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Sensing for intracellular thiols by water-insoluble two-photon fluorescent probe incorporating nanogel

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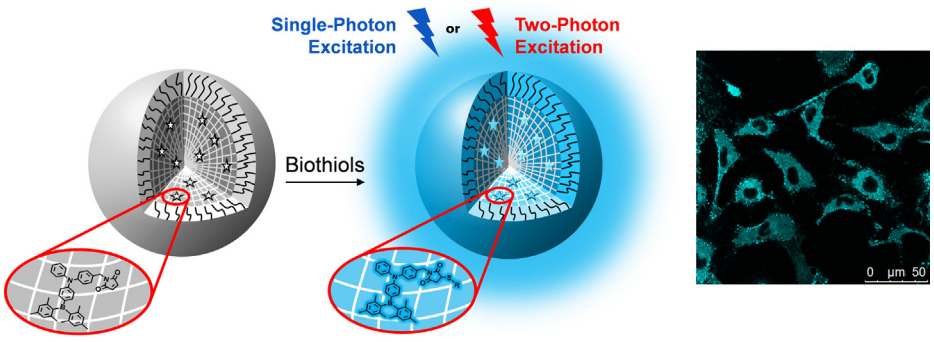
HIGHLIGHTS

- A novel "turn-on" two-photon fluorescent probe based on a π-conjugated triarylboron luminogen was designed and synthesized.
- Fast, selective and sensitive detection of biothiols in 100% aqueous solution by simply loaded on a nanogel.
- Single-photon and two-photon fluorescent bioimaging of biothiols in NIH/3T3 fibroblasts.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel "turn-on" two-photon fluorescent probe containing a π-conjugated triarylboron luminogen and a maleimide moiety DMDP-M based on the photo-induced electron transfer (PET) mechanism for biothiol detection was designed and synthesized. By simply loading the hydrophobic DMDP-M on a cross-linked Pluronic® F127 nanogel (CL-F127), a probing system DMDP-M/CL-F127 was established, which shows quick response, high selectivity and sensitivity to cysteine (Cys), homocysteine (Hcy) and glutathione (GSH) in aqueous phase. The DMDP-M/CL-F127 system presented the fastest response to Cys with a rate constant of 0.56 min^{-1}, and the detection limit to Cys was calculated to be as low as 0.18 μM. The DMDP-M/CL-F127 system has been successfully applied to the fluorescence imaging of biothiols in NIH/3T3 fibroblasts either with single-photon or two-photon excitation because of its high biocompatibility and cell-membrane permeability. The present work provides a general, simple and efficient strategy for the application of hydrophobic molecules to sensing biothiols in aqueous phase, and a novel sensing system for intracellular biothiols fitted for both single-photon and two-photon fluorescence imaging.

1. Introduction

Biothiols, such as cysteine (Cys), homocysteine (Hcy) and glutathione (GSH), play vital roles in many biological processes

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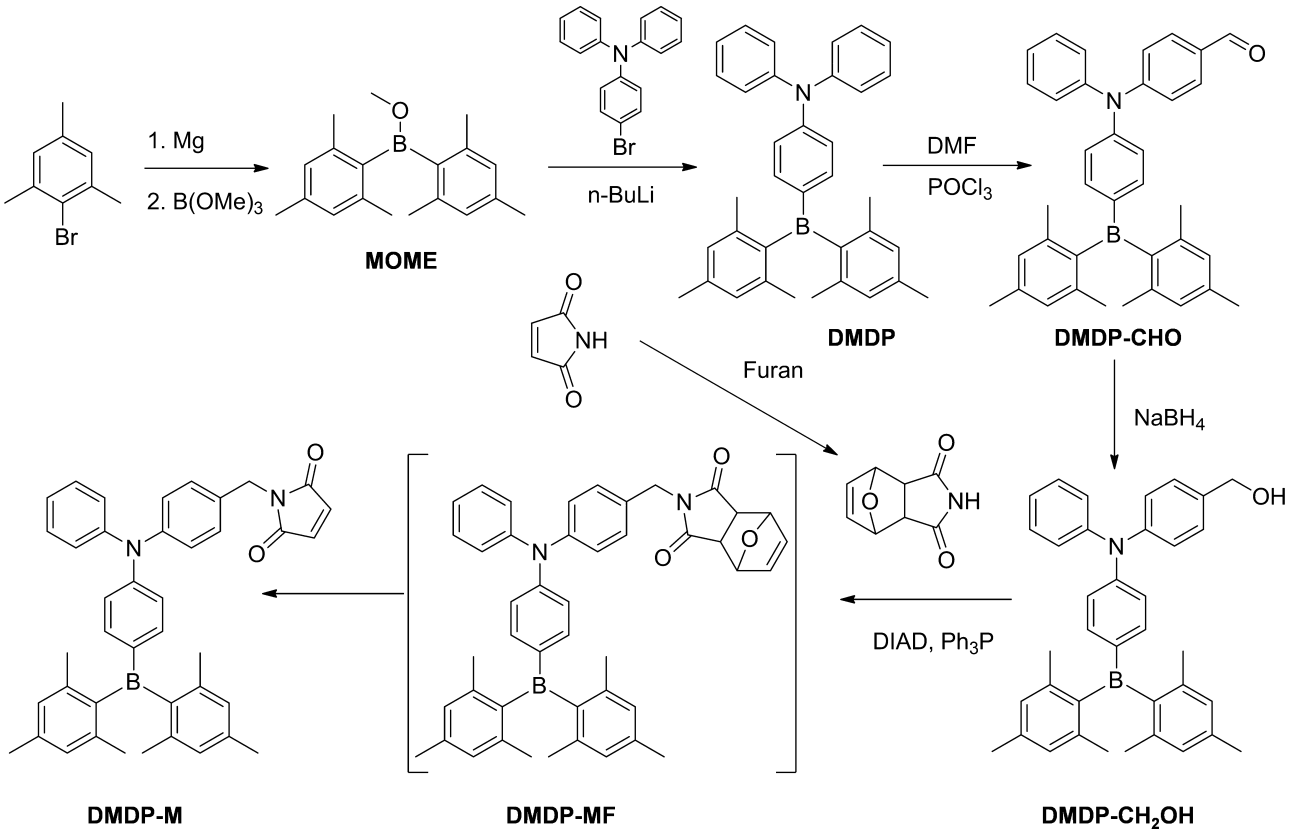
including maintenance of intracellular redox activities [1], xenobiotic metabolism [2], intracellular signal transduction [3], and gene regulation [4]. An aberrant level of biothiols in organisms is directly related to many diseases [5], including cancer [6], Alzheimer's disease [7], cardiovascular disease [8], neural tube defect [5], inflammatory bowel disease [9], and osteoporosis [10]. Lots of schemes, such as high-performance liquid chromatography (HPLC) [11], capillary electrophoresis [12] and fluorescent sensors have been established to detect biothiols *in vitro* or *in vivo*. Of these methods, fluorescent probes have shown advantages owing to their high sensitivity and specificity, fast response, and potential for real-time detection. Considerable effort has been devoted to novel fluorescent probes for thiols. For applying them to biosystems, the hydrophilic fluorescent probes have been synthesized by covalent methods [13–18]. Although some of them have been successfully applied to probe biothiols in aqueous phase, covalent synthesis processes for hydrophilic fluorescent probes are usually costly due to labor, time and energy-consumption. In comparison with the covalent methods, the noncovalent methods exhibit merits such as tunability, simplicity and convenience, for application of hydrophobic fluorescent probes in aqueous phase. The employment of surfactant micelle has allowed the application of hydrophobic fluorescent probes for thiols in aqueous system [19]. However, the surfactant micelle is not suitable for application in biosystems because it tends to dissociate in low concentrations (below the critical micelle concentration, CMC). Developing novel fluorescent probe systems suited for thiol detection in biosystems still remains a great challenge. Recently, we found that the hydrogels can be developed as a platform for the application of hydrophobic components in aqueous system [20,21]. Furthermore, it has been proved that nanogels are capable of being a delivery medium to load hydrophobic fluorescent probes for sensing intracellular pH values [22]. All these examples bring us an opportunity to overcome the challenge. More importantly, most of these fluorescent probes require a rather short excitation wavelength (<525 nm) which limits their application in biological research because of a great interference from background emissions and photodamage. Near-infrared (NIR) excitation within the range of the optical window in tissues (approximately 700–1000 nm), in which the absorption and scattering by tissues are low, enables deeper tissue penetration and reduces photoxicity. Therefore, the probe with two-photon fluorescence is considered to be an attractive solution to surmount the drawbacks of single-photon excitation [23].

The interest in efficient fluorescent probes for biothiols urges us to find a simple way to develop a two-photon novel fluorescent probe for imaging intracellular biothiols. π-Conjugated triarylboron derivatives containing electron donor groups exhibit intramolecular charge transfer (ICT) property and a large dipole moment as a result of strong electron deficiency of boron, offering them strong environmental responsiveness and large two-photon absorption cross section [24]. Furthermore, π-conjugated triarylboron derivatives show high fluorescence quantum yield and excellent photostability. Triarylboron luminogens, therefore, have promising applications in diverse areas, such as temperature measurement [24–26], adenosine triphosphate (ATP) detection [27], fluoride detection [28] and two-photon absorption antenna [29]. Considering outstanding features of π-conjugated triarylboron derivatives, we designed and synthesized a "turn-on" two-photon fluorescent probe DMDP-M (Scheme 1) based on a π-conjugated triarylboron luminogen 4-(dimesitylboryl)-*N*,*N*-diphenylaniline (DMDP) bearing a maleimide moiety linked by methylene spacer for selective detection of thiols. By loading on CL-F127, a nanogel based on cross-linked Pluronic® F127, the probe can be used to detect thiols with high selectivity and sensitivity in aqueous system and living cells.



Scheme 1. Synthetic route of DMDP-M.