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Controllable Cycloadditions between 2*H*-(Thio)pyran-2-(thi)ones and Strained Alkynes: A Click-and-Release Strategy for COS/H_2S Generation

Qi Cui, Tony W. Pan, Meg Shieh, Shane S. Kelly, Shi Xu, Wei-Jun Qian, and Ming Xian*

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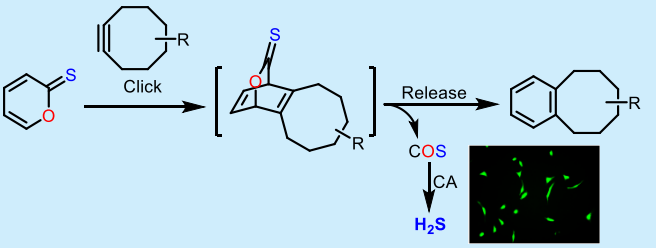
ABSTRACT: In this work, we carried out computational studies to predict the cycloaddition efficiency of strained alkynes with 2*H*-pyran-2-one and its three sulfur-containing analogues: 2*H*-pyran-2-thione, 2*H*-thiopyran-2-one, and 2*H*-thiopyran-2-thione. It was predicted that the decreased aromaticity of the substrate would yield higher reactivity. Experimental studies confirmed the calculation results, and 2*H*-pyan-2-thiones were found to be the most reactive substrates. This reaction proceeded effectively in aqueous buffers and in cellular environments. It also produced COS as the byproduct, which could be converted into hydrogen sulfide (H_2S) in the presence of carbonate anhydrase. This click-and-release approach may serve as a unique way to deliver COS/H_2S to specific locations.



Hydrogen sulfide (H_2S) is an important signaling molecule generated in mammalian cells by enzymes, including cystathionine *β*-synthase (CBS), cystathionine *γ*-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3-MST). The production of endogenous H_2S and the exogenous administration of H_2S have been demonstrated to exert protective effects in a number of pathologies such as hypertension, atherosclerosis, and coronary heart disease.[1−4] H_2S relaxes vascular smooth muscle to promote vasodilation and reduce blood pressure. Additionally, H_2S acts as a potent antioxidant and, under chronic conditions, upregulates antioxidant defenses. Therefore, chemicals that can precisely supply H_2S in biological systems are useful research tools and potential therapeutics. In the past decade, research on novel H_2S-releasing compounds (also known as H_2S donors) has received considerable attention, and many different H_2S donors have been developed (some examples are shown in Scheme 1a).[5,6] The release of H_2S from those compounds is controlled by cellular factors (such as pH, biothiols, enzymes, etc.) or biologically compatible triggers (e.g., light). These donors can be considered H_2S prodrugs. Bio-orthogonal "click"-type reactions that utilize two unnatural reaction partners to react under physiological conditions are another useful strategy in the design of prodrugs.[7] Utilizing such reactions in the design of H_2S donors would offer advantages, including a high degree of spatial control, tunable release kinetics, and zero consumption of endogenous species. However, "click" reaction-based H_2S donors are currently still very limited (Scheme 1b). In 2017, Pluth et al. reported a reaction between *trans*-cyclooctene (TCO) and tetrazine, which led to the release of COS.[8] Because COS is a known H_2S precursor in the presence of carbonate anhydrase (CA), a ubiquitous enzyme in mammalian cells, this was the first example of a "click" reaction-based H_2S donor. However, due to a rapid redox reaction between H_2S and tetrazine, this strategy cannot be used for the cellular delivery of H_2S. In 2021, Liang et al. reported another elegant reaction between 1,3-dithiolium-4-olate (DTO) and strained alkynes to release COS (and H_2S).[9] A limitation of this strategy is the synthetic complexity of DTO. Additionally, the poor solubility of DTO in aqueous solutions necessitated the use of large amounts of an organic solvent (DMSO) in the studies. As such, investigations on bio-orthogonal "click"-type reactions to release COS/H_2S are important. Herein, we report a novel click-and-release strategy to achieve this goal based on water-soluble and readily available 2*H*-pyran-2-thiones and analogues. Both COS and CS_2 can be released by this method (Scheme 1c).

2*H*-Pyran-2-one **1** is a well-known building block in organic synthesis.[10] It is known that **1** can react with alkynes through a rate-determining Diels−Alder reaction to form an unstable non-aromatic intermediate **I** via **TS1**, followed by a retro Diels−Alder reaction to provide a benzene derivative **II** and release a molecule of CO_2 as the byproduct (Scheme 2). We expected that if one of the oxygen atoms in **1** was replaced with

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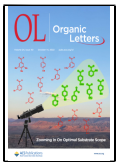
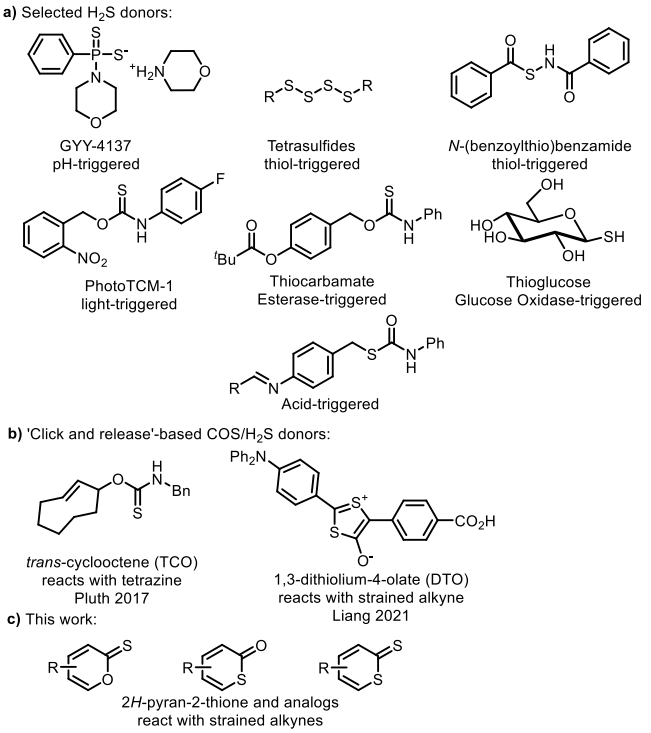
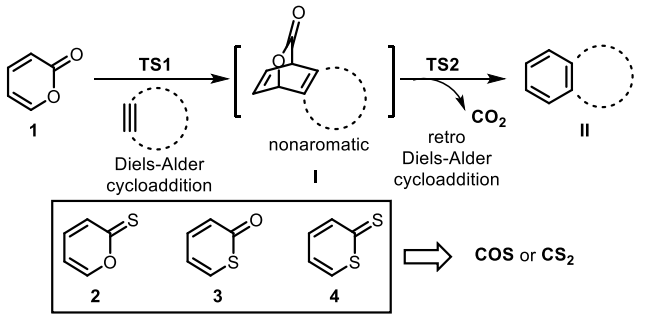


Scheme 1. Design of Novel COS/H_2S Donors



Scheme 2. Design of COS/H_2S and CS_2 Donors by a "Click-and-Release" Strategy



sulfur, the resulting compounds (e.g., 2*H*-pyran-2-thione **2** and 2*H*-thiopyran-2-one **3**) may undergo similar reactions with alkynes to release COS, thus acting as "click-and-release"-type COS/H_2S donors. Similarly, if both oxygen atoms in **1** were replaced with sulfur, the analogue (2*H*-thiopyran-2-thione **4**) would be a CS_2 donor.

In this process, the first [4+2] cycloaddition step destroys the aromaticity of the heterocyclic substrates. We thus envisioned that there would be a negative correlation between their aromaticity and reactivity, where stronger aromaticity would cause lower reactivity in the [4+2] cycloadditions. Using quantitative DFT tools (for more detailed calculation data, see the Supporting Information), the nucleus-independent chemical shift (NICS)[11] and activation Gibbs free energies ($\Delta G^{\ddagger}_{TS1}$, using bicyclo[6.1.0]nonyne BCN **5** as the alkyne component) of these four heterocycles (**1**−**4**) were calculated, allowing for a comparison between their aromaticity and reactivity (Figure 1). For thiocarbonyl compounds, 2*H*-pyran-2-thione **2** was less aromatic than its thiopyran analogue, **4** (−2.05 ppm vs −2.29 ppm), which had a lower activation Gibbs free energy (19.2 kcal/mol vs 23.6 kcal/mol). **2** was also less aromatic with an

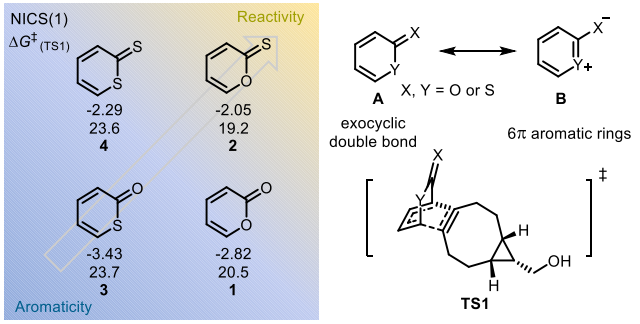


Figure 1. Theoretical studies of heterocycles **1**−**4**. Gibbs free energies of all of the intermediates and transition states computed at the SMD(water)/M06-2X/6-311G(d) level. NICS was computed at the B3LYP/6-31+G(d) level, based on the optimized structures.

activation energy lower than those of the other two carbonyl-based analogues, **1** and **3**. Therefore, 2*H*-pyran-2-thione **2** was the least aromatic compound and should exhibit the highest [4+2] cycloaddition reactivity among this group. The intrinsic reason is that O is more electronegative than S. When O is on the six-membered ring and S is on the exocyclic double bond, 6π aromatic structure **B** would be more disfavored than non-aromatic structure **A** compared to its analogues, **1**, **3**, and **4**. It should be noted that while there are no examples of **2** reacting with alkynes, some DA examples of **2** reacting with maleimide are known.[12]

We next carried out experiments to verify our predictions. Indeed, compound **2** could react with BCN **5** (in methanol) effectively to give cycloaddition product **6a** in high yield (90%) at room temperature (Table 1). With electron-withdrawing substituents (**2b** and **2c**), the reaction was even faster and could be completed within 10 min. On the contrary, electron-donating groups (**2d**) led to slower and less productive reactions. DFT calculations were also performed to analyze the substitution effects. The activation Gibbs free energies of **2b** and **2d** were 16.4 and 22.7 kcal/mol, respectively, demonstrating the reaction was an inverse electron demand Diels−Alder (iEDDA) reaction. We were curious to determine the reactivity of 2*H*-thiopyran-2-ones and prepared substrate **3b** with an electron-withdrawing group (EWG), which should be more reactive than **3** ($\Delta G^{\ddagger}$ of **3b**, 21.0). Nevertheless, the reaction of **3b** with **5** was found to be much slower than that of its thione analogue, **2b**. We also tried the reactions of 2*H*-thiopyran-2-thione **4** and its EWG-containing analogues **4b** ($\Delta G^{\ddagger}$ = 20.3), **4e**, and **4f** ($\Delta G^{\ddagger}$ = 18.4). These substrates showed improved reactivity versus that of 2*H*-thiopyran-2-one **3** but reactivity significantly lower than that of 2*H*-pyran-2-thiones **2**. These results demonstrated that sulfur-containing heterocycle substrates **2**−**4** could undergo cycloadditions with BCN, and their reactivity trend followed our calculated predictions.

Because 2*H*-pyran-2-thiones **2** were the most reactive substrates among these sulfur-containing heterocycles, we further compared the reaction kinetics of **2** and **2b** with non-sulfur substrate **1**. The rate constant (k_2) of **2** was measured by following its UV absorption changes (λ = 369 nm) and determined to be 7.35×10^{-2} M^{-1} s^{-1}, ~3.7-fold faster than that of **1** in PBS buffer (Table 2). We also attempted to measure the kinetics of **2b** in PBS buffer but found that **2b** was unstable in aqueous solutions. As such, we had to use MeOH for its kinetic measurement, and its rate constant (k_2) was 1.12×10^{-1} M^{-1} s^{-1} when the reaction was monitored by ^{1}H NMR.

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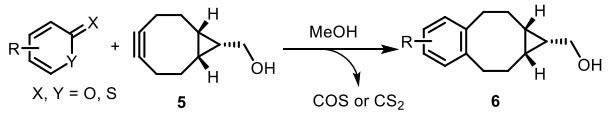
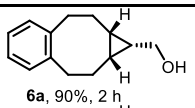
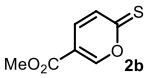
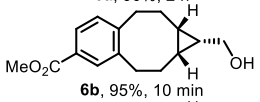
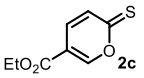
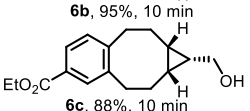
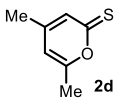
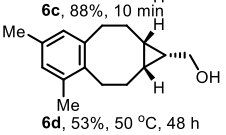
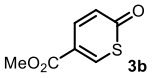
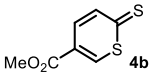
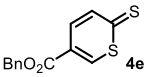
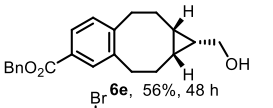
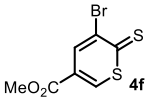
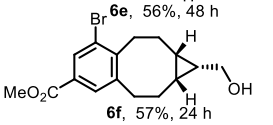
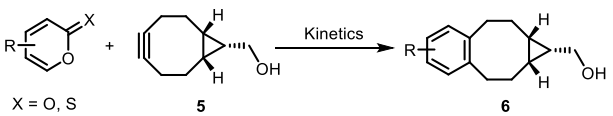
Table 1. Reaction Scope of Heterocycles with BCN 5



Entry [a]	Substrate	Product (Isolated yield)	ΔG‡(TS1)
1	 2	 **6a**, 90%, 2 h	19.2
2	 **2b**	 **6b**, 95%, 10 min	16.4
3	 **2c**	 **6c**, 88%, 10 min	
4	 **2d**	 **6d**, 53%, 50 °C, 48 h	22.7
5	 **3b**	**6b**, 24%[b], 48 h	21.0
6	 **4**	**6a**, 31%[b], 50 °C, 48 h	23.6
7	 **4b**	**6b**, 62%, 48 h	20.3
8	 **4e**	 **6e**, 56%, 48 h	
9	 **4f**	 **6f**, 57%, 24 h	18.4

[a]Heterocycles (0.12 mmol, 1.2 equiv) reacted with BCN **5** (0.1 mmol, 1.0 equiv) in MeOH (1 mL) at room temperature. [b]NMR yield, using 1,3,5-trimethoxybenzene as the internal standard.

Table 2. Kinetic Studies of 1, 2, and 2b with BCN 5



substrate	λ_{max} (nm)	k_2 (M^{-1} s^{-1})	$\Delta G^{\ddagger}_{TS1}$ (kcal/mol)
1[a]	289	1.99×10^{-2}	20.5
2[a]	369	7.35×10^{-2}	19.2
2b[b]	–	1.12×10^{-1}	16.4

[a]Substrate **1** or **2** (50 μM) and BCN **5** (1 mM) reacted in PBS buffer (pH 7.4 with 10% DMSO); the kinetics were determined by the UV–vis spectrum. [b]Substrate **2b** (7.5 mM) and BCN **5** (8.3 mM) reacted in CD_3OD; the kinetics were determined by the ^1H NMR spectrum.

The reaction between 2*H*-pyran-2-thiones and BCN should also produce COS, a CA-triggered H_2S precursor.[13] This was verified by a fluorescent probe SeSP1, which specifically detects H_2S.[14] As shown in Figure 2, only when **2**, **5**, and CA were present was strong fluorescence observed. Other combinations (e.g., **2** and CA, **5** and CA, or **2** and **5**) gave much weaker signals. These results demonstrated that 2*H*-pyran-2-thiones were cycloaddition-triggered COS/H_2S donors. We also verified CS_2 release from the reaction between

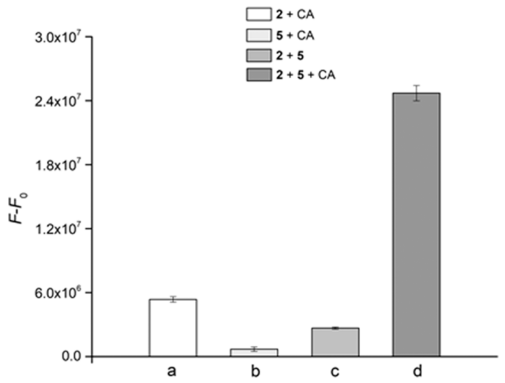


Figure 2. H_2S release detected by SeSP1. Each group was incubated in PBS buffer (10 mM, pH 7.4) at rt for 2 h. Then, SeSP1 (10 μM) and CTAB (100 μM) were added. Fluorescence responses were recorded after 30 min: (a) **2** (20 μM) and CA (50 μg/mL), (b) **5** (100 μM) and CA (50 μg/mL), (c) **2** (20 μM) and **5** (100 μM), and (d) **2** (20 μM), **5** (100 μM), and CA (50 μg/mL). The experiments were performed in triplicate, and results are expressed as means ± the standard deviation (n = 3).

2*H*-thiopyran-2-thiones and BCN by GC-MS (see the Supporting Information). However, CS_2 is a known poor substrate for CA,[15] so we did not observe obvious H_2S formation from the reaction in the presence of CA.

To investigate the compatibility of the cycloaddition of **2** in biological environments, we carried out cell imaging studies using fixed HeLa cells and monitored the formation H_2S from the reactions using SeSP1 (Figure 3). Strong green fluorescence was noted with **2**, **5**, and CA treatment (Figure 3a), while very weak fluorescence was observed in the controls [**2** and CA or **2** only (Figure 3b,c)].

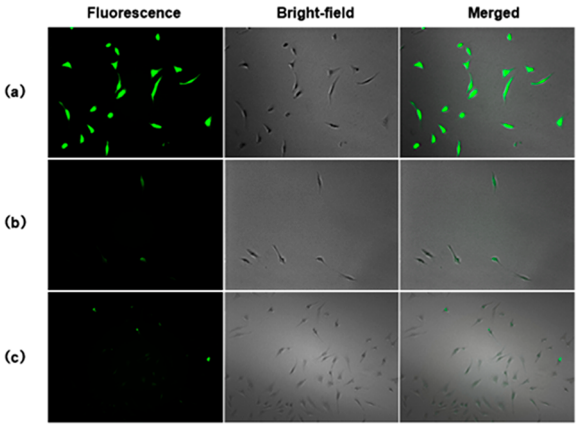


Figure 3. Fluorescence images of H_2S in fixed HeLa cells. Cells were first treated for 2 h at rt with (a) **2** (10 μM), **5** (100 μM), and CA (50 μg/mL), (b) **2** and CA, and (c) **2** only. The cells were then incubated with CTAB (100 μM) and SeSP1 (10 μM) for 30 min before images were recorded.

To further demonstrate the biocompatibility of the cycloaddition, we carried out the reaction using a protein-based model system. Briefly, lysozyme C was first treated with *p*-nitrophenol-BCN carbonate to give BCN-modified lysozyme (Lyso-BCN).[16] Then, Lyso-BCN was incubated with **2** in PBS buffer to give the click-and-release product Lyso-Prod. The protein sample was digested with trypsin and subjected to LC-MS/MS analysis. As expected, the peptide with the desired

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modification was identified (Figure 4). These results proved this "click"-like reaction has a potential application for bioconjugation.

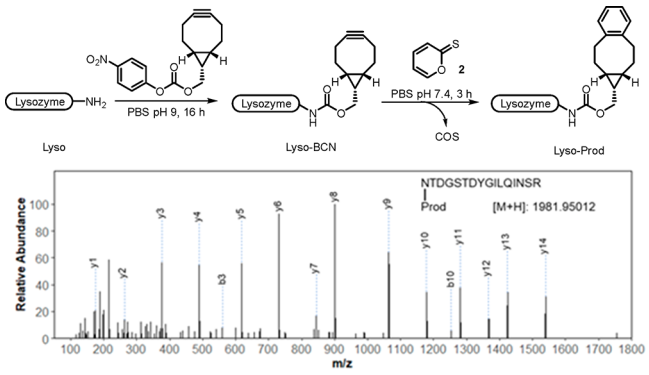

Figure 4. MS/MS spectrum of a cycloaddition product-labeled peptide (NTDGSTDYGILQINSR).

In summary, computational studies were carried out to predict the cycloaddition efficiency of BCN with 2*H*-pyran-2-one and its sulfur-containing analogues. It was predicted that the less aromatic the substrate, the more reactive it will be. This was demonstrated by experimental studies, and 2*H*-pyran-2-thiones were found to be the most reactive in this series of substrates. This reaction proceeded effectively in aqueous buffers and in cellular environments. It also produced COS as the byproduct, which could be converted into H_2S in the presence of CA. This click-and-release approach may serve as a unique way to deliver H_2S to specific locations, and this is currently under investigation in our lab.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.2c02819.

General information, syntheses of substrates, general procedure and reaction products, kinetic studies, fluorescence studies, procedures for cell imaging, protein mass spectroscopy, computational studies, references, and NMR spectra of new compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Ming Xian − *Department of Chemistry, Brown University, Providence, Rhode Island 02912, United States;* orcid.org/0000-0002-7902-2987; Phone: 401-863-3588; Email: ming_xian@brown.edu

Authors

Qi Cui − *Department of Chemistry, Brown University, Providence, Rhode Island 02912, United States*

Tony W. Pan − *Department of Chemistry, Brown University, Providence, Rhode Island 02912, United States*

Meg Shieh − *Department of Chemistry, Brown University, Providence, Rhode Island 02912, United States;* orcid.org/0000-0002-0483-5000

Shane S. Kelly − *Biological Sciences Division, Pacific Northwest National Laboratory, Richland, Washington 99352, United States*

Shi Xu − *Department of Chemistry, Brown University, Providence, Rhode Island 02912, United States;* orcid.org/0000-0002-4325-6503

Wei-Jun Qian − *Biological Sciences Division, Pacific Northwest National Laboratory, Richland, Washington 99352, United States;* orcid.org/0000-0002-5393-2827

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.orglett.2c02819

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Wang, R. Physiological implications of hydrogen sulfide: a whiff exploration that blossomed. *Physiol. Rev.* **2012**, *92*, 791−896.

(2) Wallace, J. L.; Wang, R. Hydrogen sulfide-based therapeutics: exploiting a unique but ubiquitous gasotransmitter. *Nat. Rev. Drug. Discovery* **2015**, *14*, 329−345.

(3) Paul, B. D.; Snyder, S. H. H_2S signalling through protein sulfhydration and beyond. *Nat. Rev. Mol. Cell Biol.* **2012**, *13*, 499−507.

(4) Bos, E. M.; van Goor, H.; Joles, J. A.; Whiteman, M.; Leuvenink, H. G. D. Hydrogen sulfide: physiological properties and therapeutic potential in ischaemia. *Br. J. Pharmacol.* **2015**, *172*, 1479−1493.

(5) (a) Zheng, Y.; Yu, B.; De La Cruz, L. K.; Roy Choudhury, M.; Anifowose, A.; Wang, B. Toward hydrogen sulfide based therapeutics: critical drug delivery and developability issues. *Med. Res. Rev.* **2018**, *38*, 57−100. (b) Powell, C. R.; Dillon, K. M.; Matson, J. B. A review of hydrogen sulfide (H_2S) donors: Chemistry and potential therapeutic applications. *Biochem. Pharmacol.* **2018**, *149*, 110−123. (c) Zhao, Y.; Biggs, T. D.; Xian, M. Hydrogen sulfide (H_2S) releasing agents: chemistry and biological applications. *Chem. Commun.* **2014**, *50*, 11788−11805.

(6) (a) Song, Z. L.; Zhao, L.; Ma, T.; Osama, A.; Shen, T.; He, Y.; Fang, J. Progress and perspective on hydrogen sulfide donors and their biomedical applications. *Med. Res. Rev.* **2022**, *42*, 1930−1977. (b) Ni, X.; Kelly, S. S.; Xu, S.; Xian, M. The path to controlled delivery of reactive sulfur species. *Acc. Chem. Res.* **2021**, *54*, 3968−3978. (c) Gilbert, A. K.; Zhao, Y.; Otteson, C. E.; Pluth, M. D. Development of acid-mediated H2S/COS donors that respond to a specific pH window. *J. Org. Chem.* **2019**, *84*, 14469−14475.

(7) (a) Zheng, Y.; Ji, X.; Yu, B.; Ji, K.; Gallo, D.; Csizmadia, E.; Zhu, M.; Choudhury, M. R.; De La Cruz, L. K.; Chittavong, V.; Pan, Z.; Yuan, Z.; Otterbein, L. E.; Wang, B. Enrichment-triggered prodrug activation demonstrated through mitochondria-targeted delivery of doxorubicin and carbon monoxide. *Nat. Chem.* **2018**, *10*, 787−794. (b) Ji, X.; Pan, Z.; Yu, B.; De La Cruz, L. K.; Zheng, Y.; Ke, B.; Wang, B. Click and release: bioorthogonal approaches to "on-demand" activation of prodrugs. *Chem. Soc. Rev.* **2019**, *48*, 1077−1094.

(8) Steiger, A. K.; Yang, Y.; Royzen, M.; Pluth, M. D. Bio-orthogonal "click-and-release" donation of caged carbonyl sulfide (COS) and hydrogen sulfide (H_2S). *Chem. Commun.* **2017**, *53*, 1378−1380.

(9) Chen, Y.; Zhao, R.; Tang, C.; Zhang, C.; Xu, W.; Wu, L.; Wang, Y.; Ye, D.; Liang, Y. Design and development of a bioorthogonal, visualizable and mitochondria-targeted hydrogen sulfide (H_2S) delivery system. *Angew. Chem., Int. Ed.* **2022**, *61*, e202112734.

(10) (a) Kranjc, K.; Kocevar, M. Diels−Alder reaction of highly substituted *2H*-pyran-2-ones with alkynes: reactivity and regioselectivity. *New J. Chem.* **2005**, *29*, 1027−1034. (b) Reed, J. A.; Schilling, C. L., Jr.; Tarvin, R. F.; Rettig, T. A.; Stille, J. K. Diels-Alder reactions of 2-pyrones. Direction of the addition reaction with acetylenes. *J. Org. Chem.* **1969**, *34*, 2188−2192. (c) Cole, C. J.; Fuentes, F. L.; Snyder, S. A. Asymmetric pyrone Diels−Alder reactions enabled by dienamine catalysis. *Chem. Sci.* **2020**, *11*, 2175−2180.
(11) Chen, Z.; Wannere, C. S.; Corminboeuf, C.; Puchta, R.; Schleyer, P. v. R. Nucleus-independent chemical shifts (NICS) as an aromaticity criterion. *Chem. Rev.* **2005**, *105* (105), 3842−3888.
(12) Shusherina, N. P.; Pilipendo, V. S. Reaction of 2-thiopyridones and 2-thiopyrones with N-phenylmaleimide. *Zh. Org. Khim.* **1978**, *14*, 895.
(13) (a) Levinn, C. M.; Cerda, M. M.; Pluth, M. D. Development and application of carbonyl sulfide-based donors for H_2S delivery. *Acc. Chem. Res.* **2019**, *52*, 2723−2731. (b) Steiger, A. K.; Zhao, Y.; Pluth, M. D. Emerging roles of carbonyl sulfide in chemical biology: sulfide transporter or gasotransmitter? *Antioxid. Redox Signal.* **2018**, *28*, 1516−1532.
(14) Wang, Y.; Yang, C. T.; Xu, S.; Chen, W.; Xian, M. Hydrogen sulfide mediated tandem reaction of selenenyl sulfides and its application in fluorescent probe development. *Org. Lett.* **2019**, *21*, 7573−7576.
(15) Yu, B.; Yuan, Z.; Yang, X.; Wang, B. Prodrugs of persulfides, sulfur dioxide, and carbon disulfide: important tools for studying sulfur signaling at various oxidation states. *Antioxid. Redox Signal.* **2020**, *33*, 1046−1059.
(16) Kumar, G. S.; Racioppi, S.; Zurek, E.; Lin, Q. Superfast Tetrazole−BCN Cycloaddition Reaction for Bioorthogonal Protein Labeling on Live Cells. *J. Am. Chem. Soc.* **2022**, *144*, 57−62.

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