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Three new water-soluble fluorescent organic nanoparticles with embedded structure: Structure-activity relationship and two-photon bio-imaging application

Xiaoping Gan[a,b,c,1], Haiyan Wang[a,1], Lanmei Lu[b], Hong Li[a], Kang Wang[a], Lin Kong[a], Fei Li[a], Yupeng Tian[a], Jieying Wu[a], Hongping Zhou[a,*]

[a] College of Chemistry and Chemical Engineering, Anhui University and Key Labotatory of Functional Inorganic Materials Chemistry of Anhui Province, Anhui Province Key Laboratory of Chemistry for Inorganic/Organic Hybrid Functionalized Materials, 230601 Hefei, PR China
[b] School of Science, Anhui Agricultural University, 230036 Hefei, PR China
[c] Co-operative Innovation Research Center for Weak Signal-Detecting Materials and Devices Integration, Anhui University, Hefei 230601, PR China

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ABSTRACT

Three benzothiazole-based two-photon absorption (2PA) chromophores (**B1-3**) were synthesized and the optical properties were investigated. The results illustrated the largest fluorescence quantum yield and effective 2PA cross-sections reached to 0.81 for compound **B1** and 1013 GM for compound **B3** in tetrahydrofuran solutions, respectively. Furthermore, the water-soluble fluorescent organic nanoparticles (FONs) possessing an embedded structure were prepared from the three compounds and Pluronic F127, which were successfully applied on two-photon nuclear envelope imaging.

1. Introduction

In recent years, two-photon fluorescence bio-imaging attracted intensive attention in optical diagnosis and treatment as a consequence of less photo bleaching, higher spatial resolution and deeper light penetration [1–3]. However, to obtain high quality of two-photon imaging pictures, there must be an excellent two-photon developer which mainly depends on its large effective two-photon cross-section (δ, two-photon cross-section (σ) times fluorescence quantum yield (φ)) and good biocompatibility [4,5]. Generally speaking, the former depends on its two-photon absorption (2PA) cross-section and/or fluorescence quantum yield, and the latter is mainly affected by the solubility, especially the water-solubility [6]. Thus, design and synthesis two-photon molecules to possess large effective 2PA cross-sections is an important method to expand 2PA materials applied in the bio-imaging domain.

However, organic molecules owning large effective 2PA cross-section usually required a large conjugate system, which leads to a large molecule volume, thereby resulted in poor solubility [7,8]. The formation of fluorescent organic nanoparticles (FONs) is an effective method [9] to promote solubility. Various FONs including fluorescent conjugated polymers [10], self-assembled fluorescent nanoparticles [11] and aggregation-induced-emission (AIE) or aggregation induced emission enhancement (AIEE) materials [12,13] have been successfully prepared. For example, Wei et al., [14] reported a series of FONs based on cyan-substituted diarylethlene derivatives possessing AIEE properties assisted with Pluronic F127, which could disperse in water and be applied in bio-imaging of A549 cells successfully. Nevertheless, encapsulating AIE or AIEE molecules to form FONs usually did not emit two-photon fluorescence due to the aggregation caused two-photon fluorescence quenching. Thus, utilizing 2PA molecules having large 2PA cross-sections to form FONs but with an embedded structure could retain their two-photon fluorescence performance.

In order to obtain large 2PA cross-sections, branched molecules usually act as templates due to the synergistic enhancement effect [15] of the branch number. While, because of the existence of symmetry breaking, fluorescence quantum yields decreased with increasing branch number [16], which resulted in the diminution of the effective 2PA cross-sections. Thus, an excellent fluorophore which could bring efficient fluorescence emission ability in multi-branched molecules was a key factor. Benzothiazole usually act as an electron acceptor and chromophore [17]. In 2010, Peter Hrobárik et al., [18] reported a series of benzothiazole based two-photon fluorescence molecules which all possessed large 2PA cross-sections in THF solution with significant

* Corresponding author.
E-mail address: zhpzhp@263.net (H. Zhou).
[1] These authors contributed equally to this work and should be considered as co-first authors.

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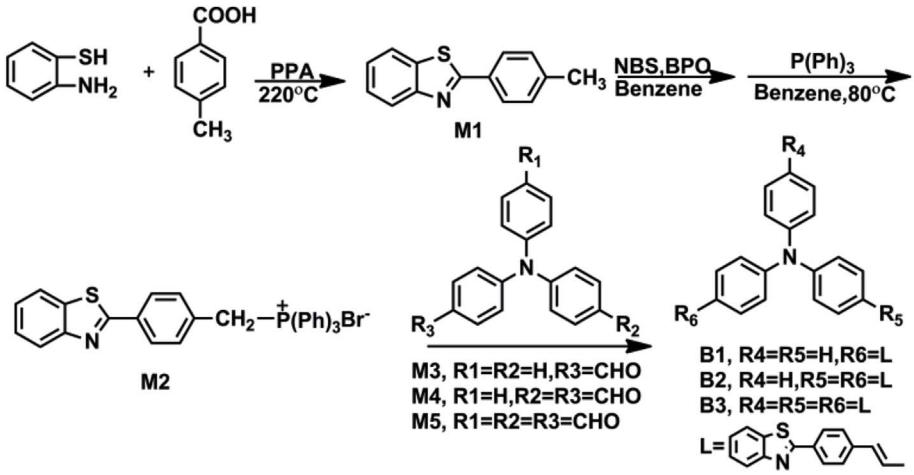

Scheme 1. Synthetic route to **B1-3**.

enhanced fluorescence quantum yields (from 0.45 to 0.57). Lorenzo Echevarria et al., [19] also reported a simple benzothiazole dimethyl aniline derivative which possessed excellent fluorescence emission ability, whose fluorescence quantum yield in ethanol was up to 0.9. All the results demonstrated that benzothiazole was an excellent fluorophore. Unfortunately, the reported molecules all emitted strong fluorescence in organic solutions rather than in aqueous solution.

Based on the above, we introduced benzothiazole as electron-withdrawing group and fluorophore into the triphenylamine core and constructed a series of branched triphenlyamine benzothiazole derivatives (Scheme 1). Then we systematically researched their structure-property relationship, especially the two-photon excited fluorescence emission abilities and fluorescence quantum yields accompanied with the change of branch number. Meanwhile, we prepared three water-soluble FONs (**B1-3@F127**) using Pluronic F127 as co-solvent, and applied the FONs on two-photon nuclear envelope imaging in Hela cells successfully.

2. Experimental section

2.1. General procedures

All commercially available chemicals were of analytical grade. Every solvent was purified as conventional methods beforehand. IR spectra were recorded with a Nicolet FT-IR NEXUS 870 spectrometer (ATR) in the 4000 - 400 cm^{-1} region. Melting points (uncorrected) were determined on an XT4 MP Apparatus (Taike Corp., Beijing, China). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV 400 Ultrashield spectrometer using dimethylsulfoxide-d_6 (DMSO-d_6) or $CDCl_3$ as solvent. Chemical shifts were reported in parts per million (ppm) relative to internal TMS (0 ppm) and coupling constants are in Hz. Splitting patterns were described as singlet (s), doublet (d), triplet (t), quartet (q), or multiplet (m). The mass spectra were obtained on a LTQ-Orbitrap XL mass spectrometer.

2.2. Optical measurements

2.2.1. One-photon absorption and emission spectra

The one-photon absorption (OPA) spectra were recorded on a Hitachi F-3600 spectrophotometer. The one-photon excited fluorescence (OPEF) spectra were performed using a Hitachi F-7000 fluorescence spectrophotometer. In the measurements of emission and excitation spectra, the pass width was 5 nm for all compounds. OPA and OPEF of **B1-3** were measured in six organic solvents with different polarities at the concentration of 1×10^{-5} mol L^{-1}. The quartz cuvettes used were of 1 cm path length.

2.2.2. Two-photon absorption cross sections (σ_{2PA})

2PA cross-sections (σ) of the three compounds were obtained by the TPEF method [20] with a femtosecond laser pulse and Ti: sapphire system (700–1080 nm, 80 MHz, 140 fs) as the light source. The samples were dissolved in tetrahydrofuran at a concentration of 1.0×10^{-3} mol. L^{-1}. The intensities of TPEF spectra of the reference and the sample were determined at their optimal excitation wavelength. Thus, σ_{2PA} of the samples was determined by Eq (1):

$$\sigma_{2PA} = \sigma_{ref} \frac{\Phi_{ref}}{\Phi} \frac{c_{ref}}{c} \frac{n_{ref}}{n} \frac{F}{F_{ref}} \quad (1)$$

Where the *ref* subscripts stands for the reference molecule (here fluorescein in the aqueous NaOH solution at concentration of 1.0×10^{-3} M was used as reference). c is the concentration of the solution, n is the refractive index of the solution, F is the TPEF integral intensities of the solution emitted at the exciting wavelength, and Φ is the fluorescence quantum yield. The σ_{ref} value of reference was taken from the literature [21]. Then the effective 2PA cross-sections (δ) of the samples were calculated by the formula $\delta = \sigma \times \Phi$.

2.2.3. Fluorescence lifetime

For time-resolved fluorescence measurements, the fluorescence signals were collimated and focused onto the entrance slit of a monochromator with the output plane equipped with a photomultiplier tube (HORIBA FluoroMax-4P). The decays were analyzed by 'least-squares'. The quality of the exponential fits was evaluated by the goodness of fit (χ^2).

2.2.4. Cytotoxicity assays in cells

To ascertain the cytotoxic effect of the three compounds, the 5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide (MTT) assay [22] was performed. Hela cells were trypsinized and plated to ~70% confluence in 96-well plates for 24 h before treatment. Prior to the treatment of the compounds, the Dulbecco's modified eagle medium (DMEM) was removed and replaced with fresh DMEM, and aliquots of the compound stock solutions (1 mM DMSO) were added to obtain final concentrations of 5, 10, 20, and 40 μM. Meanwhile, the FONs/water solution (10 μM, 2 mL) was added to another well which had no fresh DMEM. The treated cells were incubated for 24 h at 37 °C and under 5% CO_2. Subsequently, the cells were treated with 5 mg mL^{-1} MTT (40 μL/well) and incubated for an additional 4 h (37 °C, 5% CO_2). Then, DMEM was removed, the formazan crystals were dissolved in DMSO (150 μL/well), and the absorbance at 570 nm was recorded. The cell viability (%) was calculated according to the following equation:

$$\text{Cell viability \%} = OD_{570}\,(\text{sample})/OD_{570}\,(\text{control}) \times 100$$

Where OD_{570} (sample) represents the optical density of the wells