



Hydrosoluble two-photon absorbing materials: A series of sulfonated organic inner salts in biological imaging application



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ABSTRACT

Designed to achieve high two-photon absorptivity dyes, a novel series of D- π -A type hydrosoluble inner salts (**SL1**, **SL2**, **SL3** and **SL4**) based on pyridinium have been synthesized and fully characterized. Photophysical properties including linear absorption, one-photon excited fluorescence and two-photon absorption properties were systematically investigated. These results show that there is a shift in the absorption and emission maxima with variation of solvent polarity. Furthermore, the chromophore **SL4** shows the strongest intensity of two-photon excited fluorescence among the four chromophores and the largest two-photon absorption cross-section in the near infrared region (\sim 960 nm), indicating the steric bulk of the electron-donating terminal group plays an important role in two-photon absorption behavior. In addition, **SL4** displays two-photon absorption activity when dissolved in water, which can be effectively used in two-photon bioimaging for its reasonable two-photon absorption cross-section. **SL4**-labeled cells revealed a distinct vesicular localization, most likely representing lysosomal compartments.

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1. Introduction

Two-photon absorption (2PA) is a nonlinear process that involves the somewhat unusual capability of a molecule to absorb two photons simultaneously in order to populate an energy level within the molecule with energy equal to the sum of the energies of the two photons absorbed [1]. In the past two decades, the availability of stable and high-peak-power lasers has further incited the momentum to explore two-photon-related technologies. Many potential 2PA-based applications in the emerging fields of photonics and biophotonics have been proposed and explored, including data processing, upconverted lasing, three-dimensional fluorescence microscopy, biological imaging and so on [2–6]. Among these applications, bioimaging as fluorescence probes in living cells has recently been received much attention [7–10]. Importantly, near infrared (IR) excitation causes less damage to the sample than traditional UV–Vis and yields no endogenous contribution to the fluorescence [11]. Consequently, two-photon laser

scanning microscopy becomes a powerful technique for the study of dynamic processes and live-cell imaging [12] due to two-photon excitation far more efficient than mono-photon excitation for live-cell imaging and even more interestingly for intravital microscopy [13]. Therefore, significant effort has been devoted to building novel two-photon absorbing molecules with biocompatibility [14–17].

There has been an explosion of academic interest in organic nonlinear optical materials. For those 2PA-based applications, the demand for rationally designed organic compounds that exhibit sufficiently large 2PA within the desired spectral region is consequently escalating. The combination of several structural parameters, such as the efficiency of intramolecular charge-transfer and/or the effective size of the π -conjugation domain within a molecule, is closely related to molecular 2PA [18–21]. Stilbazolium salts are the best studied amongst such materials, it was previously studied by Marder's group [22], and then they were systematically investigated by Coe's group [23], Prasad's group [24] and ours [25], very recently, it was studied by Zheng's group [26]. However, most known two-photon absorbing stilbazolium salts are not water soluble, a major obstacle for bioimaging applications, necessitating strategies to enhance their biocompatibility.

Considering above, we designed a new series of organic inner salts bearing water solubilizing sulfonated groups contain the

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cation covalently linked to the anion in order to form biocompatible molecules. In addition, we launched a program changing the terminal amino substituents aimed at enhancing the two-photon cross-section. Furthermore, the impetus for further improving water solubility is compelling two methoxyethoxyethyl groups into the molecular skeleton, which may provide the possibility for acting as a ion probe or sensing to polarity of the solvents as well.

2. Experiments

2.1. General

All chemicals used were of analytical grade and the solvents were purified by conventional methods before use. The ^1H NMR spectra were performed on Bruker 400 MHz spectrometer with TMS as the internal standard, coupling constants J are given in Hertz. Elemental analysis was performed on Perkin–Elmer 240 instrument. Mass spectra were determined with MALDI-TOF-MS. IR spectra were recorded on NEXUS 870 (Nicolet) spectrophotometer in the $400\text{--}4000\text{ cm}^{-1}$ region using a powder sample on a KBr plate.

2.2. X-ray structural determinations

Single crystals of the chromophores **SL1** and **SL2** used in X-ray determination were obtained by slow evaporation of methanol. X-ray diffraction data of them were collected on a Bruker Smart 1000 CCD area detector diffractometer. Both of the radiation sources were MoK_α ($\lambda = 0.71073\text{ \AA}$). Empirical absorption correction was applied to the data. The structures were solved by direct methods and refined by full-matrix least-squares methods on F^2 . All the nonhydrogen atoms were located from the trial structure and then refined anisotropically with SHELXTL using the full-matrix least-squares procedure. The hydrogen atom positions were geometrically idealized and generated in idealized positions and fixed displacement parameters. Cambridge Crystallographic Data Center (CCDC) as supplementary publication numbers CCDC **SL1**-93044, **SL2**-929073.

2.3. Optical measurements

The one-photon absorption (OPA) spectra were recorded on a SPECORD S600 spectrophotometer. The one-photon excited fluorescence (OPEF) spectra measurements were performed using a Hitachi F-7000 fluorescence spectrophotometer. OPA and OPEF of **SL1–SL4** were measured in solvents of different polarities with the concentration of $5.0 \times 10^{-6}\text{ mol L}^{-1}$. The quartz cuvettes used are of 1 cm path length. The fluorescence quantum yields (Φ) were determined by using Rh6G (in ethanol, $\Phi = 0.94$) as the reference according to the literature method [27]. Quantum yields were corrected as follows:

$$\Phi_s = \Phi_r \left(\frac{A_r \eta_s^2 D_s}{A_s \eta_r^2 D_r} \right)$$

where the s and r indices designate the sample and reference samples, respectively, A is the absorbance at λ_{exc} , η is the average refractive index of the appropriate solution, and D is the integrated area under the corrected emission spectrum [28].

The TD-DFT {B3LYP[LANL2DZ]} calculations were performed on the optimized structure. All calculations were performed with the G03 software, the TD-DFT calculation of the lowest 25 singlet–singlet excitation energies were calculated with a basis set composed of 6–31 G(d) for C N H O S atoms.

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 HuoroMax-4P). The decays were analyzed by ‘least-squares’. The quality of the exponential fits was evaluated by the goodness of fit (χ^2).

Two-photon absorption (2PA) cross-sections (δ) of the samples were obtained by two-photon excited fluorescence (TPEF) method [29] at femtosecond laser pulse and Ti: sapphire system (680–1080 nm, 80 MHz, 140 fs) as the light source. The sample was dissolved in different solvents at a concentration of $1.0 \times 10^{-3}\text{ mol L}^{-1}$. The TPEF intensity of the reference and the sample were determined at their excitation wavelength. Thus, 2PA cross-section (δ) of samples was determined by:

$$\delta = \delta_{\text{ref}} \frac{\Phi_{\text{ref}} c_{\text{ref}} n_{\text{ref}} F}{\Phi c n F_{\text{ref}}}$$

where the ref subscripts stand for the reference molecule (here Rh6G in ethanol solution at concentration of $1.0 \times 10^{-3}\text{ mol L}^{-1}$ was used as reference). δ is the 2PA cross-sectional value, 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 δ_{ref} value of reference was taken from the literature [30].

2.4. Cell image

HepG2 cells were luminescently imaged on a Zeiss LSM 710 META upright confocal laser scanning microscope using magnification 40 \times and 100 \times water-dipping lenses for monolayer cultures. Image data acquisition and processing was performed using Zeiss LSM Image Browser, Zeiss LSM Image Expert and Image J.

2.5. Synthesis

A series of substituted (*p*-aminostyryl)-1-(3-sulfooxypropyl)pyridinium inner salts as a new class of two-photon absorption dyes have been designed (shown in Fig. 1), and synthesized by the following reactions (shown in Fig. 2).

2.5.1. Preparation of 4-methyl-*N*-(3-sulfooxypropyl)pyridinium (1)

It was synthesized according to the literature method [24(c)]. White powder product was collected. Yield 91%. ^1H NMR (DMSO- d_6 , 400 MHz), δ (ppm): 7.98 (d, 2H), 7.05 (d, 2H), 3.68 (t, 2H), 2.83 (t, 2H), 1.68 (s, 3H), 1.25 (m, 2H).

2.5.2. Preparation of substituted aminobenzaldehydes

4-(*N,N*-dialkylamino)benzaldehyde was synthesized according to the literature method [25d,31]. At room temperature, the

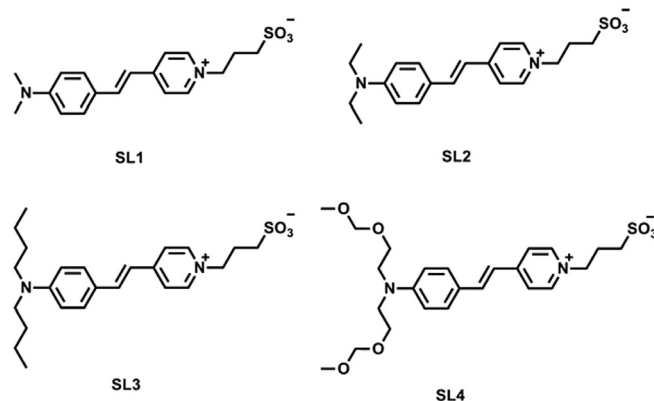


Fig. 1. The structures of target compounds.

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