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A water-soluble D–π–A chromophore based on dipicolinic acid: Synthesis, pH-dependent spectral properties and two-photon fluorescence cell imaging

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ABSTRACT

The presence of two carboxylic acid groups and two hydroxyl groups within the novel dye, (E)-4-(4-bis(2-hydroxyethyl)amino)styryl)pyridine-2,6-dicarboxylic acid, resulted in good water solubility. The compound's UV absorption and photoluminescence spectra were each highly pH sensitive in acidic solutions and two isobestic points were observed. The TPA cross-section coefficient and two-photon fluorescence cell imaging of the dye were investigated.

1. Introduction

The determination of pH is of great importance because it usually plays a crucial role in a variety of systems. There are many methods for pH measurement at present [1–8]. Of the several methods available to determine pH, optical methods have several advantages including rapid response time, high signal-to-noise ratio and excellent pH-sensitivity [9].

Nonlinear excitation of fluorophores is intensively used in recent years in microscopy techniques for biological applications because of several advantages [10–15]. The excitation and fluorescence emission are confined to the focal point because of the squared power dependence of the absorption with the light intensity. Excitation wavelengths are shifted to the near-infrared region, avoiding limitations of UV lasers and optics, and providing a deep penetration necessary to imagine thick biological specimens. There is no background, because Rayleigh and Raman scattering occur at much longer wavelength than the collected fluorescence, and the out-of-plane excitation of chromophores is not possible. Also, out-of-focus photobleaching is eliminated. For a decade, chromophores have been specially optimized for such applications with a high σ_2 value and good water solubility [1,16–18].

Pyridine-2,6-dicarboxylic (dipicolinic) acid and its derivatives are highly useful tridentate ligands. They form nine-coordinate luminescent complexes with lanthanides, providing high quantum yields of phosphorescence and are used as luminescent tags, labels, and barcodes [19–22]. We have previously reported a series of D–π–A chromophores containing dipicolinates [23]. Among them, compounds PASPD (trans-dimethyl-4-[4′-(N,N-diphenylamino)-styry1]-pyridin-2,6-dicarboxylate) and MASPD (trans-dimethyl-4-[4′-(N,N-dimethylamino)-styry1]-pyridin-2,6- dicarboxylate) show strong two-photon absorption. However, they are hydrophobic, thereby limiting their applications in aqueous biological environments. To solubilize MASPD into water, acetyloxyethyl groups instead of methyls were introduced at the nitrogen atom in the aniline fragment to be hydrolyzed as a hydrophilic group in the later stage of the synthesis and two carboxylic acid groups were introduced by hydrolysis of the two methoxycarbonyl groups in pyridine ring. The synthetic route to target **6** is shown in Scheme 1. As expected, compound **6** exhibits good water solubility and strong two-photon absorption. Compound **6** also exhibited absorption and PL pH sensitivity. Therefore, the potential of **6** as a pH indicator and the two-photon fluorescence (2 PF) biological imaging property were investigated and are reported herein.

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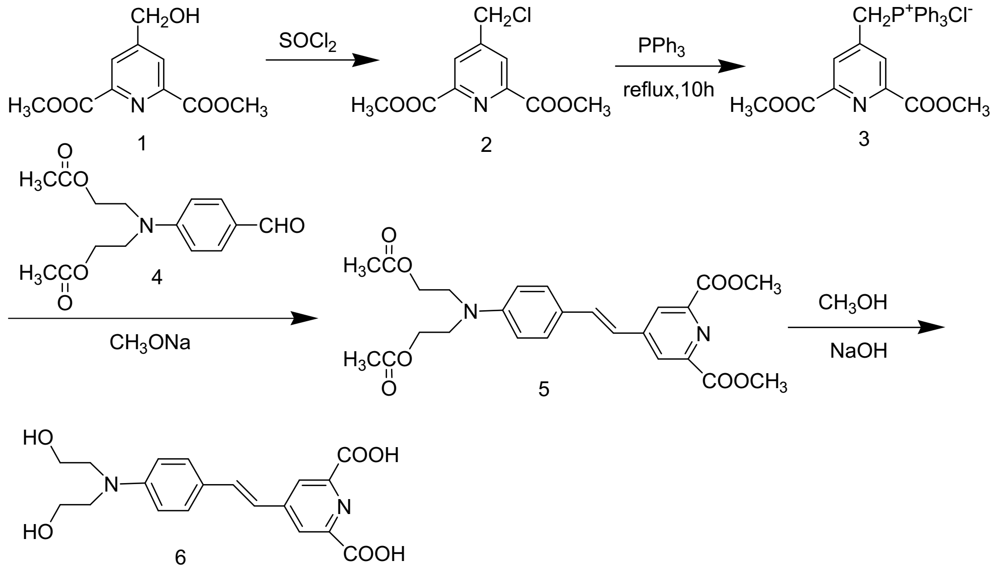

Scheme 1. Synthesis of compound **6**.

2. Experimental

2.1. Instruments and reagents

Melting points were determined with an XT4A apparatus and are uncorrected. Mass spectral studies were carried out using VG12-250 mass spectrometer. ^{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$_4$Si standard. IR spectra were obtained on a Nicolet Avatar 370 DTGS spectrometer. Elemental analyses were performed by Atlantic Microlab. Steady-state emission and excitation spectra were recorded on Perkin Elmer LS55 instrument. Visible absorption spectra were determined on Perkin Elmer Lambda 35 spectrophotometer. All the pH values were measured with a model PHS-3C meter (Shanghai Leici Equipment Factory, China). A Ti:sapphire mode-locked fs laser system with regenerative amplifier (Spectra-Physics) was employed to investigate the TPA properties. The open-aperture Z-scan technique was used to measure the TPA cross-section. In our measurements, the laser beam with the pulse energy about 0.75 μJ was focused by a lens of 10 cm focal length on the solution filled in a 1 mm cell, and the transmitted light behind the solution was detected by a photodiode connected to a Lock-in amplifier. Two-photon excited luminescence images were taken on an Ultimal IV confocal microscope equipped with a femtosecond Ti:sapphire laser.

All reagents and solvents were commercial reagents of analytical grade and were used as received. Further purification and drying by standard methods were employed and they were distilled prior to use when necessary. Silica gel (200–300 meshes) was used for column chromatography. The pH titration was run in water. In a typical experiment, a solution of the dye containing small amounts of 1 M HCl was prepared. Diluted NaOH solution was added to achieve the appropriate pH change. The overall volume change did not exceed 2%.

2.2. Synthesis

2.2.1. 4-(chloromethyl)pyridine-2,6-dicarboxylate (**2**)

To a solution of compound 1 (0.5 g, 2.20 mmol) in anhydrous CHCl$_3$ (5 mL) and sulfuryl dichloride [ed. Note: toxic; highly corrosive; reacts violently with water; incompatible with acids, alcohols, bases, metals, amines, moisture] (0.2 mL, 2.82 mmol) was added dropwise under nitrogen atmosphere at −5 °C with continuous stirring for up to 40 min. Excess solvent was removed under reduced pressure and the crude product was purified by recrystallization from ethanol to give the solid **2** 0.4 g (1.65 mmol) after drying in vacuum. Yield: 75%; m.p. 168–170 °C. IR (KBr), ν/cm^{-1}: 3079, 2959, 2836, 1725, 1710, 1380, 1257, 1125, 798. EI-MS: m/z (M$^+$) 243. ^{1}H NMR (400 MHz, CDCl$_3$), δ: 8.27(s, 2H, Py–H), 4.45 (s, 2H, –CH$_2$Cl), 4.06 (s, 6H, –OCH$_3$).

2.2.2. (E)-dimethyl 4-(4-(bis(2-acetoxyethyl)amino)styryl)pyridine-2,6-dicarboxylate (**5**)

A solution of triphenylphosphine [ed. Note: toxic; incompatible with oxidizing agents and acids] (0.5 g, 1.91 mmol) and compound **2** (0.3 g, 1.24 mmol) in benzene (10 mL) was refluxed for 10 h, then cooled to the room temperature. The reaction mixture was filtered to obtain the colourless solid **3** 0.21 g (0.415 mmol). Yield:34%.

To the absolute methanol (2 mL) solution of compound **3** (0.094 g, 0.186 mmol) and compound **4** (0.05 g, 0.171 mmol), 2 mL absolute methanol solution of sodium methanolate (12 mg, 0.222 mmol) was added dropwise and stirred up to 6 h under nitrogen atmosphere at −5 °C. The solution was concentrated under reduced pressure and a whitish solid **5** 0.06 g (0.124 mmol) was obtained by chromatography eluting from ethyl acetate and petroleum ether (1:5, v/v). Yield: 73%; m.p. 250 °C. EI-MS: m/z 484 (M$^+$). ^{1}H NMR (CDCl$_3$, δ ppm): 8.35 (s, 2H, Py–H), 7.50 (d, 2H, J = 8.4 Hz, Ph–H), 7.10 (d, 1H, J = 8.4 Hz, C=CH), 6.80 (d, 2H, J = 8.8 Hz, Ph–H), 6.60(d, 1H, J = 8.4 Hz, C=CH), 4.30 (m, 4H,–CH$_2$OAc), 4.00(s, 6H, –OCH$_3$), 3.70 (m, 4H, –NCH$_2$), 2.10(s, 6H, –OCOCH$_3$). ^{13}C NMR (CDCl$_3$, δ ppm): 171.15, 165.75, 165.48, 149.56, 148.72, 147.50, 135.89, 129.35, 127.94, 124.57, 122.37, 112.25, 111.88, 61.42, 53.36, 49.80, 21.11. Calculated for $C_{25}H_{28}N_2O_8$: C, 61.97; H, 5.83; N, 5.78. Found: C, 61.88; H, 5.85; N, 5.76.

2.2.3. (E)-4-(4-bis(2-hydroxyethyl)amino)styryl)pyridine-2,6-dicarboxylic acid (**6**)

0.021 g compound 5 (0.0433 mmol) and 10 mg NaOH were added to 10 mL methanol and the mixture was stirred at room temperature for 4 h. The solvent was then removed under reduced