A visible-light-responsive DiSCn(3)-type fluorescent probe for the rapid, sensitive, and specific detection of tin(II) ions in aqueous solution

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Abstract
Visible-light-excited fluorescent probes with excellent water solubility are of significant research importance in the biomedical field. In this work, a series of visible-light-responsive carbocyanine fluorescent compounds [DiSCn(3)] are synthesized from 2-methylbenzothiazole and are employed as fluorescent probes for detecting tin(II) ions. Upon addition of tin(II) ions to an aqueous solution of compounds DiSCn(3)-3 and 4, both exhibit considerable changes in their absorption and fluorescent intensity, respectively. These spectral changes may correspond to the binding between tin(II) ions and the sulfur atom in the benzothiazole unit to form a metal complex. This indicates that DiSCn(3)-3 and 4 can be used as promising fluorescent chemical sensors for detecting tin(II) ions in aqueous solution via visible-light excitation.

Keywords
carbocyanine, fluorescent probe, tin(II) ions, visible-light excitation, water solubility

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Introduction
Nowadays, metal ions have a significant impact in terms of environmental pollution and human health, respectively. Hence, effective detection methods for metal ions are necessary. With the development of coordination chemistry and...
probe technique, different instrumental methods, functional nucleic acids (FNAs)-based sensors,1,2 electrochemical biosensors,3 aggregation-induced emission (AIE)-based luminescence probes,4 cellulose-based sensors,5 nanomaterials,6-8 colorimetric sensors,9 metal-organic frameworks (MOFs)10 and so on,11,12 have been applied to metal ion detection in recent decades.

As a heavy metal ion, the presence of too much tin(II) ions in the body is harmful to human beings.13,14 Hence, the toxicological effects of tin(II) ions have been widely studied for about half a century. In terms of the lethal median dose in rats, McRae et al.15 found that a dose of 8 mg Sn²⁺ kg⁻¹ produced significant mortality and gross kidney damage. The outcome mentioned above was largely attributed to the tissue distribution of ⁹⁹ᵐTc-pertechnetate altered in rat bodies after treated with tin(II) ions. Meanwhile, tin(II) ions are also widely used for the preparation of ⁹⁹ᵐTc radio-pharmaceuticals16–18 and their non-radioactive preeminent kits.19 Moreover, stannous chloride spectrophotometry, as a method for the determination of phosphorus, has been widely applied in different environments.20–22

Carbocyanine fluorescent compounds [DiSCₙ(3)] can be used as simple, rapid, and specific DNA detection reagents. They represent an important alternative test method compared with existing detection methods. The sequence-specific accelerated photobleaching reaction of these compounds in the presence of a peptide nucleic acids (PNA) probe and a genomic DNA target forms a very interesting and previously unreported photochemical basis for a sensitive, rapid, simple, and potentially inexpensive method of DNA detection.23–26 However, there are no reports regarding the treatment of these [DiSCₙ(3)] compounds with different metal ions, especially tin(II) ions, in aqueous solution as fluorescent probes with visible-light excitation.

A series of carbocyanine-type fluorescent compounds [DiSCₙ(3)] have been synthesized from 2-methylbenzothiazole. Their fluorescent intensity changes revealed that the fluorescent compounds DiSCₙ(3)-3 and 4 had unique selectivity toward tin(II) ions in aqueous solution. Therefore, these two compounds can be used for the detection of tin(II) ions via fluorescence sensor studies.

Results and discussion

Organic synthesis

The synthetic route to the target compounds DiSCₙ(3)-1 to 4 is depicted in Scheme 1. The key intermediate compounds I-1 to 4 were obtained by quaternization of 2-methylbenzothiazole with the corresponding alkyl iodide or sultone derivatives.27–29 Thiacarbocyanines containing symmetric pendant N-alkyl or N-alkylsulfonic acid chains (DiSCₙ(3)-1 to 4) were prepared from N-substituted benzothiazolium salts (I-1 to 4) with a reactive methyl group at position 2.

Optical properties of DiSCₙ(3)-1 to 4

Both the UV-Vis absorption and fluorescence spectra of DiSCₙ(3)-1 to 4 (1 × 10⁻⁵ mol L⁻¹) were determined in methanol or water with a quartz cell (cross section = 1.0 cm × 1.0 cm). The fluorescence emission spectra were recorded by excitation at the maximum absorption wavelength according to their corresponding UV-Vis absorption spectra.30,31 The compounds were excited at around 518 and 556 nm (scanned with 5.0 nm excitation and 5.0 nm emission slit width) (Table 1). The advantages of visible-light excitation and water solubility impart the compounds with potentially promising properties for applications in both cell and in vivo imaging.

The results shown in Table 1 reveal similar excitation wavelengths, emission wavelengths, and stokes shift for compounds DiSCₙ(3)-1 to 4. The imperceptible changes in the fluorescence and UV-Vis absorption spectra are

![Scheme 1. Synthesis of DiSCₙ(3)-1 to 4. Reagents and conditions: (a) I-1: CH₃I, EtOH, r.t., 0.5 h, then 80 °C, 24 h; I-2: CH₃CH₂I, EtOH, r.t., 0.5 h, then 80 °C, 30 h; I-3: 1,3-propane sultone, 135 °C, 8 h; I-4: 1,4-butane sultone, 135 °C, 8 h; (b) I-1 to 4: triethoxymethane, dry pyridine, reflux, 2–18 h.](image)

| Compound       | Solvent | Excitation (nm) | Emission (nm) | Stokes shift (nm) |
|----------------|---------|-----------------|---------------|-------------------|
| DiSCₙ(3)-1     | MeOH    | 518/555         | 571           | 53/16             |
| DiSCₙ(3)-2     | MeOH    | 520/556         | 576           | 56/20             |
| DiSCₙ(3)-3     | Water   | 515/556         | 574           | 59/18             |
| DiSCₙ(3)-4     | Water   | 519/556         | 576           | 55/20             |
probably due to the presence of the same thiacarbocyanine backbone in these four compounds, with similar π-conjugation length throughout the whole molecule. The autocorrelation of the fluorescence intensity trajectories for immobilized molecules of DiSCn(3)-3 and 4 revealed two components in their corresponding system. This phenomenon could be ascribed to intersystem crossing and trans-cis isomerization. This contributed to the two peaks in the UV-Vis absorption spectra at around 520 and 556 nm. Therefore, further investigations are necessary to study the differences in the optical characteristics of these four compounds.

**Response of the compounds to metal ions**

The response properties of DiSCn(3)-1 to 4 (1 × 10^{-5} mol L^{-1}) to various metal ions were investigated by fluorescence emission spectroscopy with a quartz cell (cross section=1.0 × 1.0 cm). All the compounds (1 × 10^{-5} mol L^{-1}) were treated with different metal ions, as shown in Figure 1. As reference compounds DiSCn(3)-1 and 2 did not dissolve in water very well, the spectral responses to various metal ions were mainly examined using DiSCn(3)-3 and 4.

As shown in Figure 1, tin(II) ions quenched the fluorescence intensity of DiSCn(3)-3 and 4 most efficiently. This unique response provides a rapid, sensitive, and specific detection method for Sn^{2+}. By contrast, other metal ions, such as Hg^{2+}, Fe^{3+}, Co^{2+}, and Zn^{2+}, gave no significant changes in fluorescence intensities at the equilibrium states. The above results indicate that DiSCn(3)-3 and 4 specifically bind to tin(II) ions to form a metal complex. Therefore, these two compounds can be applied as fluorescent probe sensors for the detection of tin(II) ions in water. Furthermore, DiSCn(3)-4 was more sensitive than DiSCn(3)-3 for detecting tin(II), and there was almost no influence for the detection of Al^{3+}, at least as far as Figure 1 shows. Next, the spectrograms of the above compounds were recorded after adding tin(II) ions.

![Figure 1](image1.png)

**Figure 1.** The selectivity of DiSCn(3)-3 (left) and 4 (right) toward various metal ions. The concentration of these two compounds was 1 × 10^{-5} mol L^{-1}. The concentrations of the metal ions were 1.5 × 10^{-5} mol L^{-1}. All measurements were carried out on equilibrium states.

![Figure 2](image2.png)

**Figure 2.** Fluorescence emission spectra of DiSCn(3)-3 in water on increasing the concentration of tin(II) ions with excitation at 515 nm (left). Variation of the UV-Vis absorption spectra of DiSCn(3)-3 in water on increasing the concentration of tin(II) ions (right).
Spectral properties of DiSCn(3)-3 to 4

Due to the pendent sulfonic groups, compounds DiSCn(3)-3 and 4 dissolved in water very well, and the spectral properties of these two compounds were analyzed in aqueous solution.

As shown in Figure 2 (left), the fluorescence spectra were measured during dropwise addition of a stannous chloride solution an aqueous solution of the prepared compounds. Because no metal ion response was observed on excitation at 556 nm, the fluorescence spectra of the compounds were obtained on excitation at 515 nm. The fluorescent intensity of compound DiSCn(3)-3 was centered at 574 nm and decreased dramatically on increasing the concentration of tin(II) ions from 0 to $3.0 \times 10^{-5}$ mol L$^{-1}$. This result suggests that a chelate complex was formed between tin(II) ions and DiSCn(3)-3.

The process described above can also be monitored by UV-Vis absorption spectroscopy. As shown in Figure 2 (right), the maximum absorption of DiSCn(3)-3 in aqueous solution occurred at around 556 and 515 nm. Significant decreases of the absorption intensities at 556 and 515 nm were also observed upon adding increasing amounts of tin(II) ions. Two isosbestic points at 490 and 575 nm indicated that two components were present in the system. Hence, the interaction between DiSCn(3)-3 and tin(II) ions could also be determined easily from the UV-Vis absorption measurements.

Similar to DiSCn(3)-3, the fluorescence intensity of DiSCn(3)-4 centered at 576 nm also decreased dramatically on increasing the concentration of tin(II) ions from 0 to $3.0 \times 10^{-5}$ mol L$^{-1}$ (Figure 3, left). Meanwhile, significant decreases of the absorption intensities at 556 and 519 nm were also observed by UV-Vis absorption spectroscopy upon adding increasing amounts of tin(II) ions.

The decreasing intensity of the fluorescence and UV-Vis absorption spectra as well as the formation of two isosbestic points at 487 and 579 nm in the UV-Vis absorption spectra on dropwise addition of tin(II) ions indicated that two components existed in the system. Hence, the interaction between DiSCn(3)-4 and tin(II) ions is well illustrated by both the UV-Vis absorption and fluorescence spectral measurements. These measurements suggest the formation of a chelate complex between tin(II) ions and DiSCn(3)-4. In other words, the formation of a metal complex between DiSCn(3)-4 and tin(II) ions resulted in significant spectral changes.

A plausible complexation mechanism for DiSCn(3)-3 and 4 upon addition of SnCl$_2$

The proposed mechanism for the metal complex formation is shown in Scheme 2. The prepared probe, which has two different resonance structures, forms a chelate complex with tin(II) ions in the most stable conformation. The equilibrium between compounds DiSCn(3)-3 and 4 with tin(II) ions can be directly detected through fluorescence quenching and the formation of isosbestic points in the UV-Vis absorption spectra. Based on coordination chemistry theory, the plausible mechanism postulates that the sulfur atom in compounds DiSCn(3)-3 and 4 coordinates with the tin(II) ions to form a chelate ring that induces the fluorescence quenching process. In addition, DiSCn(3)-3 and 4 dissolve in water very well with the help of the pendent sulfonic groups. This study indicates that DiSCn(3)-3 and 4 can be used to detect tin(II) ions with high selectivity and sensitivity in aqueous solution with visible-light excitation.

Conclusion

In summary, a series of novel fluorescence probes have been synthesized from 2-methylbenzothiazole and have been applied as fluorescence probes for detecting tin(II) ions in aqueous solution. The fluorescent compounds DiSCn(3)-3 and 4 could be used to detect tin(II) ions with high sensitivity and selectivity with visible-light excitation.

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Figure 3. Fluorescence emission spectra of DiSCn(3)-4 in water on increasing the concentration of tin(II) ions with excitation at 519 nm (left). Variation of the UV-Vis absorption spectra of DiSCn(3)-4 in water on increasing the concentration of tin(II) ions (right).
Meanwhile, they showed remarkable response variations in their absorption and emission spectra, respectively. We expect that the present research will be applicable for the detection of tin(II) ions in different environmental systems. Furthermore, specific DNA detection could also be accomplished by compounds $\text{DiSCn(3)-3}$ and $\text{4}$.

**Experimental**

All chemicals and reagents were of the highest purity available and were purchased from Tianjin Jiangtian Chemical Technology Co., Ltd., and used without further purification. 2-Methylbenzothiazole, iodomethane, iodoethane, 1,3-propanesultone, 1,4-butane sultone, and triethoxymethane were purchased from Aladdin Reagent Database Inc.

$^1$H NMR spectra were recorded on a Bruker DRX-400 AVANCE spectrometer. CDCl$_3$ and D$_2$O were used as the solvents. Steady-state fluorescence spectra were recorded on a Hitachi F-4500 spectrophotometer. UV-Vis absorption spectra were measured on a Shimadzu UV-3390 spectrophotometer. Elemental analysis was obtained on a Vario MAX CHN apparatus and mass spectrometry was accomplished on a Xevo G2 Q-tof HRMS apparatus.

**DiSCn(3)-1 to 4; typical procedure**

**2,3-Dimethylbenzothiazole-3-ium iodide (I-1).** A solution of 2-methylbenzothiazole (5.00 g, 34.0 mmol) and iodomethane (4.97 g, 35.0 mmol) was stirred in ethanol (10.0 mL) at room temperature for 0.5 h. The mixture was then refluxed for 24 h. After cooling to room temperature, the resulting crystalline product was collected by filtration under reduced pressure and washed with diethyl ether until the solution became colorless. The purified product (yellow powder) was collected by filtration under reduced pressure.\textsuperscript{27–29} Yield: 86%, m.p. 239–242 °C. $^1$H NMR (400 MHz, D$_2$O): $\delta$ = 3.04 (s, 3H, -CH$_3$), 4.11 (s, 3H, N-CH$_3$), 7.67 (t, $J$ = 7.6 Hz, 1H, ArH), 7.77 (t, $J$ = 7.6 Hz, 1H, ArH), 7.97 (d, $J$ = 8.4 Hz, 1H, ArH), 8.07 (d, $J$ = 8.0 Hz, 1H, ArH). HRMS-ESI: $m/z$ [M + H]$^+$ cged for C$_9$H$_{11}$INS: 291.9657; found: 291.9654.

**2-Methyl-3-ethylbenzothiazole-3-ium iodide (I-2).** The product (yellow powder) was prepared using a similar procedure.\textsuperscript{27–29} Yield: 85%, m.p. 179–181 °C. $^1$H NMR (400 MHz, D$_2$O): $\delta$ = 1.44 (t, $J$ = 7.2 Hz, 3H, -CH$_2$CH$_3$), 3.05 (s, 3H, -CH$_3$), 4.64 (m, 2H, -CH$_2$), 7.67 (t, $J$ = 3.6 Hz, 1H, ArH), 7.77 (t, $J$ = 3.6 Hz, 1H, ArH), 8.04 (m, 2H, ArH). HRMS-ESI: $m/z$ [M + H]$^+$ cged for C$_{10}$H$_{13}$INS: 305.9813; found: 305.9808.

**2-Methyl-3-(3-sulfopropyl)benzo[d]thiazol-3-ium iodide (I-3).** A solution of 2-methylbenzothiazole (1.492 g, 10.0 mL) and 1,3-propanesultone (4.48 g, 36.7 mmol) was refluxed for 8 h under a nitrogen atmosphere. After cooling to room temperature, the resulting product was collected by filtration under reduced pressure and washed with isopropanol three times. The purified product (silver-gray solid) was collected by filtration under reduced pressure.\textsuperscript{27–29} Yield: 88%, m.p. > 300 °C. $^1$H NMR (400 MHz, D$_2$O): $\delta$ = 2.29 (m, 2H, -CH$_2$), 3.03 (t, $J$ = 7.2 Hz, 2H, -CH$_2$), 3.09 (s, 3H, -CH$_3$), 4.79 (t, $J$ = 8.2 Hz, 2H, -CH$_2$SO$_3^-$), 7.68 (t, $J$ = 8.0 Hz, 1H, ArH), 7.79 (t, $J$ = 7.8 Hz, 1H, ArH), 8.08 (m, 2H, ArH). HRMS-ESI: $m/z$ [M + H]$^+$ cged for C$_{11}$H$_{15}$INO$_3$S$_2$: 399.9538; found: 399.9535.

**2-Methyl-3-(4-sulfobutyl)benzo[d]thiazol-3-ium iodide (I-4).** The product (gray solid) was prepared using a similar procedure.\textsuperscript{27–29} Yield: 90%, m.p. 283–286 °C. $^1$H NMR (400 MHz, D$_2$O): $\delta$ = 1.86 (m, 2H, -CH$_2$), 2.06 (m, 2H, -CH$_2$), 2.91 (t, $J$ = 7.6 Hz, 2H, -CH$_2$SO$_3^-$), 3.11 (s, 3H, -CH$_3$), 4.69 (t, $J$ = 8.0 Hz, 2H, -CH$_2$SO$_3^-$), 7.70 (t, $J$ = 8.0 Hz, 1H, ArH), 7.80 (m, 2H, ArH). HRMS-ESI: $m/z$ [M + H]$^+$ cged for C$_{12}$H$_{17}$INO$_3$S$_2$: 413.9695; found: 413.9691.

**3-Methyl-2-((1E,3E)-3-(3-methylbenzo[d]thiazol-2(3H)-ylidene)prop-1-en-1-yl)benzo[d]thiazol-3-ium iodide (DiSCn(3)-1).** A solution of 2,3-dimethylbenzothiazole-3-ium iodide (I-1)
(0.57 g, 3.50 mmol), triethoxymethane (1.07 g, 7.22 mmol), and dry pyridine (13 mL) was refluxed for 22 h. After cooling to the room temperature, diethyl ether was added and the resulting mixture was refrigerated to allow complete precipitation. The brown crystalline product was collected by filtration under reduced pressure, washed with diethyl ether, and recrystallized from dry acetonitrile. The brown crystalline product was collected by filtration. The symmetric tricarbocyanine dyes DiSCn(3)-2 to 4 were prepared using a similar procedure, with reaction time of 15–24 h.

3-Ethyl-2-((1E,3E)-3-(3-ethylbenzo[d]thiazol-2(3H)-ylidene)prop-1-en-1-yl)benzo[d]thiazol-3-ium iodide (DiSCn(3)-2). Brown solid, yield: 48%, m.p. 158–160 °C. 'H NMR (400 MHz, CDCl3), δ = 1.55 (t, J = 7.2 Hz, 6H, -CH3), 4.48 (m, 4H, -CH2-), 7.35 (m, 4H, =CH-, ArH), 7.39 (s, 1H, =CH-), 7.40 (t, J = 7.6 Hz, 2H, ArH), 7.66 (d, J = 8.0 Hz, 2H, ArH), 7.75 (d, J = 8.2 Hz, 2H, ArH), IR (KBr, cm⁻¹): 952, 1067, 1122, 1137, 1196, 1214, 1237, 1254, 1292, 1330, 1375, 1421, 1445, 1501, 1533, 1587, 1628, 3078, 3133, 3374, 3442. HRMS-ESI: m/z [M + H]+ calcd for C21H21IN2O6S4: 492.0191; found: 492.0187.

3-(3-Sulfopropyl)-2-((1E,3E)-3-(3-(3-sulfopropyl)benzo[d]thiazol-2(3H)-ylidene)prop-1-en-1-yl)benzo[d]thiazol-3-ium iodide (DiSCn(3)-3). Brown solid, yield 61%, m.p. 198–200 °C. 'H NMR (400 MHz, CDCl3), δ = 1.75 (m, 4H, -CH2-), 4.48 (t, J = 7.2 Hz, 4H, -CH2-), 5.07 (t, J = 7.2 Hz, 4H, -CH2-), 7.35 (m, 3H, =CH-, ArH), 7.40 (d, J = 8.0 Hz, 2H, =CH-), 7.51 (t, J = 7.2 Hz, 2H, ArH), 7.67 (d, J = 7.6 Hz, 2H, ArH), 8.54 (d, J = 8.0 Hz, 2H, ArH), IR (KBr, cm⁻¹): 953, 1068, 1121, 1137, 1216, 1237, 1330, 3347, 3442. HRMS-ESI: m/z [M + H]+ calcd for C23H26IN2O6S4: 680.9718; found: 680.9715.

3-(4-Sulfobutyl)-2-((1E,3E)-3-(3-(4-sulfobutyl)benzo[d]thiazol-2(3H)-ylidene)prop-1-en-1-yl)benzo[d]thiazol-3-ium iodide (DiSCn(3)-4). Brown solid, yield 63%, m.p. 248–250 °C. 'H NMR (400 MHz, D2O), δ = 1.49 (m, 4H, -CH2-), 1.66 (m, 4H, -CH2-), 2.79 (t, J = 4.4 Hz, 4H, -CH2-), 3.53 (t, J = 4.2 Hz, 4H, -CH2-), 4.44 (s, 1H, =CH-), 5.91 (s, 1H, =CH-), 6.67 (s, 1H, =CH-), 7.02 (m, 4H, ArH), 8.03 (d, J = 8.0 Hz, 2H, ArH), 8.34 (t, J = 8.0 Hz, 1H, ArH), 8.73 (t, J = 8.0 Hz, 1H, ArH), IR (KBr, cm⁻¹): 952, 1067, 1122, 1137, 1217, 3373, 3443. HRMS-ESI: m/z [M + H]+ calcd for C27H32IN5O6S5: 709.0031; found: 709.0028.

Declaration of conflicting interests
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