Dyes and Pigments 159 (2018) 166–172

Contents lists available at ScienceDirect

Dyes and Pigments

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

A viscosity sensitive azide-pyridine BODIPY-based fluorescent dye for imaging of hydrogen sulfide in living cells

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ARTICLE INFO

Keywords:
Fluorescent probe
BODIPY
H_2S
Viscosity
Cell imaging

ABSTRACT

A viscosity sensitive BODIPY fluorescent probe **BDP-N_3** with high selectivity for detecting H_2S was synthesized and designed. The probe exhibited weak fluorescence in non-viscous media. With the increase of viscosity, the fluorescence intensity and decay time of the probe was enhanced significantly, which proved that it acted as a molecular rotor and could be utilized for the detection of changes in viscosity. Additionally, the probe showed a fast 'turn-on' fluorescence response to H_2S with high selectivity. The H_2S concentration was directly proportional to fluorescence intensity at 515 nm. Furthermore, cellular imaging experiments revealed that the probe was cell membrane permeable and could be applied for sensing of both H_2S and viscosity in a biological system.

1. Introduction

Among the reactive sulfur species family, hydrogen sulfide (H_2S) is connected with various physiological and pathological functions. As the third gaseous signal molecule, H_2S was regarded as the biologically active gas to regulate immune, endocrine, cardiovascular, neuronal, and gastro-intestinal systems [1–4]. Generally, in most mammalian tissues and organisms, H_2S can be endogenously metabolized by at least certain enzymes such as cystathionine γ-lyase (CSE), cystathionine β-synthase (CBS), and 3-mercaptopyruvate sulfur-transferase (MST) [5]. Abnormally low or high endogenous levels of H_2S will result in severe diseases like diabetes, Alzheimer's disease, Down syndrome, liver cirrhosis, and hypertension [6–16]. In addition, H_2S is a famous inducer of apoptosis. It initiates the vital apoptotic enzymes caspase-3, caspase-8, and caspase-9 and induces cell death by causing cytoplasmic shrinkage and nuclear condensation which finally result in an increase of intracellular viscosity. From another point, as an important factor in the process of diffusion controlled processes, viscosity plays a primary role in varied biological activities, as well as in chemistry and other fields, and normally dominates the effective of mass transport of reagents [17,18]. In biological systems, viscosity as an important fundamental and structural sensitive physical parameter is related to diffusion-mediated cellular processes, such as signal transduction and transportation of small solutes, macromolecules, protein-protein interactions, and other cellular organelles in living cells [19]. It has been described that local viscosity in cells varies from 1 to 400 cP, abnormal change in the microviscosity of biological systems affects normal cellular functions and triggers a range of diseases [20], such as atherosclerosis [21], and even cell malignancy [22]. While methods to measure the bulk macroscopic viscosity are well developed, imaging local microscopic viscosity remains a challenge. Thus, simultaneous detection of viscosity and H_2S needs further investigation in the biological and medical fields.

Numerous studies have been carried out to explore the detection of H_2S including electrochemical, colorimetric and gas chromatographic techniques (GC), monobromobimane method (MBB) [23–27]. However, many of these techniques required complicated sample preparation or destroying the biological tissues, and thus could not be applied in the biological systems. Therefore, fluorescent techniques with their simplicity, high sensitivity, selectivity, and real-time capability to detect intracellular H_2S raised lots of interest. The design strategies of H_2S fluorescent probes is mainly based on its nucleophilicity and strong reducing property, such as reducing nitro/azanol to amines [28–31], Michael addition reactions, and thiolysis reactions [32–34]. Recently a tremendous number of fluorescent probes have been reported for the detection of H_2S but most of them are limited to the single function, and their practical application only detected the exogenous of H_2S in solution. Furthermore, few probes can test a biological sample of both H_2S and viscosity [35–43]. Accordingly, we were then interested to design a probe which can detect exogenous and endogenous H_2S as well as measure viscosity in cellular systems.

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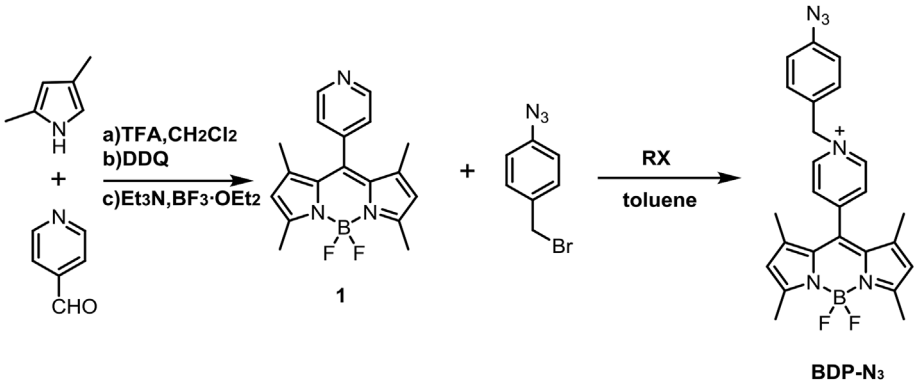
https://doi.org/10.1016/j.dyepig.2018.06.029
Received 19 May 2018; Received in revised form 18 June 2018; Accepted 19 June 2018
Available online 20 June 2018
0143-7208/



Scheme 1. Synthesis of the probe.

To address this question, we assumed that if we could design a compound that has effective H_2S reaction site and molecular rotation properties then it will be able to perform dual functions. Induction of apoptosis with dexamethasone in turn reflects the molecular rotation properties restricted in the viscous medium created during apoptosis process. Meanwhile, we recognized that BODIPY (4,4-difluoro-4-bora-3a, 4a-diaza-*s*-indacene) dyes are well-known to the excellent photophysical and photochemical properties, extremely stable and particularly insensitive to the polarity of solvents as well as to pH [44]. BODIPY analogue have been applied in the fields of probes and protein marker [45]. An azide group is introduced in pyridine BODIPY derivative, as it is known to undergo reduction in the presence of H_2S. Herein, we synthesized such a novel fluorescent probe **BDP-N$_3$** (Scheme 1) based on a BODIPY derivative incorporating quaternized 4-pyridinium group for multiply detecting intracellular H_2S and viscosity. Because H_2S related to gaseous signal transduction, and viscosity influenced diffusion in biological processes, we anticipate that the current probe **BDP-N$_3$** can be used to detect H_2S and viscosity in living systems for more insight into the role of both in medical issue.

2. Experimental

2.1. Materials and instruments

All reagents and solvents were purchased from commercial suppliers and used without further purification. Deionized water was used throughout all experiments. The solutions of anions were prepared from their sodium salts. The stock solutions of probe **BDP-N$_3$** were prepared in DMSO. SNP solution (20 mM) was prepared by dissolving SNP (8.8 g) in DMSO (2 mL). Chromatography was carried out on silica gel using silica gel GF254 plates with a thickness of 0.20–0.25 mm. A pH meter (Mettler Toledo, Switzerland) was used to determine the pH. Ultraviolet–visible (UV–vis) spectra were recorded on a Cary 50 Bio UV–visible spectrophotometer. Fluorescence spectra were measured on Hitachi F-7000 fluorescence spectrophotometer. All fluorescence and UV–vis spectra data were recorded at 2 min after the analytes addition. A PO-120 quartz cuvette (1 cm) was purchased from Shanhai Huamei Experiment Instrument Plants, China. ^{1}H NMR and ^{13}C NMR experiments were performed with a Bruker AVANCE-600 MHz NMR spectrometer, respectively (Bruker, Billerica, MA). Coupling constants (J values) are reported in hertz. ESI determinations were carried out on AB Triple TOF 5600plus System (AB SCIEX, Framingham, USA). FLIM images were obtained using FL920 transient fluorescence spectrometer purchased from Edinburgh Instruments Co., Ltd, United Kingdom. The ability of **BDP-N$_3$** reacting to H_2S in the living cells was measured by a Zeiss LSM880 Airyscan confocal laser scanning microscope.

2.2. Synthesis of *BDP-N$_3$*

Compound **1**: 4-Pyridinecarboxaldehyde (9.0 mmol, 0.96 g) was stirred with 2,4-dimethylpyrrole (19.4 mmol, 1.85 g) in deoxygenated CH_2Cl_2 (150 mL). One drop of TFA was added and the mixture was stirred overnight under Ar at room temperature. The red solution was treated with DDQ (9.0 mmol, 2.04 g), stirring was continued for 4 h followed by the addition of Et_3N (15 mL). After 15 min, $BF_3{\cdot}Et_2O$ (15 mL) was added at 0 °C, and the mixture was stirred at room temperature for further 3 h. After washing with saturated aqueous $NaHCO_3$, the organic phase was separated, dried with Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (CH_2Cl_2/Petroleum ether, 1/1, v/v, as eluent) to afford the desired 5,5-difluoro-1,3,7,9-tetramethyl-10-(pyridin-4-yl)-5H-4λ^4,5λ^4-dipyrrolo [1,2-*c*:2′,1′-*f*] [1–3]diazaborinine (compound **1**) as red powder (0.35 g, 12%). m.p. 241–243 °C. Elemental Analysis: Found C, 66.3; H, 5.4; N, 12.7%. Molecular formula: $C_{18}H_{18}BF_2N_3$, requires: C, 66.5; H, 5.6; N, 12.9%. ^{1}H NMR ($CDCl_3$, 600 MHz): δ (ppm): δ 8.79 (d, J = 5.8 Hz, 2H), 7.35 (d, J = 5.8 Hz, 2H), 6.01 (s, 2H), 2.56 (s, 6H), 1.41 (s, 6H). ^{13}C NMR ($CDCl_3$, 150 MHz): δ (ppm): 156.6, 150.1, 144.2, 142.5, 137.3, 130.2, 123.5, 121.8, 14.7 (Fig. S1).

Compound **BDP-N$_3$**: Compound **1** (0.06 mmol, 0.02 g) and 1-azido-4-(bromomethyl)benzene (0.6 mmol, 0.12 g) were dissolved in toluene (20 mL), and then the mixture was refluxed at 110 °C for 12 h. After filtration, the precipitate was filtered, washed with toluene and dried in vacuo to afford deep red powder 1-(4-azidobenzyl)-4-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ^4, 5λ^4-dipyrrolo [1,2-*c*:2′,1′-*f*] [1–3]diazaborinin-10-yl)pyridin-1-ium bromide (compound **BDP-N$_3$**) (0.018 g, 62%). m.p. > 300 °C. Elemental Analysis: Found C, 65.4; H, 5.1; N, 18.2%. Molecular formula: $C_{25}H_{24}BF_2N_6$, requires: C, 65.6; H, 5.3; N, 18.4%. ^{1}H NMR ($CDCl_3$, 600 MHz): δ (ppm): δ 9.40 (d, J = 6.2 Hz, 2H), 8.48 (d, J = 6.7 Hz, 2H), 7.60 (d, J = 8.4 Hz, 2H), 6.27 (s, 2H), 5.95 (s, 2H), 2.50 (s, 6H), 1.35 (s, 6H). ^{13}C NMR ($CDCl_3$, 150 MHz): δ (ppm):156.7, 151.1, 145.9, 142.2, 140.5, 134.4, 130.7, 130.5, 128.8, 128.6, 122.3, 119.8, 62.9, 14.5, 14.2 (Fig. S2). MS (ESI): found: *m/z* 457.2131; Molecular formula: $[C_{25}H_{24}BF_2N_6]^+$, requires: 457.2118, (Fig. S3).

2.3. Solutions preparation and UV–vis and fluorescence measurements

The stock solution of the probe, **BDP-N$_3$**, was prepared with a concentration of 2 mM in DMSO. The stock solutions of H_2S (2 mM) was prepared in deionized water, sodium hydrosulfide solid was added to aqueous solution to prepare a H_2S solution. Reagents with analytical grades and demineralized water were used for preparing the solutions. Stock solutions (2 mM) of F^-, Cl^-, Br^-, I^-, NO_3^-, NO_2^-, AcO^-, HCO_3^-, CO_3^{2-}, SCN^-, SO_3^{2-}, SO_4^{2-}, $S_2O_3^{2-}$, ClO_4^-, PO_4^{3-}, Cys, Hcy, GSH, were prepared by direct dissolution of proper amounts of sodium salts. A test solution was prepared by placing appropriate