Two-photon fluorescent probe for silver ion derived from twin-cyano-stilbene with large two-photon absorption cross section

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\textbf{A R T I C L E  I N F O}

Article history:
Received 29 May 2010
Received in revised form 19 August 2010
Accepted 7 September 2010
Available online 17 September 2010

Keywords:
Silver ion
Fluorophore
Two-photon absorption cross section
Sensor
Twin-cyano-stilbene

\textbf{A B S T R A C T}

A two-photon sensor for silver ion derived from twin-cyano-stilbene as the fluorophore is reported. The sensor exhibits remarkably large two-photon absorption cross sections ($\delta_{\text{TPA}}$) whose value is as high as 950 GM in MeCN, high sensitivity to and selectivity for silver ion, and emits strong two-photon fluorescence when excited by 790 nm laser photons. The sensor can be used to detect trace Ag\textsuperscript{+}, and its binding constant (log $K$) for Ag\textsuperscript{+} is as high as 5.76 ± 0.05. The successful applications of the sensor provide a brilliant prototype for the development of ideal two-photon fluorescence probes.

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1. Introduction

Chemosensors that convert molecular recognition into highly sensitive and easily detected signals have been actively investigated in recent years. Recently, two-photon laser scanning microscopy (TPLM) has been greatly developed in combination with suitable two-photon (TP) chemosensors to image the distribution of the guest molecules and ions in cellular processes with deeper penetration, better localized excitation, much less photodamage and photobleaching, smaller absorption coefficient of light in tissue, lower tissue autofluorescence, and higher 3-D spatial selectivity than what the single-photon technique can attain [1–5].

However, at present, the fluorophores being used for TPLM are largely those that have been developed for one-photon excitation [6–9]. Most of these conventional fluorophores have relatively small values of the two-photon absorption cross section, $\delta_{\text{TPA}}$ (∼10 GM; 1 GM = $1 \times 10^{-50}$ cm$^4$ s photon$^{-1}$ mol$^{-1}$), or their optimal excitation wavelengths do not fall into the transmissive window for biological tissues, which lies between ∼600 and 1300 nm [10,11]. TPLM could greatly benefit from the development of chromophores with large $\delta_{\text{TPA}}$ (>1000 GM) that can be excited efficiently in this range.

For the maximum utilization of TPLM, it is essential to develop efficient two-photon probes, especially those with large $\delta_{\text{TPA}}$.

Although one after another two-photon sensors for fluoride [12], metal ions [13–18], pH [19], lipid rafts [20], glucose [21] and thiols [22] have been developed, most of their $\delta_{\text{TPA}}$ values are not very satisfactory.

As we all know, Ag\textsuperscript{+} exhibits a rich biological chemistry, serving as a widely used antibacterial agent [23,24], a transcriptional initiator in plants [25] and mammals [26,27], a specific target of plasmid-conferrered resistance [28–30], and a redox-inactive probe for Cu\textsuperscript{2+}/+ sites in metalloproteins [31–33]. Silver can inactivate sulfhydryl enzymes and also combine with amine, imidazole and carboxyl group of various metabolites such as high molecular weight proteins and metallothionein in tissue of cytosol fractions. Repeated exposure of animals to silver may produce anemia, cardiac enlargement, growth retardation, embryotoxicity and degenerative changes in the liver [34,35].

Only a two-photon fluorescence sensor for silver ions [14] has been reported. In order to improve the comprehensive performance including large $\delta_{\text{TPA}}$, small molecular volume and good cell permeability, it is necessary to develop new two-photon fluorescence sensors for silver ions.

An efficient two-photon probe for Ag\textsuperscript{+} should have high selectivity for Ag\textsuperscript{+} ions, significant two-photon cross section and high photostability. In this context, we extend our earlier work [14,15] and present a new two-photon probe for Ag\textsuperscript{+} ions (1) derived from 4-methyl-2,5-dicyano-4′-amino stilbene (DSC) as a two-photon fluorophore with remarkably large $\delta_{\text{TPA}}$ [15] and 6-aryl-3,9-dithia-6-azaundecane (TAM) as the Ag\textsuperscript{+} chelator. DCS is a push–pull chromophore with the strong electron donor of N,N′-dialkylylamino...
group at its extremity and two cyano groups on its aromatic ring. On one hand, amino group can increase $\delta_{TPA}$ value up to an order of magnitude [36]. On the other hand, cyano groups can dramatically boost the $\delta_{TPA}$ value of molecules [37], remarkably decrease transition energy, stabilize resonance structures and increase the excited state dipole moment. DCS has been successfully employed in the design of two-photon fluorescence (TPF) probe for Hg$^{2+}$ ions [15], and TAU is a well-known receptor for Ag$^+$ because of its high selectivity toward silver ion [38,39]. Herein, we have synthesized compound 1 (Fig. 1) as a novel two-photon fluorescence sensor for silver ions whose $\delta_{TPA}$ is comparatively large, and its photophysical properties during the binding to metal ions events was studied using UV–Vis spectrometry, fluorescence spectroscopy, and two-photon excitation spectroscopy.

2. Experimental

2.1. Materials and methods

NMR spectra were recorded on a VARIAN INOVA 400 MHz NMR spectrometer. Mass spectral determinations were made on a GC-TOF mass spectrometry (Micromass, UK). Fluorescence measurements were performed on a PTI-C-700 Felix and Time-Master system. Fluorescence quantum yields were measured using standard protocols established by Xu and Webb [41], using a mode-locked Ti:sapphire laser that delivers 400 fs pulses at 76 MHz. Fluorescence quantum yields were measured using standard methods [40] on air-equilibrated samples at room temperature. Quinine bisulfate in 0.05 mol L$^{-1}$ H$_2$SO$_4$ (Φ = 0.546) was used as a Ref. [40]. Two-photon-excited fluorescence (TPF) action cross-section spectra were measured according to the experimental protocol established by Xu and Webb [41], using a mode-locked Ti:sapphire laser that delivers $\sim$80 fs pulses at 76 MHz. Fluorescence ($10^{-4}$ mol L$^{-1}$ in 0.1 mol L$^{-1}$ NaOH), whose TPF action cross-sections are well-known [41], served as the reference. The quadratic dependence of the fluorescence intensity on the excitation intensity was verified for each data point, indicating that the measurements were carried out in intensity regimes in which saturation or photodegradation does not occur. The measurements were performed at room temperature on air-equilibrated solutions ($10^{-5}$ mol L$^{-1}$). The experimental uncertainty on the absolute action cross-sections determined by this method has been estimated to be $\pm$20% [41]. Absorption spectra were measured on a HP-8453 spectrophotometer. Solvents were generally dried and distilled prior to use. Reactions were monitored by thin-layer chromatography on Merck silica gel 60 F$_{254}$ pre-coated aluminum sheets. Column chromatography: Merck silica gel Si 60 (40–63 μm, 230–400 mesh). The pH-dependent fluorescence studies were performed according to the literature [42].

2.2. Synthesis

2.2.1. 2-(2-{{4-[bis-(2-ethylsulfanyl-ethyl)-amino]-phenyl}-vinyl}-5-methylterephthalonitrile (1)

Aldehyde 9 (339 mg, 1.14 mmol), and NaH (33 mg 1.37 mmol) were dissolved in 10 mL of tetrahydrofuran (THF), and the solution was cooled to 0 °C. To this solution, phosphonate 6 (333 mg, 1.14 mmol) in 10 mL of THF was added dropwise, and the reaction mixture was stirred for 12 h at 0 °C. Water was added to the reaction mixture, and the product was extracted with ethyl acetate. The organic layer was dried with MgSO$_4$ followed by evaporation of the solvent. The crude product was separated by column chromatography with a gradient of hexane in dichloromethane (20–0%) and ethyl acetate in dichloromethane (0–20%). Yield 388 mg (0.89 mmol, 78%). The resulting solid was recrystallized from acetone to give yellow powder.

IR (KBr) cm$^{-1}$: 2236 (C=N) and 1654 (C=O). HRMS (EI) m/z: 435.1808 (Calcd. for C$_{25}$H$_{29}$N$_3$S$_2$: 435.1803).$^{1}$H NMR (CHCl$_3$/d$_2$, 400 MHz) ppm: 7.964 (s, 1H), 7.536 (s, 1H), 7.466 (d, $J$ = 8.8 Hz, 1H), 7.191 (d, $J$ = 16.0 Hz, 1H), 7.119 (d, $J$ = 16.4 Hz, 1H), 6.675 (d, $J$ = 8.8 Hz, 2H), 3.594 (t, $J_1$ = $J_2$ = 7.6 Hz, 4H), 2.955 (s, 3H), 2.545 (s, 3H), 1.290 (t, $J_1$ = $J_2$ = 7.6 Hz, 6H).$^{13}$C NMR (CHCl$_3$/d$_2$, 100 MHz) ppm: 147.754, 139.851, 139.129, 134.745, 134.352, 129.258, 128.740, 124.066, 117.419, 117.351, 117.021, 116.930, 113.883, 111.847, 51.592, 31.585, 28.956, 26.477, 20.041, 15.122. Elemental analysis: Calcd. for C$_{25}$H$_{29}$N$_3$S$_2$: MW 435.18: C, 68.92; H, 6.71; N, 9.65; S, 14.72%. Found: C, 68.97; H, 6.75; N, 9.61; S, 14.67%.

3. Results and discussion

3.1. Design and synthesis of 2-(2-{{4-[bis-(2-ethylsulfanyl-ethyl)-amino]-phenyl}-vinyl}-5-methylterephthalonitrile (1)

2,5-dimethyl-terephthalonitrile (4) [43], 2,5-dibromo-p-xylene (3) [43], and 4-[bis-(2-chloro-ethyl)-amino]-benzaldehyde (8) [44], 2-bromomethyl-5-methylterephthalonitrile (5) [15], 1-diethylphosphorylmethyl-4-methyl-2,5-dicyanobenzene (6) [15], 4-[bis-(2-ethylsulfanyl-ethyl)-amino]-benzaldehyde (9) [14] were synthesized according to literature procedures. Sensor 1 (Scheme 1) was obtained in modest yield by condensation of 6 with 9.

3.2. Selectivities of Sensor 1 for metal ions in one- and two-photon excited fluorescence

To obtain insight into the binding properties of 1 toward metal ions, the fluorescent spectrum changes were investigated upon addition of various metal perchlorates (Ag$^+$, Ca$^{2+}$, Cd$^{2+}$, Cr$^{3+}$, Fe$^{3+}$, Co$^{2+}$, Ni$^{2+}$, Fe$^{2+}$, Na$^+$, Cu$^{2+}$, Zn$^{2+}$, Mn$^{2+}$, Mg$^{2+}$, Hg$^{2+}$, Pb$^{2+}$, K$^+$, and Ba$^{2+}$) to MeCN solutions of 1, respectively. The experimental results suggest that 1 shows a notable selectivity to Ag$^+$ ion. As depicted in Fig. 2, 1 shows scarcely any response with other metal ions and weak complexation with Hg$^{2+}$, Pb$^{2+}$, and Cu$^{2+}$. Similar selectivities were also confirmed in the corresponding one-photon excited fluorescence (OPEF) (cf. Supporting information (SI), Figure S1(a)) and two-photon excited fluorescence (TPF) (SI, Figure S1(b)) experiments. The highly selective recognition of 1 for silver ion can be attributed to two factors. On one hand, sulfur atom and Ag$^+$ are typical “soft base” and “soft acid”, respectively, and the very strong affinity between them is quite natural. On the other hand, the nitrogen atom properties and the number of sulfur atoms in open chain monoaazadithiacrown ether may play an important role in the affinities of nitrogen and sulfur atoms to heavy-metal-ion. The extent of two-photon fluorescence (TPF) suppression by the Ag$^+$ is somewhat smaller than that of the one-photon process (Fig. 1). Also, the two-photon process shows better selectivity for Ag$^+$. Nevertheless, the trends observed in both experiments are more or less the same.
Notably, upon complexation with Ag⁺, the absorption maximum wavelength, \( \lambda_{\text{max (abs)}} \) of 1 blue-shifted from 400 nm to 366 nm, and its intensities decreased gradually (Fig. 3). The weak absorption band at 298 nm increased progressively, but its absorption peak faded. The binding constants of 1 for Ag⁺, expressed as log \( K \), were determined from the absorption–titration curves to be 5.65 \( \pm \) 0.03 at 20 °C (SI, Figure S2), which is comparable to those previously reported [14, 45].

As shown in Figs. 3 and 4, the OPEF and TPEF spectra of 1 also displayed obvious changes when Ag⁺ was added. The emission band of 1 at 612 nm progressively decreased upon the addition of Ag⁺ to the solution in OPEF and TPEF, which indicates that Ag⁺ can induce decoupling of a key nitrogen from the rest of the fluorophore \( \pi \)-electron system upon charge transfer from tertiary nitrogen atom to Ag⁺ in the ground state. 1 exhibits comparatively high fluorescence quantum yields in toluene (\( \Phi = 0.88 \)) and MeCN (\( \Phi = 0.53 \)). The binding constants of 1 for Ag⁺, expressed as log \( K \), were determined from the one- and two-photon emission–titration curves to be \( 5.72 \pm 0.07 \) and \( 5.76 \pm 0.05 \) at 20 °C in MeCN (SI, Figures S3 and S4), respectively. Hence, 1 can serve as a good sensor for Ag⁺ applied to OPEF and TPEF detection.

For the complexation ratio between the ligand and the metal ion, a Job plot experiment was conducted by varying the concentration of both 1 and Ag⁺. Solutions of 1 and Ag⁺ in MeCN in different mole fractions were prepared by mixing 1 and Ag⁺ in MeCN in appropriate ratios while maintaining the total concentration to 1 mM. The absorbance of each solution at 400 nm was measured. The concentration of 1–Ag⁺ complex for each solution was calculated by using the UV absorption data and the binding constants (Figure S2). The plot of [complex] vs. the mole fraction of Ag⁺ shows a maximum when the mole fraction is 0.5, and the appearance of isosbestic point is at 360 nm, indicating the formation of a typical ligand–metal complex ratio of 1:1 (Fig. 5).
3.5. H NMR Spectroscopic Studies of 1 in DMSO-d6 in the presence of different concentrations of Ag⁺ ions

Three H NMR spectra of 1 (9.2 mM) in DMSO-d6 in the absence of (a) and presence of 1 (b) and 10 (c) equiv silver ions were presented in Fig. 6 and Table 1 [SI, Figures. S7–S9] [46]. Except for the peak of Hg (Ar–N–CH), all the peaks of other 10 types of H (Ha–Hf, Hh–Hk) experienced clear downfield shifts upon the complexation of 1 with Ag⁺.

As far as the entire conjugated system, including two conjugated aromatic rings and a conjugated ethylene bridging group (C–C) was concerned, when tertiary nitrogen atom of aromatic amine chelates Ag⁺, its lone-pair electrons went into the nonbonding orbit of silver ion, which in turn weakened its overlap with the large π-electron cloud in the conjugated aromatic rings. That is to say, the electron density in the entire conjugated system was remarkably reduced, which gave rise to a deshielding effect and resulted in an increase in the chemical shifts of H (Hₐ–Hₜ). Of course, this type of deshielding effect in the entire conjugated system could also transfer to alpha-hydrogen (Ar–C–H), but this transfer was extremely weak. Therefore, the chemical shift of alpha-hydrogen (Hj) just had a very minor increase.

As for Hg, when the content of silver ions varied from 0 to 1 equiv, its chemical shift did not change at all, indicating that the deshielding effect of Hg before the complexation of 1 with Ag⁺ was so strong that its peak hardly shifted further downfield, probably due to the strong electron-withdrawing ability of the ortho-cyano and -ethylene bridging group. However, upon the content of silver ions increases from 1 to 10 equiv, the peak of Hg centered at 8.424 ppm shifted downfield to 8.433 ppm, which might be attributed to a deshielding effect, arising from the decrease of the electron density in the conjugated aromatic ring caused by the complexation of the aromatic ring with Ag⁺ in the presence of a large excess of Ag⁺. This further demonstrated that the 1:1 stoichiometry was in existence in the 1–Ag⁺ complex and consistent with Job’s plot analysis. Accordingly, NMR data could also indicate a 1:1 1–Ag⁺ coordination mode.

Interestingly, the chemical shift differences (Δδ₁a = δ₁a − δ₁b, Δδ₁c = δ₁c − δ₁b, δ₁a and δ₁c were the chemical shifts of H in the absence of and presence of 1 and 10 equiv Ag⁺, respectively) markedly decreased with increasing distance to be away from tertiary nitrogen atom in the order Hg (82 ppm) > Hh (26 ppm) > Hj (21 ppm) > Hk (10 ppm) (Table 1). This further suggested that tertiary nitrogen atom played the main role during the course of the complexation for 1 with Ag⁺.

Surprisingly, upon the increase of Ag⁺ from 0 to 1 equiv, the peak of Hg did not shift upfield but downfield. In fact, prior to the complexation of 1 with Ag⁺, the mutual overlap between the lone-pair electrons of tertiary nitrogen atom and the large π-electron cloud in the conjugated aromatic rings formed a larger π-bond, which caused the transfer

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**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>H_a</th>
<th>H_b</th>
<th>H_c</th>
<th>H_d</th>
<th>H_e</th>
<th>H_f</th>
<th>H_g</th>
<th>H_h</th>
<th>H_i</th>
<th>H_k</th>
</tr>
</thead>
</table>

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**Fig. 6.** Partial ¹H NMR spectra of 1 (9.2 mM) in DMSO-d₆ in the absence (a) of and presence of 1 (b) and 10 (c) equiv silver ions. Each ¹H NMR spectrum is measured after 5 min upon addition of silver ions.
of lone-pair electrons from tertiary nitrogen atom to the conjugated aromatic rings. Hence, the partial conjugated double linkage between tertiary nitrogen atom and its connecting aromatic carbon atom came into being, and thus, $H_g$ achieved extremely strong deshielding effect and exhibited a very large chemical shift of 3.571 ppm. Therefore, when tertiary nitrogen atom chelated $Ag^+$, the partial conjugated double bond was destroyed, and the strong deshielding effect of $H_g$ was also attenuated, which would undoubtedly reduced its chemical shift. Nevertheless, in the further course of the increase of 10 equiv $Ag^+$, the peak of $H_g$ hardly shifted, indicating that tertiary nitrogen atom could only chelate equimolar $Ag^+$.

As expectedly, two sulfur atoms displayed a very strong affinity for $Ag^+$, which was readily seen through the chemical shift differences of such hydrogens bound to thio-ethereal carbons ($S$–C–H) as $H_b$ ($\Delta \delta_{cb} = 72$ ppm) and $H_i$ ($\Delta \delta_{ba} = 56$ ppm). Whereas there was still a large change in the chemical shifts of $H_b$ ($\Delta \delta_{cb} = 55$ ppm) and $H_i$ ($\Delta \delta_{ba} = 64$ ppm) upon the increase of $Ag^+$ from 1 to 10 equiv, which was attributed to two pairs of lone-pair electrons capable of further chelating $Ag^+$. Additionally, this induction effect transferred even further to hydrogen ($S$–C–CH) bound to farther terminal carbons, and induced the increase in the chemical shift of $H_i$ ($\Delta \delta_{ba} = 9$ ppm, $\Delta \delta_{cb} = 37$ ppm).

### 3.6. Two-photon absorption cross section of 1 vs. two-photon excited wavelength

$\delta_{TPA}$ of 1 was determined by using the two-photon-induced fluorescence measurement technique [41]. Fig. 7 shows that $\delta_{TPA}$ values of 1 in toluene and in MeCN are 2410 and 950 GM at 790 nm, respectively. $\delta_{TPA}$ decreases with the change in solvent from toluene to MeCN, which should be attributed to the excited state charge separation. For (donor–bridge–acceptor, $D$–$\pi$–$A$) dipoles molecule 1, the charge separation is most favorable in a twisted conformation, where the two moieties involved in charge transfer, the donor $D$ and acceptor $A$, orbitally decoupled. This not only led to intramolecular fluorescence quenching, but also attenuated $\pi$-electron conjugated system to reduce $\delta_{TPA}$. More polar MeCN is so advantageous to balancing concentrated positive and negative charge arising from twisted conformation that the value of $\delta_{TPA}$ for probe 1 in MeCN is comparatively small. The significant decrease in $\delta_{TPA}$ with increased solvent polarity has been reported [47]. Nevertheless, the intensity of the two-photon fluorescence was strong enough for the measurement. When excess $Ag^+$ was added, $\delta_{TPA}$ decreased even further, probably because the electron-donating ability of the aromatic amino moiety is attenuated upon complexation.

### 3.7. The dependence of the up-converted fluorescence intensity on the incident intensity

To demonstrate that the fluorescence for 1 obtained at 790 nm is attributed to two-photon excitation, the experiment was conducted by changing the incident corresponding TPF intensity. The dependence of the up-converted fluorescence on the incident intensity for $1 \times 10^{-5}$ mol L$^{-1}$ of 1 is shown in Fig. 8. The nearly perfect quadratic dependence indicates that the up-converted fluorescence is induced by TPA.

### 3.8. Two-photon scanning microscopy imaging

For two-photon in vitro imaging, cells were imaged in the tissue culture chamber (5% CO$_2$, 37°C) using a Zeiss 510 LSM (upright configuration) confocal microscope equipped with a femtosecond-pulsed Ti:sapphire laser (Mira 900-F, Coherent). The excitation beam produced by the femtosecond laser, which was tunable from 700 to 980 nm ($\lambda_{ex} = 800$ nm, $\sim 1.5$ W), passed through an LSM 510 microscope with HFT 650 dichroic (Carl Zeiss, Inc.) and focused onto the coverslip-adherent cells using a 63× oil immersion objective (NA 1.4). The NLO META scan head allowed data collection in 10.7 nm windows at 630 nm, and a bypass filter of 600–650 nm was used for collection of the emission light.

After incubating epithelial cells with 1 in mixed HEPES Buffered Saline/EtOH/DMSO/CrEL (polyoxyethylene castor oil) (20/35/30/15, V/V/V/V) solution and exhaustive washing, uniform and bright orange red fluorescence was clearly seen with TP fluorescence microscopy in living cells (Fig. 9), which showed that 1 was membrane-permeable. After treatment of epithelial cells with HEPES-buffered solution (1 M HEPES, pH 7.0) containing 10 $\mu$M $Ag^+$, the fluorescence of the probe was completely quenched, which demonstrated that 1 was liable to combine with free silver ions. The result further suggested the feasibility of designing ICT-based TPE probes for silver ions, which could be applicable in biological imaging or determination (Fig. 9).

### 3.9. Applications to determination for silver content in real samples

All reagents were of the highest purity available and at least of analytical reagent grade. The standard stock solution of silver(I) was prepared by dissolving the appropriate amount of silver nitrate in double distilled water. Working solutions were prepared by
appropriate dilution of the stock solution with water.

Samples of waste water and river water were collected from Shaxi in Qujiang of Shaoguan (near the copper mine) and Bei river. Recovery experiments were also conducted by spiking the samples with appropriate amounts of silver, and determining their silver content by following method. For this purpose, standard solutions containing silver were added to samples.

0.5 g pretreated active carbon powder was added to 1000 mL waste water or river water containing silver, and then the mixture was absorptively stirred for 1.5 h. The resulting mixture was filtered to remove water, silver absorbed by active carbon was eluted by HNO3 (8 mol L\(^{-1}\), 30 mL) for 20 min, followed by removal of active carbon by filtration. The filtrate was evaporated to dryness at low heat. The residue dissolved in 20 mL of MeCN/water and filtered. Working solutions were prepared by taking a suitable aliquot of the sample if necessary and its silver content determined by following method.

The Ag\(^+\) concentration of real samples can be calculated corresponding to diverse two-photon fluorescence intensities according to equation (1).

\[
\log \left( \frac{F_{\text{max}} - F_{\text{min}}}{F - F_{\text{min}}} \right) = \log K_b + \log [M]
\]

where \(F\), \(F_{\text{min}}\) and \(F_{\text{max}}\) are the determined, minimum and maximum two-photon induced fluorescence intensities at 612 nm, respectively. \(K_b\) is the stability constant of complex, namely the binding constant, and \([M]\) is the ion concentration. The concentration of 1 for determination was \(1.0 \times 10^{-6}\) mol L\(^{-1}\).

In order to test the accuracy and applicability of the above method for the analysis of real samples, one standard method as the reference was used to assay real samples. The result being shown in Table 2 is the average of the six separate determinations. As can be seen, the silver concentration determined by the above method is in close agreement with the standard determination value, and excellent recoveries are obtained in two instances, which shows the potential usefulness of this method for environmental analysis.

### Table 2

<table>
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<th>Sample</th>
<th>Present method(^a) (µg L(^{-1}))</th>
<th>Standard method(^b) (µg L(^{-1}))</th>
<th>Added (µg)</th>
<th>Recovery (%)</th>
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<td>Waste water</td>
<td>74.1 ± 1.2</td>
<td>74.2 ± 1.8</td>
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<tr>
<td>River water</td>
<td>22.8 ± 2.1</td>
<td>22.5 ± 1.6</td>
<td>5</td>
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</table>

\(^a\) Mean ± standard deviation, \(n = 6\).

\(^b\) Potentiometric titration.

### 4. Conclusion

In summary, we have demonstrated the effectiveness of stilbene-derivatives with two cyano groups in the single aromatic ring which can contribute to very high |TPA as one- and two-photon excited fluorescent sensor for silver ion with high sensitivity and selectivity. The properties and numbers of sulfur atoms on the receptor of 3,9-dithia-6-azaundecane is believed to play very important roles for the unique selectivity of Sensor 1 for silver ion. It was confirmed that the introduction of two cyano groups into conjugative aromatic ring in the acceptor moiety of the chromophore skeleton can efficiently increase the excited state dipole moment and cause dramatic |TPA enhancement. The results provide a useful design strategy for the synthesis of new two-photon sensors for further applications.

### Acknowledgments

Financial supports from National Natural Science Foundation of China (Nos. 20772102 and 20972131) and China Postdoctoral Science Foundation funded project (No. 20100471224) is deeply acknowledged.

### Appendix A. Supplementary data


### References


