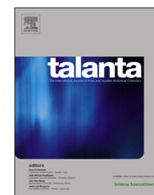
Talanta 159 (2016) 255–261

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta



Bright and photostable fluorescent probe with aggregation-induced emission characteristics for specific lysosome imaging and tracking

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ARTICLE INFO

Article history:
Received 5 April 2016
Received in revised form
11 June 2016
Accepted 15 June 2016
Available online 16 June 2016

Keywords:
Tetraphenylethene
Morpholine
Aggregation-induced emission
Lysosome-targeting
Fluorescent imaging

ABSTRACT

We develop a new lysosome-targeting AIE fluorescent probe tetraphenylethene-morpholine (TPE-MPL), by incorporating a typical lysosome-targeting moiety of morpholine into a stable tetraphenylethene skeleton. Due to both the AIE and antenna effects, TPE-MPL possesses superior photostability, appreciable tolerance to microenvironment change and high lysosome targeting ability. Our findings confirm that TPE-MPL is a well-suited imaging agent for targeting lysosome and tracking dynamic movement of lysosome. Moreover, due to its synthetic accessibility, TPE-MPL could be further modified as a dual-functional probe for lysosome, thereby gain further insight into the role of lysosome in biomedical applications.

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1. Introduction

As one of the vital organelles, lysosome plays a key role in various physiological processes, such as intracellular transport, cell antigen processing, cellular homeostasis, protein degradation and plasma membrane repair [1]. Meanwhile, increasing evidence has shown that lysosomes are also involved in various pathologies including inflammation, tumor formation, and several neurodegenerative diseases [2]. Therefore, visualization of lysosome in living cells is critical for understanding intracellular metabolism, cell membrane recycling, and evaluating drug and gene delivery systems [3–7]. Normal lysosome specific optical probes usually involve in two strategies. The first strategy often consists of a luminescent core and a side chain of a weak base to selectively accumulate in acidic organelles upon protonation [8,9]. And some of them, including DAMP, neutral red, acridine orange, and the LysoTracker probes are commercially available [10]. The other strategy utilizes fluorescence nanomaterials, such as dextran modified quantum dots [11,12], and silica nanoparticles (SiNPs) [13], which could enter living cells through endocytosis and ultimately localize within the lysosome, as it is the final compartment of the degradative endocytotic pathway. For observing the dynamic changes in a certain period of time, the probe must be photostable under the continual irradiation of light from fluorescent microscopes. However, those conventional fluorescent probes for lysosomes staining can be quickly photobleached when a harsh laser beam is used as the excitation light sources [2,9]. Photobleaching, the irreversible photodamage of a chromophore, seriously limits the application of fluorophore in biological imaging [14]. As a consequence, proposing a fluorescent probe with high photostability is urgently desired. Usually, researches used higher concentration of fluorophore to improve the photostability, unfortunately, in most cases, it didn't work because of the accompanying concentration-quenching effect [15]. Therefore, the fluorescent probes with strong photostability used for noninvasive imaging of lysosomes are highly desirable for biomedical applications.

Recently, luminogens with aggregation-induced emission (AIE) characteristics have drawn much attention. Contrary to the conventional fluorophore, AIE molecules are nearly nonfluorescent when molecularly dissolved but become highly emissive in the aggregate state, and the fluorescence increases along with the increase of fluorophore concentration [16–18]. The AIE molecules were reported to possess better photostability or higher resistance to photobleaching than that of single fluorescent molecule in dilute solutions [16]. Therefore, they have been successfully applied for long-term cell tracking in mitochondria [19], cell membrane

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http://dx.doi.org/10.1016/j.talanta.2016.06.031
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[20], lysosome and nucleus [21,22].

Herein, we have developed a new lysosome-targeting AIE fluorescent probe, by incorporating a typical lysosome-targeting moiety of morpholine into a stable tetraphenylethene skeleton with AIE spectroscopic features. We here demonstrate that tetraphenylethene-morpholine (TPE-MPL) can light up lysosome specifically in live cells with superior photostability, enabling the observation of lysosome morphological changes. Tetraphenylethene (TPE) was functionalized with morpholine (MPL) moiety, a well-established antenna for lysosome [23–25]. Meanwhile, due to the AIE effect, TPE-MPL possesses superior photostability or high resistance to photobleaching and appreciable tolerance to microenvironment change. As a result, TPE-MPL is a well-suited imaging agent for lysosome targeting and morphological change tracking with high spatial and temporal resolution. It will be a powerful tool for real-time monitor of dynamic changes of lysosome in living cells with high spatial and temporal resolution under stimulation by different medicines, and would significantly enrich our knowledge about lysosomes. To the best of our knowledge, no attempt has yet been made to combine tetraphenylethene and morpholine to fabricate bright and photostable fluorescent probe for specific lysosome imaging and tracking.

2. Experimental sections

2.1. Chemicals and instruments

4-Methylbenzophenone, zinc powder, tetrahydrofuran, titanium (IV) chloride, pyridine, *N*-bromosuccinimide (NBS), carbon tetrachloride, benzoyl peroxide, 1,4-dioxane, 3-(4,5-dimethyl-2-thiazolyl)−2,5-diphenyltetrazolium bromide (MTT), morpholine were purchased from Sigma-Aldrich. Petroleum ether (PE), dichloromethane (DCM), ethyl acetate, silica gel were obtained from Energy Chemicals (Shanghai, China). DMEM/F12 culture medium, fetal bovine serum penicillin-streptomycin, dimethyl sulfoxide (DMSO), anhydrous alcohol, trypsin-EDTA digestive juice were purchased from Sijiqing Biotech (Hangzhou, China). Ultrapure water obtained from a Milli-Q ultrapure (18.2 MΩ cm) system was used in all experiments.

^{1}H NMR spectra were recorded on a Bruker AMX-500 NMR spectrometer (Bruker). High resolution mass spectrometry (HRMS) was obtained on a micrOTOF-Q II mass spectrometer (Finnigan). The UV–vis absorption spectra were measured using a Shimadzu UV-2450 spectrophotometer (Shimadzu, Japan). The photoluminescence spectra were measured using a Hitachi F-4600 spectrophotometer (Hitachi, Japan) at an excitation voltage of 700 V with the excitation and emission slit widths at 5.0 and 5.0 nm, respectively. Cell imaging was performed with an Olympus FV500 confocal microscope (Olympus, Japan).

2.2. Synthesis of compound TPE-MPL

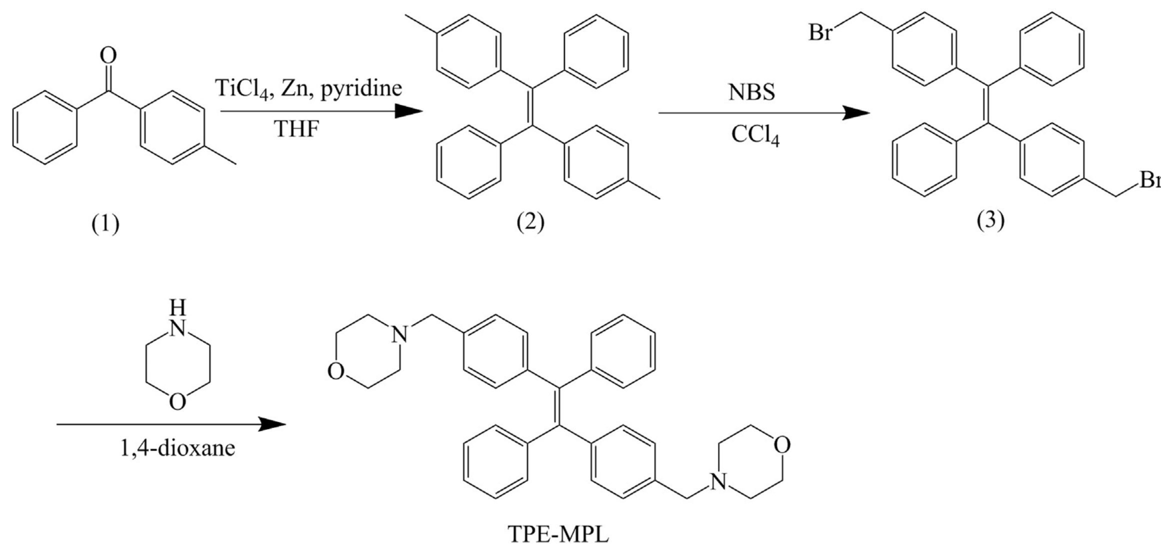
Probe TPE-MPL was synthesized as illustrated in Scheme 1. Firstly, 4-methylbenzophenone (**1**)(10.80 g, 30 mmol) and zinc powder (9.75 g, 150 mmol) were dissolved in 650 mL THF, then adding $TiCl_4$ (8.3 mL, 75 mmol) and 1.6 mL pyridine in ice-bath, and the solution was refluxed for 8 h. Afterward, the mixture solution was cooled to RT and filtered. The filtrate was evaporated and the crude product was purified on a silica-gel column using PE: DCM = 5:1 as eluent. Compound **2** was isolated as a light yellow powder in 90.6% yield (9.80 g).

Secondly, to a mixture of compound **2** (9.8 g, 27.18 mmol) and NBS (9.7 g, 54.40 mmol) in 250 mL CCl_4 was added catalytic amount of BPO (0.10 g, 0.5 mmol) at RT. The mixture was stirred and heated to reflux for 10 h. After cooling to RT, the reaction mixture was washed by saturated aqueous Na_2SO_3 and deionized water, and stirred until the organic phase was separated. After filtration and evaporation, the product was recrystallization in a mixture of DCM/PE and retained a white crystal compound **3** in 43% yield (5.93 g). ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm = 7.192–7.092 (10H, m), 7.072–6.964 (8H, m), 4.44(4H, d, *J* = 6.4 Hz).

Finally, compound **3** (0.20 g, 0.386 mmol) was dissolved in 6 mL 1,4-dioxane, then 1 mL morpholine was added into the above solution. The mixture was stirred and heated to reflux for 30 min. Afterward, the solution was poured into deionized water and extracted by DCM. The extraction liquid was evaporated and the crude product was purified on a silica-gel column using EtOAc as eluent. A white powder of TPE-MPL was obtained in 33% yield (0.128 g). ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm = 7.119–7.079(6H, m), 7.078–7.014(8H, m), 6.987(4H, d, *J* = 8.0 Hz), 3.719(8H, t, *J* = 4.0 Hz), 3.433(4H, s), 2.412(8H, s). MS: *m/z* calcd for $C_{36}H_{39}N_2O_2^{+}$[M-H^{+}]: 531.30114, found: 531.30066.

2.3. Sample preparation

TPE-MPL (0.0265 g) was dissolved in 50 mL DMSO, then the original concentration of TPE-MPL is 1.0 mM. Adding DMSO and



Scheme 1. Synthetic route to TPE-MPL.

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