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Synthesis, crystal structures, two-photon absorption and biological imaging application of two novel bent-shaped pyrimidine derivatives

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ABSTRACT

Two novel bent-shaped D–π–A–π–D chromophore molecules, 2-pyrazolyl-4,6-bis (4-*N*,*N*-ethylaminostyryl)pyrimidine (**Z1**) and 2-imidazolyl-4,6 –bis- (4-*N*,*N*- ethylaminostyryl) pyrimidine (**Z2**), were synthesized and fully characterized. It was found that the both bent-shaped chromophore molecules crystallize in the orthorhombic system, space group of $P2_12_12_1$. The photophysical properties and the connections between structure and properties of the chromophores were investigated both experimentally and theoretically. It was revealed that both absorption and single-photon excited fluorescence (SPEF) spectra show remarkable bathochromic shifts with increasing polarity of the solvents. The two chromophores have strong two-photon excited fluorescence and large two-photon absorption cross-sections in the near-infrared range. Additionally, two-photon microscopy (TPM) fluorescent imaging of BEL-7402 cells labeled with the **Z1** and **Z2** revealed their potential application as a biological fluorescent probe.

1. Introduction

Over the past decade, materials with strong two-photon absorption (TPA) effect and large TPA cross-sections have attracted growing interest due to their potential applications in the areas of optical limiting [1,2], two-photon photodynamic therapy [3,4], two-photon excited fluorescence microscopy [5,6], three-dimensional (3D) optical data storage [7–9], 3D microfabrication [10,11]. Spurred by these applications, many researchers have devoted themselves to design and synthesize novel compounds with large TPA cross-sections (δ_{TPA}) values in the field of functional materials. Some strategies have been used to design organic molecules with large δ_{TPA}, including dipoles [12–14], quadrupoles [15], octupolar molecules [16,17], dendrimers [18–20], polymers [21,22] and multi-branched molecules [23,24]. These studies reveal that the δ_{TPA} value of a compound will be increased by elongating the molecular conjugated system for charge transfer or incorporating multi-dipole or quadrupole chromophores into one molecular structure [25,26]. In addition, some studies have demonstrated that chromophores based on functionalized heteroaryl molecules with planar π-cores possess large δ_{TPA} and high fluorescence quantum yield (Φ) [15,27,28].

Pyrimidine, as a heterocycle molecule, has high electron affinity and good coplanarity making it an appropriate building block in construction of chromophores for nonlinear optical materials [29–31]. Recently, several TPA chromophores utilizing pyrimidine as an electron-withdrawing central core substituent have been synthesized, and fairly good values of TPA cross-sections have been measured for them [32,33]. Meanwhile, some of pyrimidine-based chromophores can be also used as polarity and pH sensors [34–36]. On the other hand, pyrazole and imidazole derivatives have been widely used in the field of biological [37] and supramolecular chemistry, not only because of their conjugate bases (their deprotonated forms) but also because of their various coordination modes [38–42]. At present work, we introduced a pyrazole and imidazole groups to the pyrimidine ring in high yield by a modified Ullmann reaction. By applying this strategy, two novel bent-shaped D–π–A–π–D chromophores based on pyrimidine with two-photon absorption, 2-pyrazolyl-4,6-bis(4-*N*,*N*-ethylamino- styryl)-pyrimidine (**Z1**) and 2-imidazolyl-4,6-bis(4-*N*,*N*- ethylaminostyryl)-pyrimidine (**Z2**), were prepared and characterized. The photophysical properties and the connections between structure and properties of the two chromophores were investigated both experimentally and theoretically. Furthermore, the application for bioimaging in living cells was carried out.

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2. Experiments

2.1. General

2.1.1. Materials and apparatus

All chemicals were commercially available and used without further purification. The solvents were purified by conventional methods before use. 2-Iodo-4,6-dimethyl pyrimidine was acquired from laboratory. 4-[*N*,*N*-di(ethylamino)]benzaldehyde was synthesized according to the methods reported [25]. The ^{1}H NMR and ^{13}C NMR spectra recorded on at 25 °C using Bruker Avance 400 spectrometer were reported as parts per million (ppm) from TMS. Mass spectra were determined with a Micromass GCT-MS (EI source). IR spectra were recorded on NEXUS 870 (Nicolet) spectrophotometer in the 400–4000 cm^{-1} region using a powder sample on a KBr plate.

X-ray diffraction data of single crystals were collected on CCD diffractometer. The determination of unit cell parameters and data collections were performed with Mo-K$_\alpha$ radiation ($\lambda = 0.71073$ Å). Unit cell dimensions were obtained with least-squares refinements, and all structures were solved by direct methods using SHELXS-97. The other non-hydrogen atoms were located in successive difference Fourier syntheses. The final refinement was performed by full-matrix least-squares methods with anisotropic thermal parameters for non-hydrogen atoms on F^2. The hydrogen atoms were added theoretically and riding on the concerned atoms.

Electronic absorption spectra were obtained on a UV-265 spectrophotometer. Fluorescence measurements were performed using a Hitachi F-7000 fluorescence spectrophotometer.

2.1.2. Measurements

(1) *The fluorescence quantum yields (Φ)*: the fluorescence quantum yields (Φ) were determined by using fluorescein as the reference according to the literature method [43]. Quantum yields were corrected as follows:

$$\Phi_\mathrm{s} = \Phi_\mathrm{r}\left(\frac{A_\mathrm{r}\eta_\mathrm{s}^2 D_\mathrm{s}}{A_\mathrm{s}\eta_\mathrm{r}^2 D_\mathrm{r}}\right)$$

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.

(2) *TPA cross-section (δ)*: TPEF spectra were measured using femtosecond laser pulse and Ti: sapphire system (680–1080 nm, 80 MHz, 140 fs, Chameleon II) as the light source. All measurements were carried out in air at room temperature. TPA cross-sections were measured using two-photon induced fluorescence measurement technique. The TPA cross-sections (δ) are determined by comparing their TPEF to that of fluorescein in solvents, according to the following equation [44]:

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

here, the subscripts ref stands for the reference molecule. δ is the TPA cross-section value. c is the concentration of 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 [45].

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

Z1 and **Z2** were synthesized by the reactions shown in Fig. 1.

2.2. Synthesis

2.2.1. 2-Iodo-4,6-bis(4-N,N-ethylaminostyryl) pyrimidine (*1*)

t-BuOK (0.56 g, 5 mmol), 4-[*N*,*N*-di(ethylamino)]benzaldehyde (0.39 g, 2.2 mmol) and 2-iodo-4,6-dimethyl pyrimidine (0.23 g, 1 mmol) were mixed together, and milled vigorously for about 10 min. The reaction was monitored by TLC (petroleum/ethyl acetate 4:1 v/v). After the reaction was completed, the mixture was dispersed in 200 mL distilled water. The solution was extracted with CH_2Cl_2 several times. The organic layer was washed with water, saturated brine and dried over anhydrous $MgSO_4$. After removing solvent under reduced pressure, the residue was purified by flash chromatography on silica gel using petroleum/ethyl acetate (8:1 v/v) as eluent and gave deep-red micro-crystals **1** 0.33 g, yield: 60%. FT-IR (KBr, cm^{-1}) selected bands: 2969 (s), 2901 (m), 1601 (s),

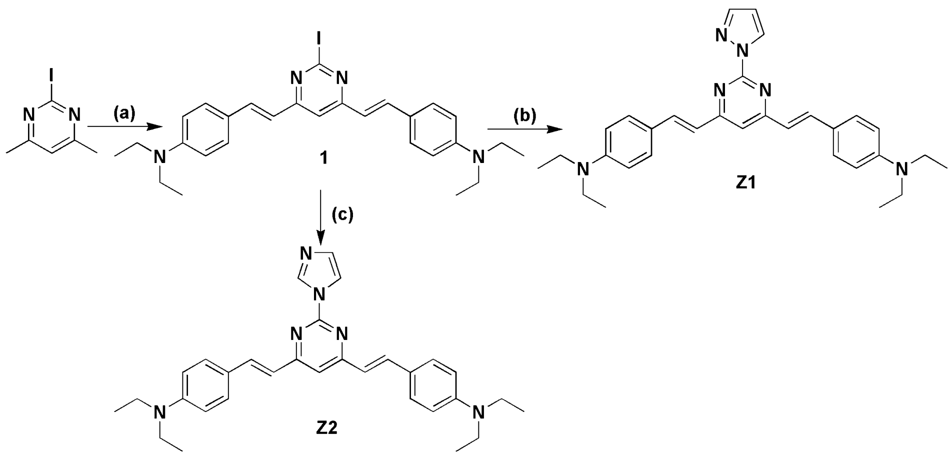

Fig. 1. Synthesis route for **Z1** and **Z2**. (a) 4-[*N*,*N*-di(ethylamino)]benzaldehyde, *t*-BuOK, grind; (b) pyrazole, CuI, *t*-BuOK, monohydrate 1,10-phenanthroline, 18-crown-6, DMF, reflux. (c) imidazole, CuI, *t*-BuOK, monohydrate 1,10-phenanthroline, 18-crown -6, DMF, reflux.