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Two-photon absorption and cell imaging of two multi-branched dyes based on curcumin

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ABSTRACT

Two novel multibranched dyes curcumin-based: 1,7-bis(4′-propyloxy-3′-methyloxy-phenyl)-3-propyloxy-5-carbonyl-1,6-heptadiene (**dye 1**) and 1,7-bis(4′-acetate ethyloxy-3′-methyloxy-phenyl)-1,6-heptadiene-4,4-diacetate ethyl-3,5-dione (**dye 2**) were easily prepared. Their one- and two-photon absorption properties have been investigated carefully. The two-photon absorption (TPA) cross sections measured by two-photon excited fluorescence (TPEF) were determined to be 1143 and 842 GM for **dye 1** and **dye 2**, respectively. Cell viability tests revealed the as-prepared compounds possessed low cytotoxicity against MCF-7 cell lines over a period of at least 24 h. In addition, *in vitro* and *in vivo* studies demonstrated the **dye 1** could enter into tumor cells, and even rapid accumulate in tumor tissues, which makes it an ideal organic material for biomedical imaging.

1. Introduction

π-Conjugated organic molecular materials with large TPA cross-sections have attracted much attention in the past twenty years because of their various applications in 3D microfabrication, 3D optical data storage, two-photon optical power limiting, two-photon cellular imaging and two-photon photodynamic therapy [1–4]. Among these applications, biological imaging in living cells and tissues as a fluorescence probe has attracted great interest by monitoring and recording molecular spatiotemporal distribution, cellular biochemistry processes, and even earlier detection of disease [5–7]. Furthermore, two-photon microscopy (TPM) technique offers many advantages over traditional confocal microscopy, such as increased penetration depth, localized excitation and prolonged observation time [2,8,9]. However, an ideal two-photon fluorescence (TPF) probe which can exactly reflect the cell environment is very rare by limitations of some essential requirements. Firstly, the fluorescence signal should not be perturbed by the cell surrounding environment. Secondly, the probe fluorescence signal should remain photostability and have no fluorescence quenching in living cell or even tissue environment in polar solvent. Thirdly, large TPA cross-section was required to improve the sensitivity. It's well-known that the conjugation length, donor and acceptor strength, and the π-center planarity are important parameters to obtain large TPA cross-sections based on the theory of Marder [10] and Perry [11]. According to this design strategy, many efficient TPA materials, including dipolar, quadrupolar, dendrimers, D–π–A–type, D–π–D–type and multibranched chromophores have been developed [12–14]. However, structurally stable TPA dyes exhibiting a large σ and also emitting strong red fluorescence via direct two-photon excitation at a longer wavelength are still scarce at present. More importantly, the synthesis of D–π–A–type, D–π–D–type and multibranched compounds were suffered from the complex procedures and low-yielding, which limited their practical application.

Curcumin is a natural yellow pigment with low toxicity and outstanding photostability [15,16]. Curcumin derivatives were reported to show good photostability and efficient two-photon exited

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fluorescence [17,18]. In this approach, the fluorophore was further modified in order to obtain better photophysical properties and biological imaging application. Namely, propyl and acetate ethyl groups were respectively connected to 4′,4′-diphenolic hydroxyl groups positions of curcumin to get two multibranched dyes curcumin-based: 1,7-bis(4′-propyloxy-3′-methyloxy-phenyl)-3-propyloxy-5-carbonyl-1,6-heptadiene (**dye 1**) and 1,7-bis (4′-acetate ethyloxy-3′-methoxy-phenyl)-1,6-heptadiene-4, 4-diacetate ethyl-3,5-dione (**dye 2**). Simultaneously, linear photophysical characterization and investigation of TPA properties are also presented as a basis for potential applications in two-photon fluorescence imaging. The synthetic route of as-prepared dyes 1–2 are presented in Scheme 1.

2. Materials and methods

2.1. Materials and general instruments

All chemicals were available commercially and every solvent was purified by conventional methods before use. Each solution used for one/two photon fluorescence measurements is freshly prepared and kept in the dark before measurement. Melting points were determined on Rigaku Thermo Plus 2 system TG8120. ^{1}H NMR spectra were performed on a Bruker 300 spectrometer with tetramethylsilane ($Si(CH_3)_4$) as the internal standard. The mass spectra were obtained on FINNIGAN LCQ Advantage MAX LC/MS (Thermo Finnigan, American). Elemental analysis was carried out with a Perkin–Elmer 240C analyzer. Single-crystal X-ray diffraction was implemented on a Seimens Smart 1000 CCD diffractometer equipped with a graphite crystal monochromator situated in the incident beam for data collection at room temperature. The determination of unit cell parameters and data collections were performed with Mo Kα radiation (λ = 0.71069 Å) (shown in Table S1, Supporting Information). Unit cell dimensions were obtained with least-square refinements, and all structures were solved by the direct method as SHELXL-97.9. The final refinement was performed by full-matrix least-square methods with anisotropic thermal parameters for non-hydrogen atoms on F^2.

2.2. Photophysical methods

The linear absorption spectra were measured on a UV-3600 spectrophotometer. The one-photon excited fluorescence (OPEF) spectra were collected on an F-7000 fluorescence spectrophotometer (Japan). The quartz cuvettes used had a 1 cm path length. The fluorescence quantum yields of target compounds in DMF were measured on a Perkin–Elmer LS-55B fluorospectrometer by comparison of the fluorescence intensities with the compound RhB ethanol solution with known quantum yield (Φ = 0.69) under identical experimental conditions. 1.0 × 10^{-5} mol/L dilute solution of obtained compound was prepared in order to avoid possible aggregation and self-absorption. The quantum yield was calculated as follow [19]:

$$\Phi_s = \Phi_r \left(\frac{Ar(\lambda r)}{As(\lambda s)}\right)\left(\frac{I(\lambda r)}{I(\lambda s)}\right)\left(\frac{n_s^2}{n_r^2}\right)\frac{\int F_s}{\int F_r} \quad (1)$$

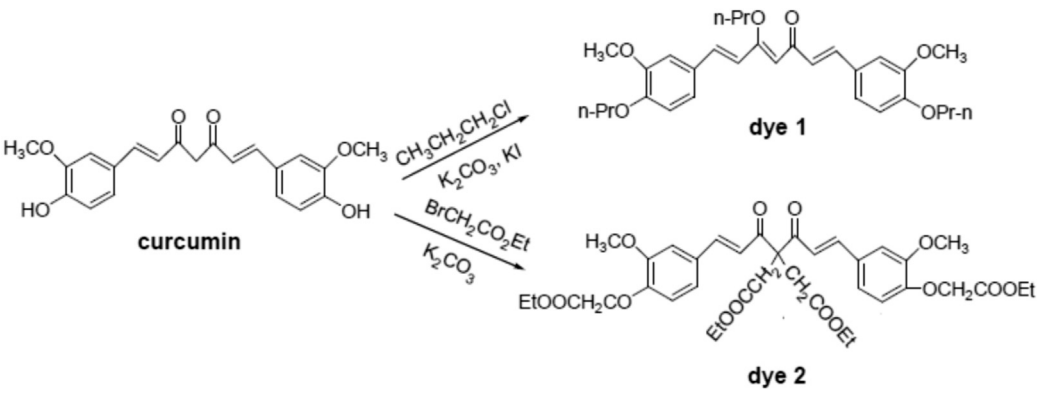
where, Φ is the quantum yield, $A(\lambda)$ is the absorbance of the solution at the exciting wavelength λ, $I(\lambda)$ is the fluorescence intensity of the solution at exciting wavelength λ, n is the refractive index, $\int F$ is the integrated area under the corrected emission spectrum, subscripts s and r refer to the sample and reference RhB solutions, respectively. The experimental errors are estimated to be ±10% from sample concentrations and instruments.

The two-photon excited fluorescence (TPEF) spectra can be measured with a certain laser beam from a mode-locked Ti:sapphire laser (Coherent Mira 900 F) as the pump source with a pulse duration of 200 fs, a repetition rate of 76 MHz, and a single-scan streak camera (Hamamatsu Model C5680-01) together with a monochromator as the recorder.

TPA cross-sections of the sample were obtained by the TPEF method with a femtosecond laser pulse and a Ti:sapphire system (670–1080 nm, 80 MHz, 200 fs) as the light source, and a single-scan streak camera (Hamamatsu, model: C5680-01) together with a monochromator as the recorder. The excitation wavelength of the sample is 760 nm. The sample was dissolved in DMF at a concentration of 1.0 × 10^{-3} mol/L with fluorescein in ethanol solution at the same concentration as the reference. The TPA cross-section σ of the sample was determined by the following equation [20,22]:

$$\sigma = \sigma_r \frac{\Phi_r}{\Phi}\frac{c_r}{c}\frac{n_r}{n}\frac{F}{F_r} \quad (2)$$

Here, the subscript r refers to the reference molecule, σ is the TPA cross-section value, the value of σ_r is taken from the literature [20,21], n is the refractive index of the solution, c is the concentration of the solution, F is the TPEF integral intensity of the emitting light at the excitation wavelength λ, and Φ is the fluorescence quantum yield.



Scheme 1. Synthetic route of the as-prepared compounds.