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A ratiometric fluorescent probe for gasotransmitter hydrogen sulfide based on a coumarin-benzopyrylium platform

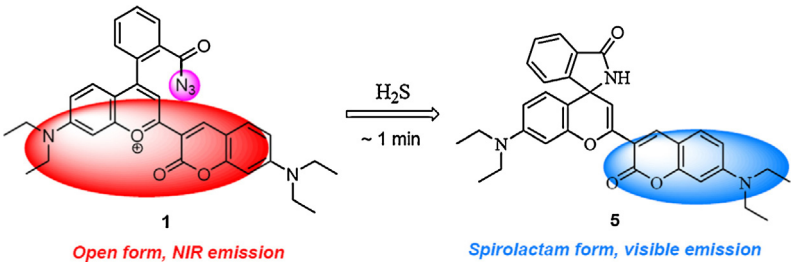
Yu-Wei Duan [a], Xiao-Feng Yang [a,*], Yaogang Zhong [b], Yuan Guo [a,*], Zheng Li [b], Hua Li [a]

[a] Key Laboratory of Synthetic and Natural Functional Molecule Chemistry of the Ministry of Education, College of Chemistry and Materials Science, Northwest University, Xi'an 710069, PR China
[b] College of Life Sciences, Northwest University, Xi'an 710069, China

HIGHLIGHTS

- A ratiometric fluorescent probe for H_2S has been developed based on coumarin–benzopyrylium platform.
- The ratiometric sensing is realized by coupling the azide-based strategy with the intramolecular spirolactamization reaction.
- The proposed probe utilizes the acyl azide as the recognition moiety and exhibits a rapid response towards H_2S (*ca.* 1 min).
- Preliminary experiments show that the proposed probe has potential to track H_2S in live cells.

GRAPHICAL ABSTRACT



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ABSTRACT

A ratiometric fluorescent probe for H_2S was developed based on a coumarin– benzopyrylium platform. The ratiometric sensing is realized by a selective conversion of acyl azide to the corresponding amide, which subsequently undergoes an intramolecular spirocyclization to alter the large π-conjugated system of **CB** fluorophore. Compared with the traditional azide-based H_2S probes, the proposed probe utilizes the acyl azide as the recognition moiety and exhibits a rapid response (~1 min) towards H_2S, which is superior to most of the azide-based H_2S probes. Preliminary fluorescence imaging experiments show that probe **1** has potential to track H_2S in living cells.

1. Introduction

Hydrogen sulfide (H_2S) is an endogenous gaseous transmitter and can be produced from cystathionine-β-synthase, cystathionine-γ-lyase, and cysteine (Cys) amino transferase in combination with 3-mercaptosulfurtransferase [1–3]. H_2S has also been known to be involved in a variety of physiological processes, such as angiogenesis, vasodilation, cardioprotection, inflammation and

* Corresponding authors. Tel.: +86 29 88302604.
E-mail addresses: xfyang@nwu.edu.cn (X.-F. Yang), guoyuan@nwu.edu.cn (Y. Guo).

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neuromodulation [4–7]. On the other hand, the abnormal H_2S level is correlated to diseases such as Alzheimer's disease, Down's syndrome, diabetes and liver cirrhosis [8–11]. Therefore, in order to understand the physiological and pathological functions of H_2S, selective tracking and quantifying of this small molecule within living biological specimens is crucial.

Fluorescence imaging through staining with a smaller probe has now been recognized as the most attractive molecular imaging techniques for in vivo monitoring and visualizing of biomolecules by virtue of its high sensitivity/selectivity, high spatiotemporal resolution and non-destructive advantage. In recent years, a number of fluorescent probes have been developed for detection and imaging of H_2S based on different sensing mechanisms [12,13], including reduction of azido [14–24] and nitro groups [25,26], unique dual nucleophilic reaction [27–34], high binding affinity with Cu^{2+} complexes [35–37], thiolysis of dinitrophenyl ether [38–40], as well as specific addition reaction towards unsaturated double bonds [41].

Among these, the strategy based on the conversion of azides to amines is particularly attractive from the selectivity point of view, as azides are widely valued for their inert chemical behavior in biological specimens such as abundant cellular thiols (glutathione and Cys), amino acids, reducing species and extracellular fluids. Unfortunately, most of azide-functionalized probes display a delayed response time [14,17,18,22,24] -except for dansyl azide [15], and thus are not suitable for real-time imaging of H_2S due to its rapid metabolism in endogenous systems. In view of these, further efforts to innovate the azide-based design strategy are still needed.

2-(7-Diethylamino-2-oxo-2H-1-benzopyran-3-yl)-4-(2-carboxyphenyl)-7-diethylamino-1-benzopyrylium (**CB**), a hybrid fluorophore of coumarin and benzopyrylium, shows a near-infrared (NIR) absorbance and fluorescence in neutral conditions and has been reported as laser dyes previously [42]. Recent studies show that **CB** has the carboxylic-acid-regulated fluorescence switching mechanism by intramolecular spirocyclization [43], which is quite similar to those of rhodamine derivatives. More significantly, unlike traditional rhodamine derivatives, which are colorless and nonfluorescent in the spirocyclic form [44],**CB** contains a 7-diethylaminecoumarin moiety and features coumarin spectral properties even in its spirocyclic form. Therefore, **CB** has become a robust platform for the development of ratiometric fluorescent probes based on altering the π-conjugation of **CB** derivatives by spirocyclic/ring-open switching mechanism, and fluorescent probes for Hg^{2+} [43] and Cys/Homocysteine (Hcy) have been reported [45].

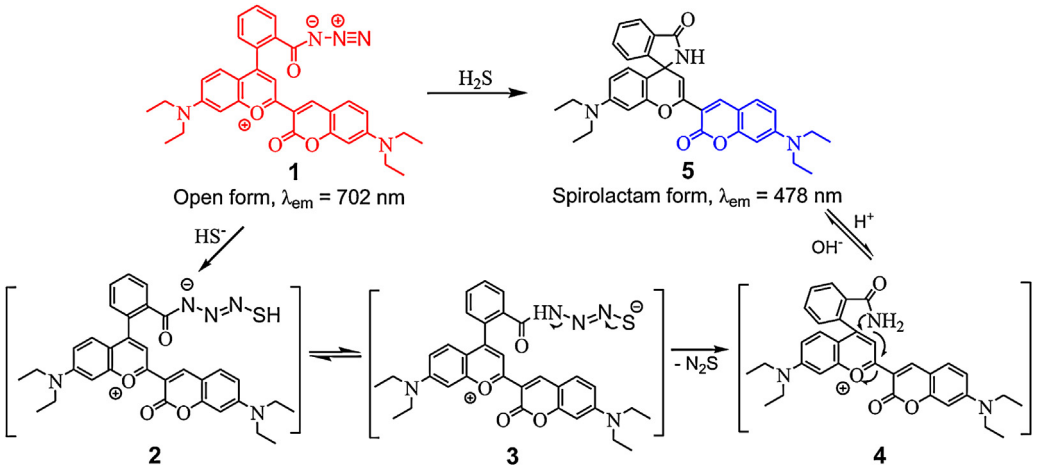
Motivated by the above facts, we design and synthesize a new NIR fluorescent probe **1** for the ratiometric sensing of H_2S by employing **CB** as the dye scaffold and acyl azide as the recognition unit. The design rationale is depicted in Scheme 1. We reasoned that HS^-, the main stable form of H_2S in the physiological condition, can selectively react with the acyl azide of **1** to afford the corresponding amide **4**. At the physiological pH, the resulting N atom of the amide group may further attack the benzopyrylium C-4 atom to undergo an intramolecular spirocyclization to give **5**. As a result, the π-conjugation system of **1** is interrupted, thereby leading to the decrease of the NIR emission. On the other hand, since **1** contains a 7-diethylaminecoumarin moiety, it can afford coumarin emission even in its spirolactam form. Therefore, two well-resolved emission peaks before and after adding H_2S could be obtained due to the distinct emission between **CB** and the produced coumarin fluorophore. Based on the above strategy, ratiometric sensing of H_2S can be realized. In addition, acyl azide, while not aryl or aliphatic azides, was selected as the recognition unit, as its structure is similar to that of sulfonyl azide (both possessing adjacent oxygen atom), thus expecting to display a very quick response to H_2S.

To test the above-mentioned possibilities, we decided to synthesize and examine the spectra profiles of **1**. Compound **1** was easily prepared by the reaction of **CB** with $POCl_3$ followed without purification by sodium azide, and its structure was characterized by IR, ^1H NMR, ^{13}C NMR and HRMS spectra.

2. Materials and methods

2.1. Synthesis of *1*

Compound **CB** was synthesized according to the reported procedure [45]. A solution of **CB** (0.537 g, 1.0 mmol) in 1,2-dichloroethane (6 mL) was stirred and $POCl_3$ (0.3 mL) was added dropwise over 5 min. The solution was refluxed for 4 h. After cooling to room temperature, the reaction mixture was evaporated in vacuo to give **CB** acid chloride. The crude acid chloride was dissolved in CH_3CN (12 mL), to which NaN_3 (0.35 g) was added and the reaction solution was stirred at 0–5 °C overnight. The reaction mixture was washed with water and dried in vacuum to afford the crude product, which was then purified by silica gel flash chromatography using CH_2Cl_2/CH_3OH



Scheme 1. The proposed sensing mechanism of probe **1** for H_2S.