Bioorganic & Medicinal Chemistry Letters 25 (2015) 175–178

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Development of photo-controllable hydrogen sulfide donor applicable in live cells

Naoki Fukushima [a], Naoya Ieda [a], Mitsuyasu Kawaguchi [a], Kiyoshi Sasakura [b], Tetsuo Nagano [b], Kenjiro Hanaoka [b], Naoki Miyata [a], Hidehiko Nakagawa [a,*]

[a] Graduate School of Pharmaceutical Sciences, Nagoya City University, 3-1, Tanabe-dori, Mizuho-ku, Nagoya-shi, Aichi 467-8603, Japan
[b] Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

ARTICLE INFO

Article history:
Received 7 November 2014
Revised 27 November 2014
Accepted 29 November 2014
Available online 8 December 2014

Keywords:
Hydrogen sulfide
Caged H_2S
Photolabile protecting group
Xanthone photocage

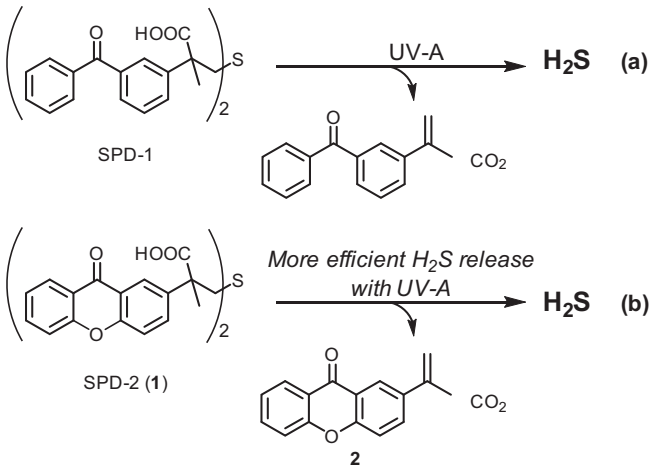
ABSTRACT

Hydrogen sulfide (H_2S) has multiple physiological roles, for example, in vasodilation and inflammation. It is a highly reactive gas under ambient conditions, so controllable H_2S donors are required for studying its biological functions. Here, we describe the design, synthesis and application of a H_2S donor (SPD-2) that utilizes xanthone photochemistry to control H_2S release. H_2S generation from SPD-2 was completely dependent on UVA-irradiation (325–385 nm), as confirmed by methylene blue assay and by the use of a H_2S-selective fluorescent probe. SPD-2 was confirmed to provide controlled H_2S delivery in live cells, and should be suitable for various biological applications.

Hydrogen sulfide (H_2S), like nitric oxide (NO) and carbon monoxide (CO), is a gaseous physiological signal transmitter[1] that has roles in multiple processes, including vascular smooth muscle relaxation,[2] neurotransmission[3] and regulation of inflammation.[4] It is biologically synthesized from L-cysteine and/or L-homocysteine by cystathionine-β-synthase (CBS),[5] cystathionine-γ-lyase (CSE)[6] and 3-mercaptopyruvate sulfur transferase (3MST) coupled with cysteine aminotransferase (CAT).[7] Recently, a novel H_2S biosynthetic pathway from D-cysteine, involving 3-MST and D-amino acid oxidase (DAO), has also been reported.[8] These enzymes have been demonstrated to influence a wide range of physiological and pathological processes.[9,10]

In biological research on H_2S, inorganic sulfide salts, such as Na_2S and NaSH have been widely used as H_2S sources. However, these sources have substantial disadvantages for examination of the physiological functions of H_2S. For example, H_2S generation is very fast, and precise control of the release rate and dosage is not feasible. These compounds cannot mimic biological H_2S release, which is thought to be relatively slow and continuous. Therefore, controllable H_2S donors are required for detailed investigation of the physiological functions of H_2S and for potential therapeutic applications. We focused on photocontrollable H_2S donors, since photolysis-induced H_2S release has the potential to allow precise control of the location, timing and dosage of release.

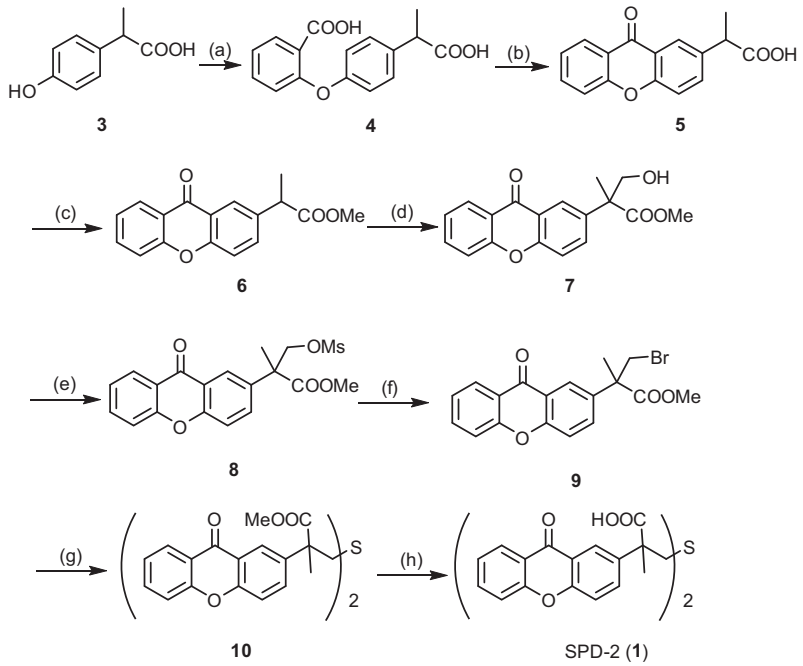
Some photolysis-inducing H_2S donors, based on geminal-dithiol structure, have already been reported.[11] However, it is difficult to control the rate of H_2S release from these donors because the formation of H_2S depends on hydrolysis of geminal dithiol in aqueous media. Recently, we have reported a H_2S derivative doubly protected with a photolabile protecting group (PPG) as a photo-activatable H_2S donor,[12] based on ketoprofenate PPG[13] which has good light-responsiveness (SPD-1, Scheme 1a). Ketoprofenate



Scheme 1. Structures and photoreactions of SPD-1 and SPD-2.

* Corresponding author.

http://dx.doi.org/10.1016/j.bmcl.2014.11.084
0960-894X/



Scheme 2. Synthesis of SPD-2. Reagents and conditions: (a) 2-iodobenzoic acid, Cs_2CO_3, CuCl, TDA-1, dioxane; (b) H_2SO_4, 85 °C, 2 steps 52%; (c) H_2SO_4, MeOH, 96%; (d) paraformaldehyde, K_2CO_3, DMSO, 56%; (e) MsCl, NEt_3, CH_2Cl_2, quant.; (f) LiBr, MeCN, 36%; (g) sodium sulfide nonahydrate, toluene, H_2O, $C_{16}H_{33}(C_4H_9)_3P^+Br^-$, 46%; (h) LiOH, THF, H_2O, MeOH, 84%. TDA-1 = tris[2-(2-methoxyethoxy)ethyl]amine, DMSO = dimethylsulfoxide, MsCl = methanesulfonyl chloride, THF = tetrahydrofuran.

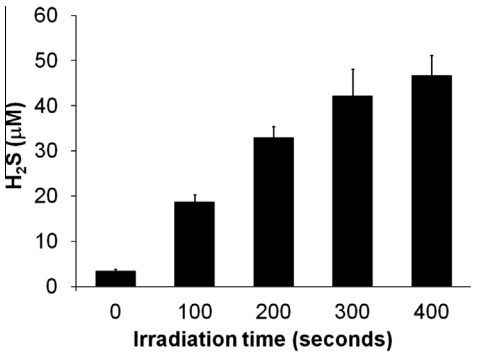


Figure 1. Release of H_2S from SPD-2 (100 μM) in PBS, pH 7.4, with 1% DMSO as detected by the methylene blue method. Formation of methylene blue after irradiation of SPD-2 for various times was determined from the absorbance at 671 nm. Photoirradiation was performed at 325–385 nm and the light intensity was set at 2.0 mW/cm^2. The data represent the average of three independent experiments with standard deviations.

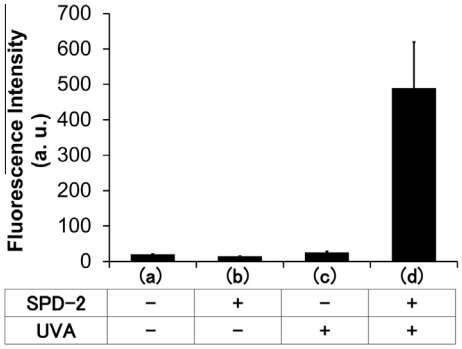


Figure 2. Fluorescence measurement for detection of H_2S generation using HSip-1. The concentration of SPD-2 was 100 μM and that of HSip-1 was 1 μM in phosphate buffer, pH 7.4, containing 1% DMSO. Fluorescence emission was measured at 516 nm with excitation at 491 nm. Measurements were made after incubation of HSip-1: (a) without SPD-2 or irradiation; (b) with SPD-2 but without irradiation; (c) without SPD-2 after irradiation for 300 s; (d) with SPD-2 after irradiation for 300 seconds. Photoirradiation was performed at 325–385 nm and the light intensity was set at 2.0 mW/cm^2. The data represent the average of three independent experiments with standard deviations.

PPG has many advantageous features, such as good water solubility, formation of benign photo-products and a higher release rate than *o*-nitrobenzyl PPG,[14] the most widely used PPG. However, SPD-1 shows maximum absorbance at 260 nm (Fig. S1b), and consequently the efficiency of H_2S release from SPD-1 is insufficient for application in living cells when UV-A (330–380 nm), widely equipped in some microscopies, is utilized as an uncaging light source. Therefore, we set out to design a new photo-induced H_2S donor suitable for cellular application. Recently, xanthone PPGs have been reported as next-generation carbanion-mediated PPGs[15] with excellent absorption in the UVA region (>320 nm). We designed a novel photo-controllable H_2S donor, SPD-2, by utilizing xanthone PPG to protect H_2S directly (Scheme 1b). SPD-2 showed superior photochemical properties to the ketoprofenate-type H_2S donor, and we confirmed that it is available for photo-controlled, site-specific H_2S release in live cells.

SPD-2 was synthesized as shown in Scheme 2. Preparation of the synthetic intermediate was performed as described previously.[15] Xanthone derivative **5** was prepared by Ullmann condensation of *o*-iodobenzoic acid and 2-(4-hydroxyphenyl)propanoic acid (**3**), followed by acid-catalyzed Friedel–Crafts type reaction. After esterification of **5**, hydroxymethylation by treatment with paraformaldehyde and K_2CO_3 gave the desired alcohol **7**. This was mesylated to obtain **8**, which was converted to the brominated derivative **9**. Then, sulfide **10** was synthesized by using sodium sulfide nonahydrate. In this reaction, 2-(prop-1-en-2-yl)-9*H*-xanthen-9-one (Scheme 1b, the proposed photoproduct **2**) was obtained as a by-product, and this was isolated for structural confirmation of the photo-product of SPD-2. Finally, hydrolysis gave the desired product SPD-2. The structure and purity of SPD-2 were confirmed by ^1H NMR, ^{13}C NMR, mass spectrometry and elemental analysis.