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Two novel dyes containing spirobifluorene and triphenylamine: Synthesis, one- and two-photon excited fluorescence and applications as probes for silver ions, water and cell imaging

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ABSTRACT

Two fluorophores, **SPF-TNS** and **SPF-TBB**, with electron-rich triphenylamine groups and Ag^+ receptor units on the respective biphenyl branches of 9,9′-spirobifluorene were synthesized. They exhibit strong one- and two-photon excited fluorescence, and the two-photon absorption cross-section values are 665 GM for **SPF-TNS** and 355GM for **SPF-TBB**. The probing behaviors toward metal ions were investigated via UV/Vis absorption spectra as well as one-photon fluorescence changes. The results indicated that **SPF-TNS** and **SPF-TBB** exhibit distinct fluorescent quenching in the presence of silver ions that can be used for highly sensitive and selective naked-eye detection of Ag^+. The one-photon excited fluorescence of **SPF-TBB** can be made to measure water content of organic solvents. The detection limits were calculated to be 0.04% for THF and 0.03% for 1,4-dioxane. The two-photon scanning microscopy experiments suggest that **SPF-TNS** is a promising label potentially applicable for the tracking of biomolecules.

1. Introduction

Two-photon excited fluorescent (TPEF) microscopy is a powerful method for three-dimensional imaging in biological systems [1]. Conventional one-photon techniques use UV or visible light to excite fluorescent molecules. The same transition can be excited by a two-photon process in which two less energetic photons are simultaneously absorbed [2]. This two-photon excitation process has a number of unique advantages, such as reduced specimen photodamage and enhanced penetration depth. For the development of TPEF-optimized molecular probes, it is important to have a significant modulation of the photophysical properties of the chromophore in response to external stimuli, such as polarity, pH, ion concentration, and temperature [3]. At the same time, the chromophore should retain a significant two-photon absorption (TPA) cross-section (σ) in a suitable excitation window for biological imaging (700–1000 nm, corresponding to an optimum combination of reduced scattering and absorption in biological samples) [4,5].

Due to the widespread applications and the broad prospects of sliver in the electronic industry, photographic and imaging industry, much attention has been paid to the negative impact of silver ions on the environment, especially on organisms. It is believed that silver ions can bind to various metabolites and enzymes such as inactivate sulphydrylenzymes. Many approaches such as fluorescence, UV–vis absorption, atomic absorption, and ICP atomic emission spectroscopy have been employed to measure trace amounts of silver ions. Among these methodologies, fluorescence spectroscopy is widely used because of its high sensitivity and facile operation. More importantly, most fluorescent sensors are ready for in vivo and in vitro cellular imaging to make the fluorescence approach superior to other analytical methods [6]. However, sensors for silver ions are comparatively scarce [1].

In addition, devising a convenient system for the detection of water content in organic solvents is attractive in various fields of chemistry due to the importance of water content in routine chemical processes including organic synthesis, liquid chromatography, and industrial processes [7,8].

Spirobifluorene appears to be a suitable fluorophore core for highly sensitive fluorescent probes because spirobifluorene-based molecules generally have high extinction coefficients and photoluminescence (PL) quantum yields [9–11]. Meanwhile, the two biphenyl branches of spirobifluorene connected at a quaternary center through σ-bonds can be independently tailored [9]. Nevertheless, there are few reports on spirobifluorene-based fluorescent probes which possess two-photon absorbing property [9,10]. We have reported previously the strong two-photon

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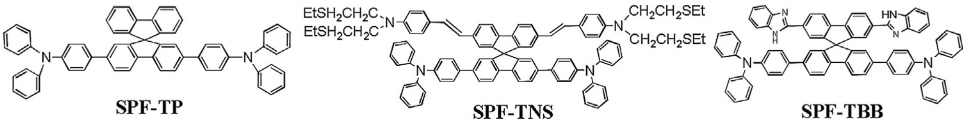

Scheme 1. The structures of **SPF-TP**, **SPF-TNS** and **SPF-TBB**.

induced fluorescence property of 2,7-bis-(4-(*N*,*N*-diphenylamino) phen-1-yl)-9,9′-spirobifluorene (**SPF-TP**, Scheme 1) [12].

Herein, we extend our earlier work and two novel chromophores, namely, 4,4′-(2′,7′-bis((E)-4-(bis(2-(ethylthio)ethyl) amino)styryl)-9,9′-spirobi[fluorene]-2,7-diyl)bis(*N*,*N*-diphenylaniline) (**SPF-TNS**, Scheme 1) and 4,4′-(2′,7′-bis(1H-benzo[d]imidazol-2-yl)-9,9′-spirobi[fluorene]-2,7-diyl)bis(*N*,*N*-diphenylaniline) (**SPF-TBB**, Scheme 1) were obtained. The synthetic pathways of **SPF-TNS** and **SPF-TBB** are shown in Scheme 2. From the view of molecular design, the benzimidazole and 6-aryl-3,9-dithia-6-azaundecane moieties were introduced to act as the Ag+ chelator [10,13]. In the structure of SPF-TBB, charge donor and acceptor parts are both contained, which results in its sensitivity to solvent polarity and water content [14]. The triarylamine-capped biphenyl branch of **SPF-TNS** and **SPF-TBB** is a donor-π-donor (D-π-D) type chromophore and expected to serve as a source of the two-photon absorption [15]. The probing behaviors toward metal ions, water content in organic solvents and the applications in two-photon fluorescence biological imaging were investigated.

2. Experimental

2.1. Materials

Compounds **1** and **4** were prepared according to literature procedures [1,16]. All chemicals were commercially available and of analytical grade. Solutions of metal ions were all prepared from various metal perchlorates. Ultrapure water was used from a Millipore water purification system.

2.2. Characterization

Melting points were determined with an XT-4A apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker DRX 400 MHz spectrometer. Chemical shifts were reported in ppm relative to a Me_4Si standard. Steady-state emission and excitation spectra were recorded on Perkin Elmer LS55 instrument. Visible absorption spectra were determined on Perkin Elmer Lambda 35 spectrophotometer. Two-photon absorption cross sections were measured via a TPEF method with femtosecond pulsed excitation. The excitation source was a mode-locked Ti:Sapphire laser (Spectra-Physics). This laser provided pulses of 100 fs of duration with repetition rate of 80 MHz at wavelength of 800 nm. The samples used for the TPA study were 10^{-4} M solutions of the molecules in tetrahydrofuran (THF). The laser beam was focused into a quartz cell of 1 cm path length by using a 5 cm focal-length lens. A half-wave plate and a polarizer were used to control the excitation intensity. The induced two- photon fluorescence was collimated by a lens at a direction perpendicular to the pump beam. To minimize the attenuation of fluorescence due to linear absorption effects, the excitation beam was focused as close as possible to the lateral wall of the quartz cell. The TPEF was then focused into the input slit of an imaging spectrographand recorded at the exit with a CCD camera. To calculate the TPEF cross sections, Rhodamine B in methanol solution (10 mM) was utilized as a reference which has a well known TPA spectrum. All the samples and standards were tested under the same experimental conditions. The TPEF cross-sections were determined by comparing their TPEF to that of fluorescein in solvents, according to the following equation:$\sigma_{TPEF} = \sigma_{ref}\ c_{ref}\ n_{ref}\ F/cnF_{ref}$, where *c* and *n* were the concentration and refractive index of the samples and reference, and *F* was the integral of the TPEF spectrum. The two photon cross-section σ was then calculated by the equation $\sigma = \sigma_{TPEF}/\Phi$, where Φ was the one-photon fluorescence quantum yield. It was assumed that the fluorescence quantum yield is the same under two-photon or one-photon excitation [17]. TPEF images were taken on an Ultimal IV confocal microscope equipped with a femtosecond Ti:sapphire laser.

2.3. Synthesis

2.3.1. Synthesis of (4-(bis(2-(ethylthio)ethyl)amino)phenyl)methanol (2)

To compound **1** (160 mg, 0.54 mmol) in ethanol (10 ml) at 0 °C was added $NaBH_4$ powder (494 mg, 40 mmol). The reaction mixture was stirred for 2 h at 40 °C; it was then quenched by adding a small amount of water. The reaction mixture was neutralized with HCl and extracted with dichloromethane (4 × 10 ml); the organic layer was then dried with $MgSO_4$ and evaporated to give an oily residue. Yield 0.086 g (0.287 mmol, 53.3%).

2.3.2. Synthesis of N,N-bis(2-(ethylthio)ethyl)-4-((iodotriphenylphosphor-anyl)methyl)aniline (3)

To a 50 ml flask, 0.86 g (2.87 mmol) **2**, 754mg(2.88 mmol) PPh_3, 478 mg (2.88 mmol) KI, 0.375 ml water, 3.75 ml $CHCl_3$, 0.7ml(11.52mmol)acetic acid were added. The mixture was refluxed for 12 h at 100 °C, followed by the removal of the solvent under reduced pressure. Dimethylbenzene was added to the residue solid, and the resulting precipitate was filtered and recrystallized from ethanol to give white solid **3** (1.2 g, yield 62.3%). ^{1}H NMR (400 MHz, $CDCl_3$ δ): 7.82-7.75 (m, 3H), 7.73–7.60 (m, 12H), 6.90 (dd, *J* = 8.0, 4.0 Hz, 2H), 6.40 (d, *J* = 8.0 Hz, 2H), 5.05 (d, *J* = 12.0 Hz, 2H), 3.46 (t, *J* = 8.0 Hz, 4H), 2.64 (t, *J* = 8.0 Hz, 4H), 2.60-2.52 (m, 4H), 1.25 (t, *J* = 8.0 Hz, 6H).

2.3.3. Synthesis of 4,4′-(2′,7′-bis((E)-4-(bis(2-(ethylthio)ethyl)amino)styryl)-9,9′-spirobi[fluorene]-2,7-diyl)bis(N,N-diphenylaniline) (**SPF-TNS**)

To a 25 ml flask, 179.2 mg (1.6 mmol) potassium *tert*-butylate, 536.8mg(0.8 mmol) **3**, 2 ml dried THF were added. The mixture was stirred under nitrogen atmosphere at 0 °C for 30 min, and then 2 ml THF solution of compound **4** (171.8 mg, 0.2 mmol) was added dropwise, after that, it was continually stirred at room temperature for 12 h. The solution was concentrated under reduced pressure and water was poured. The resulting mixture was extracted with dichloromethane. The organic layer was dried (Na_2SO_4), concentrated, and purified by column chromatography (petroleum ether/dichloromethane, 5:1–2:1, v/v) affording **SPF-TNS** as a yellow solid (160 mg, yield 58%). m.p. 165–167 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, *J* = 8.0 Hz, 2H), 7.77 (d, *J* = 8.0 Hz, 2H), 7.64 (dd, *J* = 8.0, 1.6 Hz, 2H), 7.48 (d, *J* = 8.0 Hz, 2H), 7.33 (d, *J* = 8.0 Hz, 4H), 7.28 (d, *J* = 8.0 Hz, 4H), 7.23–7.16 (m, 8H), 7.06–6.95 (m, 8H), 6.92–6.88 (m, 2H), 6.82-6.88 (m, 8H), 6.89 (s, 2H), 6.86 (d, *J* = 16.4 Hz, 2H), 6.76