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# A novel Schiff base derivative: Synthesis, two-photon absorption properties and application for bioimaging

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ABSTRACT

A novel donor-π-acceptor-π-donor type (D-π-A-π-D′) Schiff base derivative (**L**) has been designed and synthesized. The structure of **L** is confirmed by single-crystal X-ray diffraction analysis as well. The photophysical properties of compound **L** were comprehensively investigated by using both experimental and theoretical methods. The results indicate that **L** exhibits large Stokes shift and moderate two-photon action (2PA) cross-section in the near infrared (NIR) region. Furthermore, the confocal microscopy imaging study demonstrates that compound **L** could penetrate into cells and target the cellular mitochondria compartment. Due to its low cytotoxicity, compound **L** provides a promising tool for directly lighting up the mitochondria compartment in living HepG2 cells.



## 1. Introduction

In recent years, materials with large two-photon absorption (2PA) cross section (σ) have attracted considerable attention owing to the potential application including three-dimensional micro-fabrication, photodynamic therapy, optical limiting, fluorescence microscopy and biological imaging [1–10]. Thus, many researchers fabricated various compounds with 2PA properties [11,12]. Up to now, several strategies for the construction of molecules with enhancing 2PA have successfully been applied, such as effectively extending the conjugation system, enhancing the amount of branches, and improving the planarity of the chromophore [13–16]. Among them, expanding the π-conjugation is an efficient method to obtain large 2PA σ [1,17]. However, there are still some drawbacks, such as the complicated synthesis routes and the resulted high molecular weight. Therefore, simple and efficient construction of small molecules with enhanced 2PA properties is still urgently needed.

Herein, we designed a novel asymmetric D-π-A-π-D′ type Schiff base derivative (**L**) (Scheme 1), which was according to the following considerations: (i) The *N* hydroxyethylaniline and 5 (diethylamino) 2 methylphenol moiety act as donor (D), cyan group acts as acceptor (A), —C═C— and —CH═N— moiety act as the π-conjugated bridge forming a D-π-A-π-D system. (ii) Schiff base derivatives have been widely investigated due to their high synthesis flexibility, bioactivity and medicinal utility [18]. (iii) The hydroxyl group could improve the solubility of the compound [19]. (iv) Due to its high electron affinities, cyano-substituted dyes exhibit good optical properties [20,21].The resulted compound **L** was confirmed to have suitable 2PA property and excellent biocompatibility. Furthermore, **L** can be easily loaded into living cells and target mitochondria.

## 2. Experimental Sections

### 2.1. Synthesis of L

Compound **M** (0.30 g, 1 mmol) [22] was dissolved in 20 mL methanol, and then added 4 (Diethylamino)salicylaldehyde (0.19 g, 1 mmol) and two drops acetic acid. The mixture was heated to reflux for 12 h. After reaction, the mixture was cooled down to room temperature and then filtered. The crude product was washed by methanol to obtain the yellow solid. Yield: 79%. IR (KBr, $cm^{-1}$): 3497, 2973, 2903, 2207, 1623, 1582, 1522, 1430, 1398, 1357, 1266, 1243, 1190, 1132, 1072, 998, 899, 829, 786, 696, 532. $^1$H NMR (400 MHz, $d_6$-DMSO, ppm) δ: 13.57 (s, 1H), 8.75 (s, 1H), 7.86 (d, $J$ = 8.4 Hz, 2H), 7.79 (s, 1H), 7.70 (d, $J$ = 7.8 Hz, 2H), 7.41 (m, 3H), 6.80 (d, $J$ = 8.4 Hz, 2H), 6.34 (d, $J$ = 8.7 Hz, 1H), 6.08 (s, 1H), 4.78 (s, 1H), 3.58 (s, 2H), 3.44 (m, 8H), 1.13 (t, $J$ = 6.6 Hz, 9H). $^{13}$C NMR (100 MHz, $d_6$-DMSO, ppm) δ: 163.43, 149.63, 141.81, 134.30, 132.02, 131.30, 129.70, 125.84, 121.34, 120.35,

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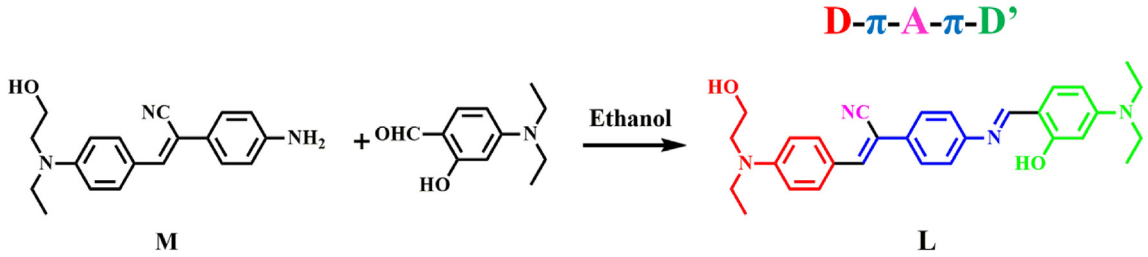


**Scheme 1.** Synthetic route of the title compound **L**.

119.38, 111.13, 101.28, 96.74, 58.31, 51.88, 44.79, 43.93, 12.52. ESI-MS (*m*/*z*): 483.27 ($[M + 1]^+$).

## 3. Results and Discussion

### 3.1. Crystal Structure

The crystal structure of **L** is displayed in Fig. 1. The crystallography data, selected bond lengths and angles of **L** are provided in Tables S1–S2.

Compound **L** belonged to orthorhombic system with Pbca space group. As displayed in Fig. 1, the dihedral angles between the plane (C30C12C14) and two neighboring planes (C1C2C9 and C27C28C21) are 28.26° and 17.28°, respectively. The feature indicated that C1C2C9 and c27C28C21 are nearly coplanar. The selected bond lengths, such as C(10)-C(11) (1.409 Å), C(11)-N(2) (1.300 Å), N(2)-C(12) (1.417 Å), C(15)-C(16) (1.475 Å), C(16)-C(18) (1.346 Å), C(18)-C(19) (1.422 Å), show that compound **L** has high delocalized π-electron system, favoring its good nonlinear optical properties. Adjacent molecules are stacked *via* O2—H2···O1 (1.834 Å) hydrogen bond interaction forming a one-dimensional chain structure as displayed in Fig. S1.

### 3.2. Photophysical Properties

The photophysical properties of **L** are investigated in six solvents, and the relevant data are listed in Table S3. Fig. 2 and Table S3 exhibited that a weak solvatochromism for the absorption bands, which indicated that there is a little difference between the ground state and excited state of dipoles. As displayed in Fig. 2, compound **L** displayed two major absorption bands between 300 and 500 nm, which can be attributed to the π–π* of the molecule mixed with intramolecular charge transfer (ICT). The TD-DFT calculations (Fig. 3 and Table S4) indicate that the HOMO-1 (H-1) is mainly located on (Z) 2 (4 aminophenyl) 3 (4 (ethyl (2 hydroxyethyl) amino) phenyl) acrylonitrile unit (denoted as $\pi_D$), and the HOMO (H) primarily localized on 4 (Diethylamino)salicylaldehyde moiety (denoted as $\pi_{D1}$). The LUMO (L) is of π* orbital largely on cyan group and Schiff base group (denoted as $\pi^*_{\text{cyan-Schiff base}}$). The low energy band at about 431 nm was assigned to $\pi_{D1}$–$\pi^*_{\text{cyan-Schiff base}}$ transition mixed ICT due to the H → L transition. The high energy band at about 368 nm mainly induced by H-1 → L transition was considered as the $\pi_D$–$\pi^*_{\text{cyan-Schiff base}}$ transition mixed ICT. Taken together, the result from the theoretical calculations is in accordance with the experiments.

From Fig. 2, the fluorescence maxima wavelength of compound **L** exhibited obvious red-shifts and the Stokes shifts also exhibit an increasing tendency with increasing the solvent polarity [22]. Compound **L** displayed relative high fluorescence quantum yields in absolute ethanol and DMSO (Table S3), which is explained that these solvents might stabilize the excited state of L. The Lippert-Mataga equation is used to evaluate the dipole moment changes of the dyes [23,24]. A linear relationship between the solvent polarity factor Δ*f* and the Stokes shifts is found. As shown in Fig. 4, the slope of **L** is 916 $cm^{-1}$. As a result, the value of $\mu_e - \mu_g$ was calculated as 5.03 D. It indicates that in the excited state the molecule has large polar structure, which provides promising linear and nonlinear optical properties.

### 3.3. Two-photon Excited Fluorescence (2PEF)

The two-photon excited fluorescence (2PEF) spectra of **L** in DMSO solution was measured from the region of 700 to 1000 nm. As displayed in Fig. S2, upon the input laser power increasing, the logarithmic plot has a slope of 1.98, indicating a two-photon excitation mechanism. The 2PA action cross section (Φσ) of **L** was measured by by using 2PEF method, where fluorescein was used as the reference. Fig. 5 exhibited that the $\Phi\sigma_{max}$ for **L** is approximately 27 GM at 740 nm, attributing to the expanded π-conjugated system of **L** between the (Z) 2 (4 aminophenyl) 3 (4 (ethyl(2 hydroxyethyl)amino)phenyl)acrylonitrile and 5 (diethylamino) 2 methylphenol groups. Besides, the $\Phi\sigma_{max}$ for compound **L** in the NIR range, which is favorable for bioimaging applications.

### 3.4. Biological Imaging Application

Before exploring its biological application, cytotoxicity test was firstly evaluated against the human cervical carcinoma cells (HepG2) *via* MTT assay. As shown in Fig. S3, with the concentration ranging from 5 to 25 μM, compound **L** remained little cytotoxicity (cell viability > 80%) over 24 h incubation, demonstrating compound **L** presents low toxicity in living HepG2 cells.

To evaluate the internalization of compound **L** in living cells, HepG2 cells was employed as a cell model and treated with compound **L** for

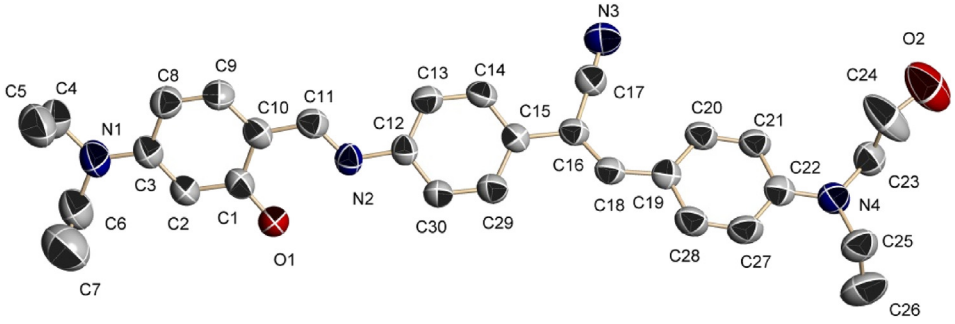


**Fig. 1.** Crystal structure of **L**, all H atoms were omitted for clarity.