systems and the solution environment. Trinucleus dimethine cyanine dyes are large conjugated systems, which contain three heteroaromatic rings joined by two vinyl chains. However the synthesis of trinucleus dimethine cyanine dyes and their applications as fluorescent probe were rarely reported.[11,12] Our previous efforts have been devoted to developing series of cyanine dyes and styryl dyes.[13-17]

In this paper, six trinucleus dimethine cyanine dyes with pyridine nucleus, four dyes of which were novel, were synthesized in water or ethanol via the condensation of appropriate heteroaromatic aldehydes with quaternary salts of heterocyclic compounds containing active methyl groups. The influence of different solvents on spectral properties of the trinucleus dimethine cyanine dyes was studied by UV-Vis and fluorescence spectroscopy. A further objective of this study was to investigate the fluorescence spectral properties of prepared trinucleus dimethine cyanine dyes both in solution and in the DNA presence, exploring their future applications as fluorescence probe.

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Experimental

General

All reagents were obtained from commercial sources and used without further purification. All chemicals were of analytical grade. Melting points were taken on a XT-4 micromelting apparatus and uncorrected. IR spectra in cm^{-1} were recorded on Shimadzu IRPrestige-21 spectrometer and Bruker Equiox-55 spectrometer. ^1H NMR spectra were recorded at 400 MHz on a Varian Inova-400 spectrometer and chemical shifts were reported relative to internal Me_4Si. ^{13}C NMR spectra were recorded at 100 MHz on a Varian Inova-400 spectrometer and chemical shifts were reported relative to internal Me_4Si. Elemental analysis was performed with Vario EL-III instrument. The electron impact (EI) mass spectra were recorded at 70 eV with a GCMS-QP2010 system equipped with the solid sample direct insertion probe. The absorption spectra were recorded on a Shimadzu UV-1700 UV-Vis spectrometer. Fluorescence measurements were carried out on a Hitachi F-4500 spectrofluorimeter.

Measurements of the spectral properties of the dyes in different solvents

The dye stock solutions (5.0×10^{-4} mol L^{-1} in dimethylsulfoxide (DMSO)) were diluted with different solvents and resulted in working solutions of dyes (2.0×10^{-5} mol L^{-1}). The absorption spectra were examined at room temperature in different solvents and recorded using 1 cm quartz cells on a Shimadzu UV-1700 UV-Vis spectrometer. Fluorescence measurements were carried out at room temperature on a Hitachi F-4500 spectrofluorimeter in 1 cm quartz cells. Fluorescence emission was excited at the maximum of the absorption. The absorption and fluorescence spectral data were listed in Table 1.

Measurements of spectral properties of the dyes in the presence of DNA

Dye stock solutions (2.0×10^{-4} mol L^{-1}) were prepared by dissolving the dyes in DMSO and further diluted with Tris-HCl buffer (pH 7.4) to result in working solutions of dyes (1.0×10^{-5} mol L^{-1}). Stock solution of DNA was prepared by dissolving salmon sperm DNA in 0.05 mol L^{-1} Tris-HCl buffer. The concentration of DNA in stock solution was 3.1×10^{-3} mol L^{-1} base pairs (bp). The fluorescence of dyes in the presence of DNA was tested by adding 0.5 mL DNA solution (3.1×10^{-3} mol L^{-1} bp) into 1.0 mL 1.0×10^{-5} mol L^{-1} working solutions of dyes. All working solutions were prepared immediately before the experiment.

The absorption spectra were examined at room temperature in different solvents and recorded using 1 cm quartz cells on a Shimadzu UV-1700 UV-Vis spectrometer. Fluorescence measurements were carried out at room temperature on a Hitachi F-4500 spectrofluorimeter in 1 cm quartz cells. Fluorescence emission was excited at the maximum of the fluorescence excitation spectrum. The absorption and fluorescence spectral date were listed in Table 2 and Table 3.

*Preparation of trimethyl pyridine quaternary salt **2a-2b***

A mixture of 3.54 g (0.033 mol) **1a** or **1b** with 4.71 g (0.033 mol) CH_3I was refluxed for 20 h. After cooling, the product was filtered off and purified by recrystallization from ethanol. Pyridine quaternary salt **2a**: 5.1 g, yield 62%, mp 130-131 °C. **2b**: 5.4 g. Yield 66%, mp 235-236 °C.

*Preparation of dyes **3a-3b***

A mixture of **2a** or **2b** (0.10 g, 0.4 mmol), indole-3-carboxaldehyde (0.15 g, 1.0 mmol) and 5 drops of piperidine was refluxed for 24 h in 8 mL ethanol. After cooling, ether was added and the product was filtered off, washed with ether and recrystallized from ethanol:water = 9:1. Yield: **3a**: 0.15 g (84%), **3b**: 0.16 g (79%).

*Preparation of dyes **3c-3d***

The dyes **3c-3d** were synthesized according to a revised literature procedure.[12] A mixture of **2a** or **2b** (0.10 g, 0.4 mmol), 1-methylpyrrole-2-carboxaldehyde (0.20 g, 1.0 mmol) and 5 drops of piperidine was refluxed for 20 h in 5 mL ethanol. After cooling, ether was added and the product was filtered off, washed with ether and recrystallized from ethanol:water = 9:1. Yield: **3c**: 0.17 g (86%), **3d**: 0.15 g (76%).

*Preparation of dyes **3e-3f***

Quaternary salts **2a** or **2b** (0.2 g, 0.8 mmol) and furaldehyde (0.62 g, 6.4 mmol) were dissolved in 18 mL H_2O. NaOH (20%, 4.6 mL) was added with stirring over 30 min. After 1 h, the product was filtered off, washed with cold water and recrystallized from water. Yield: **3e**: 0.22 g (69%), **3f**: 0.27 g (83%).

*1-Methyl-2,4-bis[2-(indole-3-yl)vinyl]pyridinium iodide (**3a**)*

Orange-red crystals with metallic luster, mp 172 °C (decomposition), ^1H NMR (DMSO-d_6, 400 MHz): δ 4.21 (s,

3H, N^+CH_3), 7.20-7.32 (m, 6H, Ar–H, CH=CH), 7.52-7.54 (m, 2H, Ar–H), 7.89 (d, 1H, *J* 6.8 Hz, pyridine–H), 7.91-7.92 (m, 1H, Ar–H), 8.10-8.19 (m, 2H, CH=CH), 8.19-8.25 (m, 3H, indole-H, CH=CH), 8.48 (s, 1H, pyridine–H), 8.54 (d, 1H, *J* 6.8 Hz, pyridine–H), 11.84 (s, 1H, indole N–H), 11.96 (s, 1H, indole N–H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 43.6, 110.2, 112.1, 113.0, 113.1, 116.7, 117.0, 117.6, 119.7, 120.0, 120.4, 120.6, 122.3, 122.4, 124.5, 131.1, 131.3, 134.3, 136.1, 136.8, 137.0, 143.5, 152.1. IR ν_{max}/cm^{-1} (KBr) 3400 (m, $\nu_{=NH}$), 3068 ($\nu_{=C-H}$), 1597, 1552 ($\nu_{C=C}$), 1517, 1492 1423 (s, $\nu_{C=C}$, $\nu_{C=N}$), 1367, 1315,1238, 1128, 1107 (δ_{CH}), 957, 744 (m, $\delta_{=CH}$). MS: EI (70 ev) *m/z* (%): 361 (58 M – CH_3I), 360 (100 M – CH_3I – H), 142, 127. UV-Vis λ_{max}/nm (methanol) 465. Found: C, 59.0; H, 4.43; N, 7.56. Calc. for $C_{26}H_{22}N_3I{\cdot}3/2H_2O$ (530.3 g mol^{-1}): C, 58.9; H, 4.72; N, 7.92%.

*1-Methyl-2,6-bis[2-(indole-3-yl)vinyl]pyridinium iodide (**3b**)*

Orange-red crystals with metallic luster, mp 206-207 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 4.12 (s, 3H, N^+CH_3), 7.21-7.29 (m, 6H, Ar–H), 7.51-7.54 (m, 2H, CH=CH), 7.85-7.92 (m, 2H, CH=CH), 8.10-8.24 (m, 5H, Ar–H, pyridine–H), 8.48-8.56 (m, 2H, pyridine–H), 11.86 (s, 1H, indole N–H), 11.97 (s, 1H, indole N–H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 44.0, 110.7, 112.5, 113.3, 113.4, 117.2, 117.4, 118.0, 120.1, 120.4, 120.9, 121.1, 122.7, 122.8, 124.7, 124.9, 131.3, 131.5, 131.7, 134.7, 136.4, 137.1, 137.2, 137.4, 143.9, 152.5. IR ν_{max}/cm^{-1} (KBr) 3389 (m, $\nu_{=NH}$), 3071 ($\nu_{=C-H}$), 1598, 1551 ($\nu_{C=C}$), 1493 1423 (s, $\nu_{C=C}$, $\nu_{C=N}$), 1313, 1273, 1237, 1189, 1128 (δ_{CH}), 956 (s, $\nu_{=CH}$), 809, 745 (m, $\delta_{=CH}$). MS: EI (70 ev) *m/z* (%): 361 (62 M – CH_3I), 360 (100 M – CH_3I – H), 142, 127. UV-Vis λ_{max}/nm (methanol) 464. Found: C, 61.50; H, 4.23; N, 8.13. Calc. for $C_{26}H_{22}N_3I$ (503.3 g mol^{-1}): C, 61.85; H, 4.40; N, 8.32%.

*1-Methyl-2,4-bis[2-(1-methylpyrrol-2-yl)vinyl]pyridinium iodide (**3c**)*

Red needle crystals, mp 180-181°C, ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.70-4.01 (m, 9H, pyrrole N–CH_3, N^+CH_3), 6.20 (s 1H, pyrrole–H), 6.24 (s 1H, pyrrole–H), 6.52 (d, 1H, *J* 15.2 Hz, CH=CH), 6.73 (s, 1H, pyrrole–H), 6.83 (s, 3H, pyrrole–H), 7.08 (d, 1H, *J* 16.0 Hz, CH=CH) 7.54 (d, 1H, *J* 16.0 Hz, CH=CH), 7.68 (d, 1H, *J* 6.8 Hz, pyridine–H), 8.03 (d, 1H, *J* 15.2 Hz, CH=CH), 8.28 (d, 1H, *J* 6.8 Hz, pyridine–H), 8.60 (s, 1H, pyridine–H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 34.0, 34.1, 44.2, 109.6, 109.7, 111.1, 111.3, 112.7, 117.6, 117.9, 119.2, 128.0, 128.1, 128.5, 130.2, 130.2, 130.4, 144.0, 151.6, 151.9. IR ν_{max}/cm^{-1} (KBr) 3440 (m, $\nu_{=NH}$), 3031 ($\nu_{=C-H}$), 1597, 1547 ($\nu_{C=C}$), 1520, 1409 (s, $\nu_{C=C}$, $\nu_{C=N}$), 1339, 1264, 1194, 1126, 1086 (δ_{CH}), 971, 888, 814, 710 (m, $\delta_{=CH}$). MS: EI(70 ev) *m/z* (%): 289 (45 M – CH_3I), 288 (100 M – CH_3I – H), 142, 127. UV-Vis λ_{max}/nm (methanol) 473. Found: C, 53.91; H, 3.57; N, 5.99. Calc. for $C_{20}H_{22}N_3I$ (431.09 g mol^{-1}): C, 53.77; H, 3.61; N, 6.27%.

*1-Methyl-2,6-bis[2-(1-methylpyrrol-2-yl)vinyl]pyridinium iodide (**3d**)*

Red needle, mp 230 °C (decomposition), ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.86 (s, 6H, pyrrole N–CH_3), 4.28 (s, 3H, N^+CH_3), 6.21-6.23 (m, 2H, pyrrole–H), 6.79 (s, 2H, pyridine–H), 6.93-6.99 (m, 4H, CH=CH, pyrrole–H), 7.50 (d, 2H, *J* 16.0 Hz, CH=CH), 8.08-8.10 (m, 3H, pyridine–H, pyrrole–H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 33.9, 40.9, 109.5, 112.4, 112.7, 121.2, 128.2, 130.3, 141.1, 153.2. IR ν_{max}/cm^{-1} (KBr) 3446 (m, $\nu_{=NH}$), 3073 ($\nu_{=C-H}$), 1604, 1559 ($\nu_{C=C}$), 1469, 1404 (s, $\nu_{C=C}$, $\nu_{C=N}$), 1335, 1307, 1228, 1189, 1144, 1090 (δ_{CH}), 948, 841, 802, 730 (m, $\delta_{=CH}$). MS: EI (70 ev) *m/z* (%): 289 (100 M – CH_3I), 288 (58 M – CH_3I – H), 142, 127. UV-Vis λ_{max}/nm (methanol) 465. Found: C, 53.88; H, 3.65; N, 6.47. Calc. for $C_{20}H_{22}N_3I$ (431.09 g mol^{-1}): C, 53.77; H, 3.61; N, 6.27%.

*1-Methyl-2,4-bis[2-(furan-2-yl)vinyl]pyridinium iodide (**3e**)*

Yellow powder, mp 254-255 °C, ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.35 (s, 3H, N^+CH_3), 6.54-6.55 (m, 2H, furan–H), 6.91-6.98 (m, 4H, furan–H, CH=CH), 7.56-7.58 (m, 2H, furan–H), 7.68-7.82 (m, 3H, CH=CH, pyridine–H), 8.14 (s, 1H, pyridine–H), 9.10 (d, 1H, *J* 6.8 Hz, pyridine–H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 44.4, 112.8, 115.0, 115.7, 119.8, 120.2, 126.4, 128.3, 144.9, 145.7, 146.0, 150.6, 150.8, 150.9. IR ν_{max}/cm^{-1} (KBr) 3474 (m, $\nu_{=NH}$), 3083 ($\nu_{=C-H}$), 1608, 1558 ($\nu_{C=C}$), 1470, 1442 (s, $\nu_{C=C}$, $\nu_{C=N}$), 1386, 1330, 1301 (δ_{CH}) 1253($\nu_{asC-O-C}$), 1069(ν_{sC-O-C}), 969, 880,751 (m, $\delta_{=CH}$). MS: EI (70 ev) *m/z* (%): 264 (11 M – CH_3I), 263 (53 M – CH_3I – H), 142, 127. MS: EI (70 ev) *m/z* (%): 264 (11 M – CH_3I), 263 (53 M – CH_3I – H), 142, 127. UV-Vis λ_{max}/nm (methanol) 397. Found: C, 53.64; H, 3.60; N, 3.60. Calc. for $C_{18}H_{16}NIO_2$ (405.02 g mol^{-1}): C, 53.35; H, 3.98; N, 3.46%.

*1-Methyl-2,6-bis[2-(furan-2-yl)vinyl]pyridinium iodide (**3f**)*

Khaki-colored powder, mp 214-215 °C, ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.37 (s, 3H, N^+CH_3), 6.54-6.55 (m, 2H, CH=CH), 6.93-6.94 (m, 2H, furan–H), 7.21-7.27 (m, 2H, CH=CH), 7.50-7.56 (m, 4H, furan–H), 8.08 (d, 2H, *J* 8.8 Hz, pyridine–H), 8.28 (t, 1H, *J* 8.8 Hz, pydidine–H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 41.1, 112.7, 115.4,

115.5, 122.8, 128.4, 142.3, 145.7, 150.6, 152.2. IR ν_{max}/cm^{-1} (KBr) 3479 (m, $\nu_{=NH}$), 3061 ($\nu_{=C-H}$), 1602, 1561($\nu_{C=C}$), 1463, 1384 (s, $\nu_{C=C}$, $\nu_{C=N}$), 1305 (δ_{CH}) 1247($\nu_{asC-O-C}$) 1068(ν_{sC-O-C}), 951, 982, 878, 757 (m, $\delta_{=CH}$). MS: EI (70 ev) *m/z* (%): 264 (8 M – CH_3I), 263 (39 M – CH_3I – H), 142, 127. UV-Vis λ_{max}/nm (methanol) 397. Found: C, 53.31; H, 3.60; N, 3.76. Calc. for $C_{18}H_{16}NIO_2$ (405.02 g mol^{-1}): C, 53.35; H, 3.46; N, 3.98%.

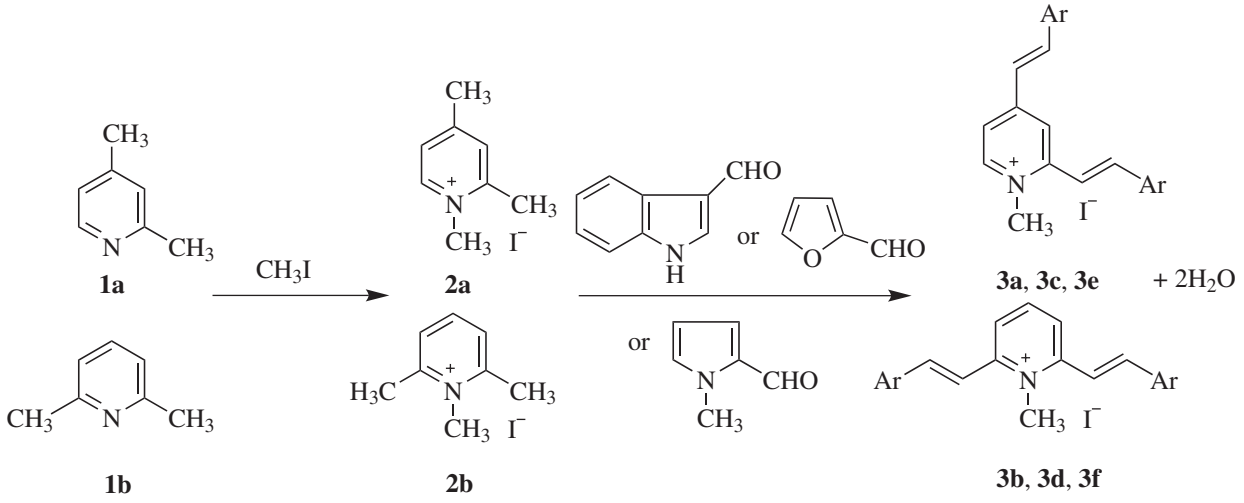
Results and Discussion

Synthesis

The trinucleus dimethine cyanine dye was synthesized via the reaction of heteroaromatic aldehydes with the quaternary ammonium salt of 1,2,4-trimethyl pyridine quaternary salt or 1,2,6- trimethyl pyridine quaternary salt (Scheme 1). In all cases investigated, we found that the formation reactions of trinucleus dimethine cyanine dyes with pyridine nucleus proceeded efficiently under catalysis of piperidine or NaOH, and the better and purer yields were obtained in ethanol for **3a**-**3d** and in water for **3e**-**3f**. For example, the yield of **3d** could reach 86% in ethanol, and 65% in water.[12] The yield of **3f** could reach 83% in water, but in ethanol **3f** could hardly be obtained. Because in organic solvents furaldehyde reacted with quaternary salt so quickly that the reaction could not be controlled, and there would be serious side reactions. In the synthesis of dyes **3a**-**3f** , the required reaction time of heterocyclic aromatic aldehydes with trimethylpyridinium iodides was furaldehyde (30 min) < 1-methylpyrrole-2-carboxaldehyde (20 h) < indole-3-carboxaldehyde (24 h), thereby the reaction activity of heterocyclic aromatic aldehydes was furaldehyde > 1-methylpyrrole-2-carboxaldehyde > indole-3-carboxaldehyde. From the structure it was also suggested that the electron-withdrawing ability of the group attached to aldehyde group was =C–O > =C–N > =C–C=, leading to the positive charge density of carbon in aldehyde group was furaldehyde > 1-methylpyrrole-2-carboxaldehyde > indole-3-carboxaldehyde. The larger the positive charge density of carbon in aldehyde group is, the higher the reaction activity of heterocyclic aromatic aldehyde with trimethylpyridinium iodides is.

Spectral properties of the dyes in different solvents

Figure 1 gives the absorption maxima (λ_{max}) of six trinucleus dimethine cyanine dyes in different solvents (their dielectric constant: $CHCl_3$ 4.9, EtOH 24.6, MeOH 32.6, dimethylformamide 38.3, H_2O 78.4). From Figure 1 it could be found that the λ_{max} of **3a**, **3b**, **3c** and **3d** was longer than the λ_{max} of **3e** and **3f**. The reason suggested here was that dyes **3a**-**3f** were all D-π-A molecules, in dyes **3a**-**3d** the electron donor was N of indole or pyrrole ring, and the electron donor was O of furan ring in dyes **3e**-**3f**, and the electron acceptor of six dyes was all N^+ of pyridinium. The stronger was electron-donating capability of electron donor, the longer was the λ_{max} of dyes under the same electron-withdrawing capability of electron acceptor. The electron-donating capability of N situated indole or pyrrole



3a, 3b: Ar = indole-3-carboxaldehyde
3c, 3d: Ar = 1-methylpyrrole-2-carboxaldehyde
3e, 3f: Ar = furaldehyde

Scheme 1. Synthesis of trinucleus dimethine cyanine dyes.

ring was stronger than that of O situated furan ring, so the λ_{max} of **3a**-**3d** was longer than the λ_{max} of **3e**-**3f**. It could be also found that with the increasing of solvent polarity the λ_{max} of the dyes decreased. The effect of the solvent polarity on the absorption maximum could be illustrated by interactions between the dye molecules and the solvents, as the interactions made the ground state of dye more stable by forming hydrogen bonds.[18,19]

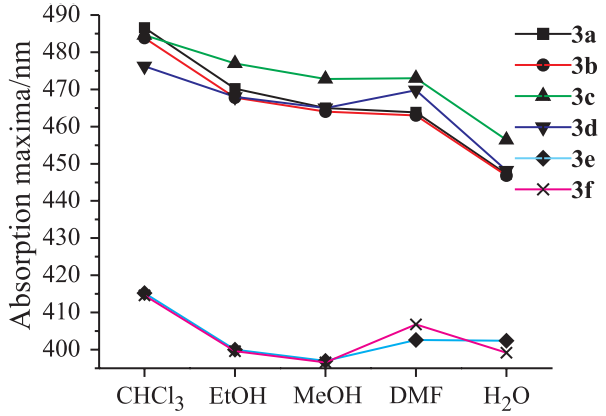


Figure 1. Solvatochromic shifts for the λ_{max} of six trinucleus dimethincyanine dyes in five kinds of solvents.

The absorption, excitation maxima (λ_{ex}), fluorescence maxima emission (λ_{em}) of the dyes are summarized in Table 1. The dyes exhibited fluorescence properties at room temperature. Their fluorescence maxima were located at 502-565 nm. Compared with the absorption maxima of the dyes, the emission spectra were shifted to the red by 69-140 nm (Stokes shift). The stokes shift of six dyes were all large, this might be attributed to an excited-state intramolecular charge transfer between the donor and acceptor in the dyes. Large stokes shift could help to reduce self-quenching and measurement error by excitation light and scattered light.[20] The fluorescence quantum yield of six dyes was in the region 0.0001-0.0781 in different solvents. From Table 1 it could be found that the order of fluorescence quantum yield was **3a**, **3b** > **3c**, **3d**. The possible reason was that the conjugated system of **3a**, **3b** was larger than that of **3c**, **3d**. We also found the order of fluorescence quantum yield was **3a** > **3b** and **3c** > **3d**. The cause lay in the fact that two D-π-A system in **3b** and **3d** was in opposite orientation, which made their conjugated system became small. It could be found that the fluorescence performance of **3e** and **3f** was complex, because oxygen of furan ring was of both electron-donating and electron-acceptor effect. And there was also influence of solvents on the fluorescence quantum yield of these dyes. Further investigation of the influence of solvents on the fluorescence quantum yield of the dyes is in progressing.

Table 1. The absorption and fluorescence spectral characteristics of dyes **3a**-**3f** in different solvents

	λ_{max}/nm	λ_{ex}/nm	λ_{em}/nm	Stokes shift	Φ_F
Methanol					
3a	465	465	550	85	0.0538
3b	464	464	551	87	0.0310
3c	473	473	559	86	0.0031
3d	465	465	556	91	0.0027
3e	397	397	517	120	0.0289
3f	397	397	508	112	0.0115
Ethanol					
3a	470	470	552	82	0.0781
3b	468	467	550	82	0.0689
3c	477	477	561	84	0.0191
3d	468	468	560	92	0.0039
3e	400	400	518	118	0.0029
3f	400	400	540	140	0.0028
Chloroform					
3a	487	487	556	70	0.0470
3b	484	484	557	73	0.0403
3c	485	484	559	74	0.0098
3d	476	476	558	82	0.0023
3e	415	415	502	87	0.0110
3f	415	415	509	94	0.0037
Dimetilformamida					
3a	464	464	557	93	0.0298
3b	463	463	556	93	0.0275
3c	473	473	563	90	0.0060
3d	470	470	565	95	0.0008
3e	403	402	532	129	0.0148
3f	407	407	518	111	0.0052
Water					
3a	447	447	554	107	0.0008
3b	447	447	553	106	0.0008
3c	456	456	562	106	0.0003
3d	448	448	541	93	0.0001
3e	402	402	529	127	0.0032
3f	399	399	515	116	0.0016

[a] The fluorescence quantum yields of the dyes were determined by the reference standard (rhodamine B Φ_F=0.49 in ethanol at 25 °C).[21]

Spectral properties of the dyes in the presence of DNA

The spectral properties of the dyes in the presence of DNA are summarized in Table 2 and Table 3. The λ_{max} of DNA-dye solution was situated at 401-458 nm and showed a slight red shift relative to the corresponding maxima of

Table 2. Spectral characteristics of dyes **3a**-**3f** in buffer

Dye	in buffer				
	λ_{max} (nm)	$\varepsilon^{free}\times10^{-4}$ (dm^3 mol^{-1} cm^{-1})	λ_{ex} (nm)	λ_{em} (nm)	Φ_F^{free}
3a	447	2.2	445	565	0.0235
3b	447	2.1	445	560	0.0041
3c	456	5.5	453	564	0.0042
3d	448	4.8	448	-	-
3e	402	3.8	406	519	0.0557
3f	399	4.2	406	504	0.0323

[a] The fluorescence quantum yields of the dyes were determined by the reference standard (rhodamine B Φ_F = 0.49 in ethanol at 25 °C).[21]

Table 3. Spectral characteristics of dyes **3a**-**3f** in presence of DNA

Dye	in DNA presence					$\Phi_F^{DNA}/\Phi_F^{free}$
	λ_{max} (nm)	$\varepsilon^{DNA}\times10^{-4}$ (dm^3 mol^{-1} cm^{-1})	λ_{ex} (nm)	λ_{em} (nm)	Φ_F^{DNA}	
3a	455	3.2	445	545	0.2242	9.5
3b	454	6.2	445	545	0.1291	31.6
3c	458	8.5	453	555	0.0791	18.9
3d	449	5.1	448	553	0.0187	-
3e	403	3.8	406	515	0.0553	1.0
3f	401	4.2	406	503	0.0336	1.0

[a] The fluorescence quantum yields of the dyes were determined by the reference standard (rhodamine B Φ_F = 0.49 in ethanol at 25 °C).[21]

free dyes in buffer. The molar extinction coefficients for dyes **3a**-**3d** were increased in the presence of DNA, and were in the range from 3.2×10^4 to 8.5×10^4 L mol^{-1} cm^{-1}. However, for dyes **3e** and **3f** the values of molar extinction coefficients were unchanged relative to free dyes, which were 3.8×10^4 and 4.2×10^4 L mol^{-1} cm^{-1}, respectively.

The fluorescence emission maxima of DNA-dyes were located at 503-555 nm, and showed a slight blue shift relative to free dyes in solution. Stokes shift values for the DNA-dyes were in the range of 97-109 nm. Moreover, the fluorescence intensity of dyes **3a**-**3c** was greatly increased in the presence of DNA. Compared with free dyes, the quantum yields of DNA-dyes were up to 9.5 times for dye **3a**, up to 31.6 times for dye **3b** (Figure 2) and up to 18.9 times for dye **3c**. Specially, the quantum yields of DNA-dye **3a** was the highest in six dyes. It was noteworthy that free dye **3d** could not be detected significantly fluorescence in buffer, but DNA-dye **3d** showed great fluorescence quantum yields. The fluorescence enhancement of trinucleus dimethine cyanine dyes bound to DNA was attributable to the fact that on photoexcitation a lack of free rotation around the internuclear bridge made isomerisation around the C-C bonds of the methine chain difficult; and subsequently nonradiative deactivation of the excited state was not possible, causing the dye to fluoresce.[22,23] Upon binding with DNA, **3a**-**3d** maintained

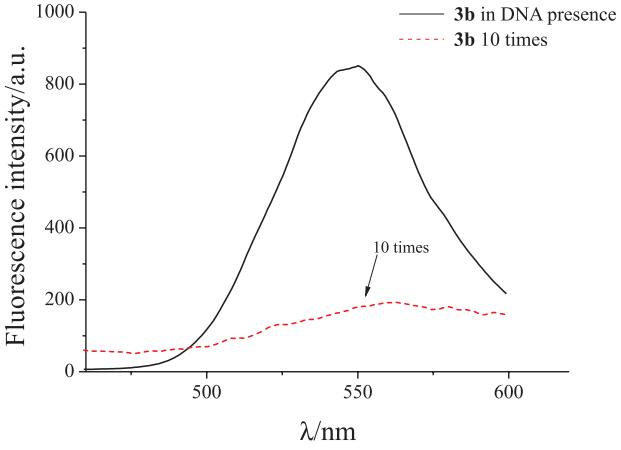


Figure 2. Fluorescence spectra of **3b** in buffer, in the presence of different concentration of DNA.

their high Stokes shift and showed a red shift, owing to a more efficient ICT in the excited state between the terminal heterocyclic aromatic and the pyridimium group.[24,25]

Conclusions

Six trinucleus dimethine cyanine dyes with pyridine nucleus were synthesized and isolated in 69-86% yield with piperidine or NaOH as catalyst.

The absorption maxima of the dyes were located at 397-487 nm in different solvents, and with the increase of solvents polarity the maximum absorption wavelength of these dyes had a blue-shift. The fluorescence maxima of the dyes in different solvents were basically located at 502-565 nm, and the fluorescence quantum yield of six dyes was in the region 0.0001-0.0781 in different solvents.

The absorption maxima of the dyes in the presence of DNA were situated at 401-458 nm and showed a slight red shift relative to free dyes. The fluorescence maxima of DNA-dyes were located at 503-555 nm, and showed a slight blue shift relative to free dyes. The fluorescent quantum yields of the DNA-dyes **3a-3c** were up to 9.5-31.6 times higher than that of free dyes. Specially, free dye **3d** emitted weak fluorescence in buffer, but DNA-dye **3d** showed great fluorescence quantum yields. Therefore dyes **3a-3d** could be proposed as fluorescent dyes for DNA detection.

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br, as PDF file.

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