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## Introduction

Hydrogen sulfide ( $H_2S$ ) has long been known as a toxic gas, but recent studies indicate that  $H_2S$  has important physiological functions, leading to its inclusion as the third gasotransmitter along with nitric oxide (NO) and carbon monoxide (CO).<sup>1-3</sup> Endogenous  $H_2S$  is enzymatically generated by cystathione  $\gamma$ -lyase (CSE), cystathione- $\beta$ -synthase (CBS) and 3-mercaptopropionate sulfurtransferase (3-MST)/cysteine aminotransferase (CAT).<sup>4</sup>  $H_2S$  influences a wide range of physiological processes in mammals, ranging from vasorelaxation,<sup>5</sup> cardioprotection,<sup>6</sup> and neurotransmission<sup>7</sup> to anti-inflammatory action<sup>8</sup> and angiogenesis.<sup>9</sup> Misregulation of  $H_2S$  is associated with numerous diseases.<sup>10,11</sup> Specially, low levels of endogenous  $H_2S$  appears to

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## Highly efficient $H_2S$ scavengers via thiolysis of positively-charged NBD amines†

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$H_2S$  is a well-known toxic gas and also a gaseous signaling molecule involved in many biological processes. Advanced chemical tools that can regulate  $H_2S$  levels *in vivo* are useful for understanding  $H_2S$  biology as well as its potential therapeutic effects. To this end, we have developed a series of 7-nitro-1,2,3-benzoxadiazole (NBD) amines as potential  $H_2S$  scavengers. The kinetic studies of thiolysis reactions revealed that incorporation of positively-charged groups onto the NBD amines greatly increased the rate of the  $H_2S$ -specific thiolysis reaction. We demonstrate that these reactions proceed effectively, with second order rate constants ( $k_2$ ) of  $>116\text{ M}^{-1}\text{ s}^{-1}$  at  $37\text{ }^\circ\text{C}$  for NBD-S8. Additionally, we demonstrate that NBD-S8 can effectively scavenge enzymatically-produced and endogenous  $H_2S$  in live cells. Furthering the biological significance, we demonstrate NBD-S8 mediates scavenging of  $H_2S$  in mice.

exhibit pro-cancer effects, whereas higher concentrations of  $H_2S$  can lead to cell apoptosis and have anti-cancer characteristics.<sup>11</sup> Complementing the importance of  $H_2S$  in mammals,  $H_2S$  in bacteria and plants also plays many important functions.<sup>12,13</sup> Due to its wide biodistribution and complex behaviors in many diseases, the physiological characters of  $H_2S$  and the molecular mechanisms in which  $H_2S$  is involved need further investigation. In addition, these factors support the development and refinement of advanced chemical tools that can visualize,<sup>14</sup> scavenge<sup>15</sup> or release<sup>16</sup>  $H_2S$  *in vivo* and in related complex environments.

Significant attention has focused on developing chemical tools for  $H_2S$  detection and delivery,<sup>14,16</sup> but much less effort has focused on reducing  $H_2S$  levels in complex environments.<sup>15,17</sup> Such  $H_2S$  elimination could be achieved by either inhibition of  $H_2S$  biosynthesis or by the selective scavenging of  $H_2S$ . Though CBS inhibitors have been reported to show anti-tumor activity, many of CBS inhibitors target the pyridoxal-5'-phosphate (PLP) cofactor and therefore have low specificity or unwanted side effects.<sup>11b</sup> Complementing these challenges, both CSE and 3-MST inhibitors are relatively underdeveloped.<sup>11a</sup> Moreover,  $H_2S$  can be produced from non-enzymatic processes,<sup>14</sup> which suggests that enzymatic inhibitors can only perturb certain pools of biological  $H_2S$  genesis. Recently, sulfonyl azides were reported as highly efficient  $H_2S$  scavengers in buffers, enzymatic systems, and living biological environments.<sup>15</sup> It should be noted that the  $H_2S$ -mediated reduction of aryl azides to amines results in generation of sulfane sulfur and polysulfides,<sup>15,18</sup> both of which are important reactive sulfur species that might complicate such scavenging.<sup>19a</sup> Considering the *in vivo* biological complex,<sup>19</sup> our goal was to develop efficient  $H_2S$  scavengers utilizing different reaction mechanisms that not only scavenge



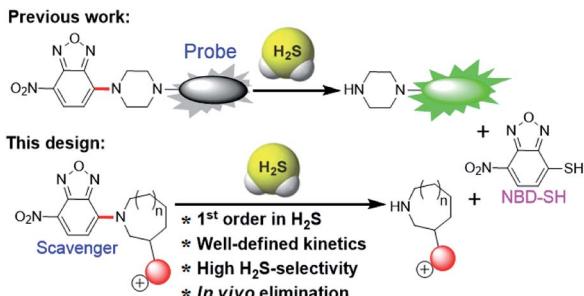


Fig. 1 (top) The thiolysis of NBD amines used previously for  $\text{H}_2\text{S}$  probe development. (bottom) Inclusion of cationic charge in the present system enables access to efficient  $\text{H}_2\text{S}$  scavengers by thiolysis of NBD cyclic amines.

$\text{H}_2\text{S}$  efficiently, but also do not generate other reactive sulfur species in the scavenging process with the long-term goal of applying these toward *in vivo* systems.

A major challenge in the development of  $\text{H}_2\text{S}$  scavengers is developing fast chemical reactions that effectively differentiate the reactivity of biological nucleophiles (e.g. biothiols) from  $\text{H}_2\text{S}$ . To this end, we as well as others have previously reported the  $\text{H}_2\text{S}$ -specific thiolysis of 7-nitro-1,2,3-benzoxadiazole (NBD) amine in 2013 (Fig. 1),<sup>20</sup> which has enabled, amongst other applications, the development of near-infrared  $\text{H}_2\text{S}$  probes for imaging of  $\text{H}_2\text{S}$  *in vivo*.<sup>20c</sup> To further advance the development of  $\text{H}_2\text{S}$  scavengers, we report here that simple modifications of the NBD electrophiles can increase the reactivity of this platform to enable efficient  $\text{H}_2\text{S}$  scavenging (Fig. 2) and apply these chemical tools to scavenge  $\text{H}_2\text{S}$  in buffer, serum, cells, and mice. We believe that the present  $\text{H}_2\text{S}$  scavengers could be useful tools to study  $\text{H}_2\text{S}$  biology as well as potential therapeutic agents in the future.

## Results and discussion

Compounds **NBD-S1** to **NBD-S8** were synthesized from commercially available reagents by simply coupling **NBD-Cl** to the desired amine or by reacting NBD-piperazine with 2-chloro-1-methylpyridinium salt (Fig. 2). Compound identity was confirmed by  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy and HRMS. All scavengers were prepared in high yield in either one- or two-step syntheses, which readily enables access to large quantities of each scavenger.

To measure the rate of the prepared compounds with  $\text{H}_2\text{S}$ , we monitored the optical response of **NBD-S1-NBD-S8** (5–10  $\mu\text{M}$ ) after addition of  $\text{H}_2\text{S}$  (6–600 equiv., using  $\text{Na}_2\text{S}$ ) in PBS buffer (pH 7.4) at 25 °C. An example of the observed reactivity is shown for **NBD-S2** (Fig. 3a), in which the starting absorbance peak at 490 nm from the NBD moiety shifts to 540 nm after reaction with  $\text{H}_2\text{S}$  due to formation of **NBD-SH**.<sup>20b</sup> The reaction kinetics were monitored by measuring absorbance data at 540 nm or 490 nm (Fig. S1†) and showed a clear isosbestic point at 510 nm, which is consistent with a clean overall transformation. The pseudo-first-order rate constant,  $k_{\text{obs}}$  was determined by fitting the intensity data to a single exponential

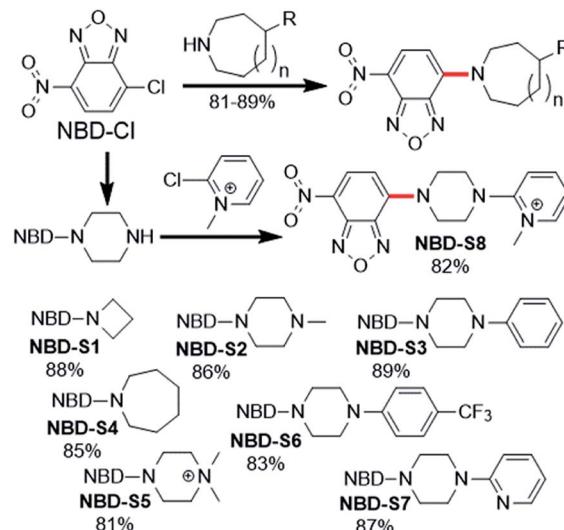


Fig. 2 Synthesis of NBD amines **NBD-S1** to **NBD-S8**. The C–N bond cleaved by  $\text{H}_2\text{S}$ -mediated thiolysis is highlighted in red in each structure.

function (Fig. 3b). Plotting  $\log(k_{\text{obs}})$  versus  $\log([\text{H}_2\text{S}])$  confirmed a first-order dependence in  $\text{H}_2\text{S}$  (Fig. 3c). The linear fitting between  $k_{\text{obs}}$  and  $\text{H}_2\text{S}$  concentrations gives the second-order rate constant ( $k_2$ ) of 23.3  $\text{M}^{-1} \text{s}^{-1}$  (Fig. 3d).

Using the above method, the  $\text{H}_2\text{S}$ -mediated thiolysis rates for the prepared NBD amines were measured, and these data are summarized in Table 1. The thiolysis rates for the four, six, and seven-membered ring amines (**NBD-S1** to **NBD-S4**) are significantly different, with the presence of the second amine group in the ring appearing to be particularly important. We hypothesized that this increased rate could be due to protonation of the

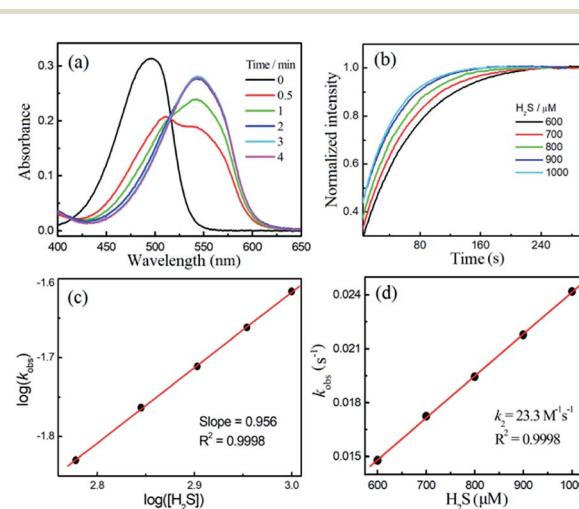


Fig. 3 (a) Time-dependent absorbance spectra of 10  $\mu\text{M}$  **NBD-S2** towards 1 mM  $\text{H}_2\text{S}$  in PBS buffer (50 mM, pH = 7.4, containing 2% DMSO) at 25 °C. (b) Time-dependent normalized signals at 540 nm of 10  $\mu\text{M}$  **NBD-S2** towards different concentrations of  $\text{H}_2\text{S}$ . (c) The reaction order of  $\text{H}_2\text{S}$  was determined as the slope of plots of  $\log(k_{\text{obs}})$  versus  $\log([\text{H}_2\text{S}])$ . (d) The  $k_2$  was determined as the slope of plots of  $k_{\text{obs}}$  versus  $[\text{H}_2\text{S}]$ .



Table 1 Kinetic parameters of the thiolysis of NBD amines at 25 °C

NBD-S1	NBD-S2	NBD-S3	NBD-S4	NBD-S5	NBD-S6	NBD-S7	NBD-S8	
$k_{\text{obs}}^a / \text{s}^{-1}$ ([H <sub>2</sub> S] <sup>b</sup> )	$4.2 \times 10^{-3}$ 6.0 mM	$2.4 \times 10^{-2}$ 1.0 mM	$2.7 \times 10^{-2}$ 1.5 mM	N.D. <sup>c</sup>	$9.1 \times 10^{-3}$ 120 μM	$2.2 \times 10^{-2}$ 1.2 mM	$3.6 \times 10^{-2}$ 2.0 mM	$9.3 \times 10^{-3}$ 120 μM
Slope <sup>c</sup>	0.937	0.956	1.039	N.D.	0.898	1.066	1.048	0.979
$k_2^d / \text{M}^{-1} \text{s}^{-1}$	0.67	23.3	18.0	< 0.01	70.2	19.7	18.8	76.2

<sup>a</sup>  $k_{\text{obs}}$ : the value was obtained by fitting the time-dependent spectra data with single exponential function. <sup>b</sup> [H<sub>2</sub>S]: the H<sub>2</sub>S concentration used at the determination of the  $k_{\text{obs}}$  value. <sup>c</sup> Slope: the slope value of the linear fitting of  $\log(k_{\text{obs}})$  versus  $\log([H_2S])$ . <sup>d</sup>  $k_2$ : the slope value of the linear fitting of  $k_{\text{obs}}$  versus [H<sub>2</sub>S]. <sup>e</sup> N.D.: no detection due to the very slow reaction.

piperazine nitrogen at physiological pH (e.g.: the  $\text{p}K_{\text{a}}$  of 1-methyl piperazine is ~9).<sup>21</sup> To verify this hypothesis, compounds **NBD-S5** to **NBD-S8** with the functionlized second amine were further tested. As expected, alkylation of the piperazine nitrogen (**NBD-S5**) or inclusion of a positively charged pyridinium (**NBD-S8**) significantly increased the thiolysis reaction rates. For example,  $k_2$  values for cationic **NBD-S5** and **NBD-S8** derivatives were  $70.2 \text{ M}^{-1} \text{s}^{-1}$  and  $76.2 \text{ M}^{-1} \text{s}^{-1}$  at 25 °C, respectively, which are significantly faster than the  $k_2$  values of the neutral **NBD-S2** and **NBD-S7** analogues. To further support these measurements, we also performed kinetic analysis using fluorescence methods (Fig. S9 and S10†) for **NBD-S5** and **NBD-S8**, which afforded  $k_2$  values of  $71.6 \text{ M}^{-1} \text{s}^{-1}$  and  $78.3 \text{ M}^{-1} \text{s}^{-1}$ , respectively, which are consistent with those from absorbance measurements. We also measured  $k_2$  for **NBD-S8** at 37 °C, which yielded a significantly higher value of  $116.1 \text{ M}^{-1} \text{s}^{-1}$  (Fig. S11†), and suggests that this compound can scavenge H<sub>2</sub>S efficiently at physiological temperatures. The formation of the amine product during the thiolysis reaction was confirmed by HPLC and HRMS analysis (Fig. S12 and S13†). Taken together, these data suggest that **NBD-S5** and **NBD-S8** are highly reactive reagents toward H<sub>2</sub>S clearance.

We also obtained crystals suitable for single-crystal X-ray diffraction studies of **NBD-S7** and **NBD-S8** (Fig. 4). In the solid state, the piperazinyl heterocycles in **NBD-S7** and **NBD-S8** are in twist-boat and chair conformations, respectively. Prior work

with electrophilic piperazine-containing cyanine GSH probes has shown that the chair conformation is favorable for the thiolysis reaction,<sup>22a</sup> which may help explain the increased reaction rate of **NBD-S8** when compared to **NBD-S7**. In addition, the positive charge of **NBD-S8** may favor reaction with anionic HS<sup>-</sup>, and prior work with probes for F<sup>-</sup> has shown increased rates for cationic versions of probes when compared to neutral counterparts.<sup>22b</sup>

We also evaluated the stability, water-solubility, and selectivity of **NBD-S5** and **NBD-S8**. The plots of absorbance intensity against the scavenger concentration (up to 100 μM) were linear (Fig. S14 and S15†), suggesting the good solubility of **NBD-S5** and **NBD-S8** in PBS buffer (50 mM, pH = 7.4, containing 2% DMSO). Both **NBD-S5** and **NBD-S8** were shown to be stable in PBS buffer, with no decomposition over 2 days (Fig. S16†). The high selectivity of the NBD amines toward H<sub>2</sub>S over biothiols were validated by HPLC analysis (Fig. S17†), suggesting the thiolysis of the NBD amines is highly H<sub>2</sub>S-specific.<sup>20c</sup> We propose that this high selectivity may originate from the intrinsic low thiolysis reactivity for C–N bond cleavage (e.g. **NBD-S1**), and that certain structural factors, such as the piperazine chair conformation and positively-charged group, may contribute to the faster reactivity with HS<sup>-</sup> (e.g. **NBD-S5** and **NBD-S8**). Therefore, **NBD-S5** and **NBD-S8** were further employed as H<sub>2</sub>S scavengers in the following studies.

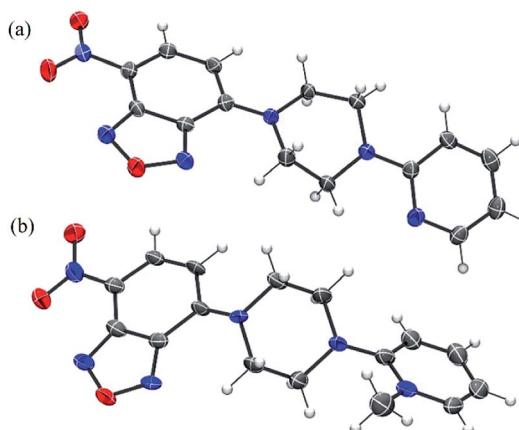


Fig. 4 ORTEP diagram (50% ellipsoid) for the molecular structure of **NBD-S7** (a) and **NBD-S8** (b). The I<sup>-</sup> counterion in (b) is omitted for clarity.

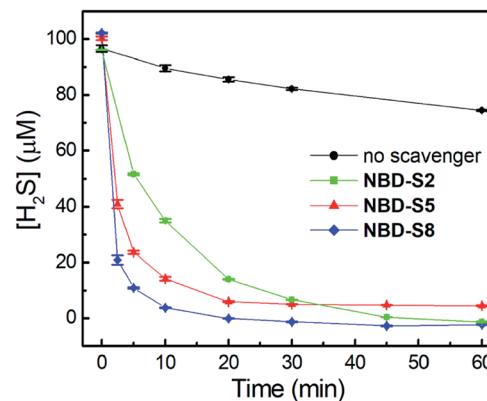


Fig. 5 H<sub>2</sub>S-scavenging curves of NBD amines or no scavenger in PBS buffer (pH 7.4) at 25 °C. Three scavengers (**NBD-S2**, **NBD-S5**, and **NBD-S8**, 110 μM) were added to H<sub>2</sub>S solutions (100 μM). The H<sub>2</sub>S concentrations were measured using MBA. The results are expressed as mean ± S.D. (n = 3).



We first tested the efficiency of  $\text{H}_2\text{S}$  clearance by NBD amines in PBS buffer (50 mM, pH 7.4), using the methylene blue assay (MBA)<sup>23</sup> to quantify  $\text{H}_2\text{S}$  concentrations (Fig. S18–S21†). The time-dependent  $\text{H}_2\text{S}$  concentrations in the absence or presence of **NBD-S2**, **NBD-S5**, or **NBD-S8** are shown in Fig. 5. The concentration of  $\text{H}_2\text{S}$  slowly decreased over time, which matches previous observations.<sup>24</sup> Under these conditions, both **NBD-S5** and **NBD-S8** can scavenge more than 50%  $\text{H}_2\text{S}$  within the first 2 min. Additionally, **NBD-S8** can scavenge more than 90%  $\text{H}_2\text{S}$  within 5 min, suggesting it is a highly efficient  $\text{H}_2\text{S}$  scavenger in aqueous buffer (pH 7.4). Based on these parameters, we used **NBD-S8** for subsequent applications. Considering  $\text{H}_2\text{S}$  is a toxic gas,<sup>2b</sup> we tested the ability of the **NBD-S8** solution to remove  $\text{H}_2\text{S}$  in gaseous sample (Fig. S22†).<sup>25</sup> The color change of **NBD-S8** solution and MBA tests of the solution clearly suggested **NBD-S8** could efficiently scavenge  $\text{H}_2\text{S}$  when the  $\text{H}_2\text{S}$ -containing gas was passed through the **NBD-S8** solution.

We further investigated the time-dependent  $\text{H}_2\text{S}$ -scavenging ability of **NBD-S8** in the presence of biologically relevant species. As shown in Fig. 6, the results suggest that the scavenging of  $\text{H}_2\text{S}$  via **NBD-S8** is not significantly influenced by the presence of millimolar biothiols or other species. The scavenging of  $\text{H}_2\text{S}$  in 10% fetal bovine serum (FBS) was also efficient. For all tested samples, **NBD-S8** could scavenge more than 90%  $\text{H}_2\text{S}$  within first 10 min. These data suggest that **NBD-S8** should be an efficient  $\text{H}_2\text{S}$  scavenger in complex biological-related systems.

Then we tested whether **NBD-S8** could scavenge  $\text{H}_2\text{S}$  in live cells. We first tested the cytotoxicity of **NBD-S8** by the MTT assay using HeLa cells which contain nearly no endogenous  $\text{H}_2\text{S}$ . The scavenger did not show cytotoxicity up to 50  $\mu\text{M}$  concentration (Fig. S23a†). Furthermore, the cell viability in the presence of 100  $\mu\text{M}$  and 200  $\mu\text{M}$  **NBD-S8** is still greater than 90% and 80%, respectively. These results suggest that **NBD-S8** is biocompatible for scavenging  $\text{H}_2\text{S}$  in living cells. To confirm that the thiolysis products of **NBD-S8** (**NBD-SH** and **S8-II**) are not cytotoxic, we

evaluated the cytotoxicity in HeLa cells for 24 hours. **NBD-SH** did not show any significant cytotoxicity below 25  $\mu\text{M}$  (Fig. S23b†). The cell viability in the presence of 25  $\mu\text{M}$  and 100  $\mu\text{M}$  **S8-II** is still greater than 90% and 80%, respectively (Fig. S23c†). We note that under physiological conditions, the concentration of free  $\text{H}_2\text{S}$  is significantly lower than 1  $\mu\text{M}$ ,<sup>19a</sup> meaning that we do not expect to generate high micromolar levels of **NBD-SH** under normal scavenging conditions.

To measure  $\text{H}_2\text{S}$  levels, we use the  $\text{H}_2\text{S}$ -responsive fluorescence probe **Cy7-NBD** (Fig. S24†).<sup>20c</sup> We used HT-29 (human colonic carcinoma cells) cells for these experiments due to recent reports that colorectal cancer cell lines exhibit increased  $\text{H}_2\text{S}$  production,<sup>11b</sup> which is likely due to the increased angiogenesis needed for cancer cell growth and proliferation.<sup>9</sup> We used non-cancerous FHC (human fetal normal colonic mucosa) cells as a negative control, which we expected to lower basal  $\text{H}_2\text{S}$  levels. To test whether **NBD-S8** could function as an efficient  $\text{H}_2\text{S}$  scavenger, we compared the fluorescence response from **Cy7-NBD** in FHC cells, HT-29 cells, and HT-29 cells treated with **NBD-S8** (Fig. 7a and S25†). We observed a significantly higher response from **Cy7-NBD** in HT-29 cells when compared to FHC cells, as expected. Upon pre-treatment of HT-29 cells with 100  $\mu\text{M}$  **NBD-S8** for 10 min, the fluorescence signals in HT-29 cells significantly decreased (Fig. 7b), which supports that **NBD-S8** can efficiently scavenge endogenous  $\text{H}_2\text{S}$  in HT-29 cells.

To further support the ability of **NBD-S8** to scavenge  $\text{H}_2\text{S}$  in live cells, we also measured  $\text{H}_2\text{S}$  levels in HeLa cells treated with

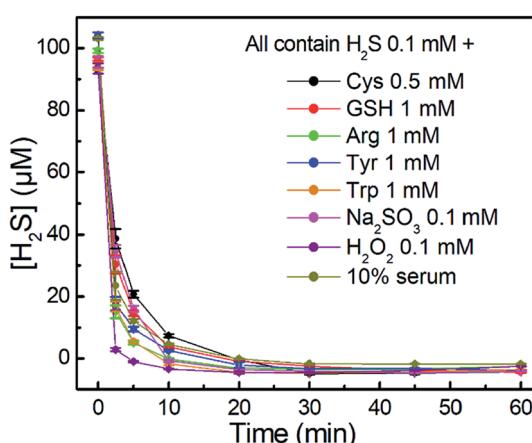


Fig. 6 Time-dependent  $\text{H}_2\text{S}$ -scavenging curves of **NBD-S8** (110  $\mu\text{M}$ ) in the presence of other species (inset) and  $\text{H}_2\text{S}$  (100  $\mu\text{M}$ ). The  $\text{H}_2\text{S}$  concentrations were measured using MBA. The results are expressed as mean  $\pm$  S.D. ( $n = 3$ ).

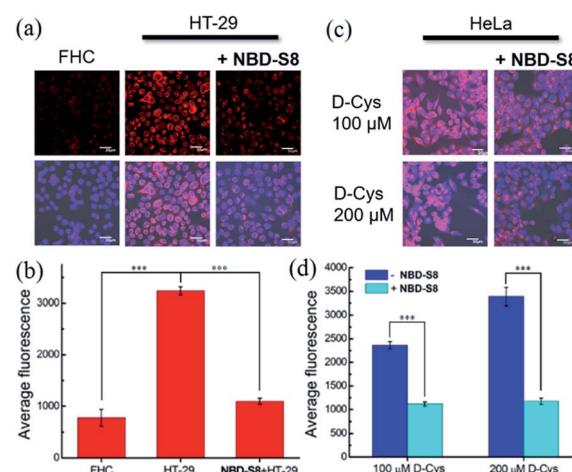


Fig. 7  $\text{H}_2\text{S}$  scavenged by **NBD-S8** in live cells. (a)  $\text{H}_2\text{S}$  levels in FHC and HT-29 cells were visualized by the fluorescence probe **Cy7-NBD** (10  $\mu\text{M}$ ), and the HT-29 cells were pre-treated with or without 100  $\mu\text{M}$  **NBD-S8**. The nuclei were stained with DAPI. Red fluorescence images (top) and overlap of blue and red fluorescence images (bottom) are shown. (b) Average fluorescence of  $N = 3$  fields of cell images in (a). (c) D-Cys-induced  $\text{H}_2\text{S}$  levels in HeLa cells were visualized by **Cy7-NBD** (10  $\mu\text{M}$ ), and the HeLa cells were treated with or without 100  $\mu\text{M}$  **NBD-S8**. Overlap of blue and red fluorescence images are shown. (d) Average fluorescence of  $N = 3$  fields of cell images in (c). All emissions were collected at blue (450–550 nm, excitation at 405 nm) and red (650–750 nm, excitation at 647 nm) channels for DAPI and **Cy7-NBD**, respectively. Error bars are means  $\pm$  S.D. \*\*\*p < 0.001. Scale bar, 30  $\mu\text{m}$ .



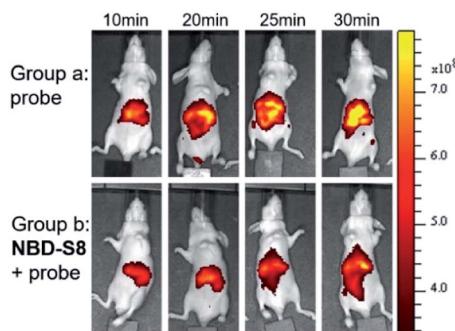


Fig. 8 Representative fluorescence images of mice which were treated with NBD-S8 and Cy7-NBD via tail vein injection. In (group a) mice were injected with Cy7-NBD (150  $\mu$ M, 200  $\mu$ L) only; whilst in (group b) mice were pretreated with NBD-S8 (100  $\mu$ M, 200  $\mu$ L) for 10 min, and then Cy7-NBD.

D-Cys (100  $\mu$ M or 200  $\mu$ M), which can increase H<sub>2</sub>S biosynthesis from the 3-MST pathway.<sup>4b,26</sup> As expected, we observed a strong red fluorescence signal from the D-Cys-treated cells in the absence of **NBD-S8** (Fig. 7c and S26†).<sup>26</sup> In contrast, co-incubation of D-Cys treated HeLa cells with 100  $\mu$ M **NBD-S8**, the cells displayed significantly lower fluorescence than that of the control cells (Fig. 7d). Taken together with the data in HT-29 cells, these data suggest that **NBD-S8** can be applied to efficiently scavenge enzymatically-produced and endogenous H<sub>2</sub>S in live cells.

Encouraged by these results, we further examined the feasibility of **NBD-S8** for H<sub>2</sub>S clearance in normal nude mice again using Cy7-NBD as an H<sub>2</sub>S reporter. Briefly, mice were intraperitoneally injected with probe only (negative control), Na<sub>2</sub>S followed by probe (positive control), or Na<sub>2</sub>S followed by **NBD-S8** for 10 min and then probe. The mice were imaged using an IVIS spectrum imaging system (PerkinElmer, USA) with a 740 nm excitation filter. As expected, the mice injected with Na<sub>2</sub>S and **NBD-S8** displayed weaker fluorescence signal than that of the mice injected with Na<sub>2</sub>S (Fig. S27†). These results implied that **NBD-S8** still functions H<sub>2</sub>S clearance *in vivo*, and its feasibility to scavenge endogenous H<sub>2</sub>S in live mice was further examined. To investigate the ability to scavenge endogenous H<sub>2</sub>S, **NBD-S8** was administered to 3 mice *via* tail vein injection. After 10 min, probe Cy7-NBD was injected to visualize the level of endogenous H<sub>2</sub>S. As a control group, another 3 mice were injected with Cy7-NBD only. As shown in Fig. 8, time-dependent fluorescence increase was detected primarily in the liver of mice (group a), which is consistent with previous results from tissue imaging.<sup>20c</sup> However, mice in (group b) exhibited comparatively lower fluorescence at each corresponding time point when compared to group a (Fig. S28†). These results preliminarily demonstrate that **NBD-S8** can scavenge endogenous H<sub>2</sub>S at the organism level.

## Conclusions

In summary, we have developed H<sub>2</sub>S scavengers based on the thiolysis of NBD amines. **NBD-S8** shows clear reaction kinetics

toward H<sub>2</sub>S with 2<sup>nd</sup> order rate constants of up to 116 M<sup>-1</sup> s<sup>-1</sup> at 37 °C and high selectivity toward H<sub>2</sub>S over other biologically relevant species. **NBD-S8** can efficiently scavenge H<sub>2</sub>S in aqueous buffer, serum, gaseous sample, and live cells. Additionally, the high selectivity and reactivity of **NBD-S8** toward H<sub>2</sub>S enables efficient H<sub>2</sub>S scavenging in live animals. Moreover, **NBD-S8** showed better stability in 5 mM GSH containing PBS buffer (pH 7.4) than that of *p*-toluenesulfonyl azide (Fig. S29†), a recently reported H<sub>2</sub>S scavenger. Considering *in vivo* biological complexity,<sup>19</sup> we propose that H<sub>2</sub>S scavengers *via* the H<sub>2</sub>S-specific thiolysis of NBD amines may represent an emerging class of efficient scavengers for investigating H<sub>2</sub>S in complex biological systems.

## Author contributions

L. Y., M. D. P., Z. X. supervised the work. I. I. synthesized the compounds; Z. C., H. Y., X. K. tested the kinetics and *in vitro* H<sub>2</sub>S scavenging; L. S., X. J., H. H. performed the bioimaging tests; I. I., H. S. solved crystal structures; S. G. B., X. J. tested the cytotoxicity; L. Y., M. D. P. wrote the manuscript; all authors involved in checking the manuscript and data.

## Ethical statement

All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Tianjin Medical University and approved by the Animal Ethics Committee of Tianjin Medical University.

## Conflicts of interest

The authors declare no competing financial interests.

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