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o,o-Difluorination of aromatic azide yields fast-response fluorescent probe for H_2S detection and for improved bioorthogonal reactions†

Jie Zhang,[a] Yasi Gao,[a] Xueying Kang,[a] Zhentao Zhu,[a] Zhiqian Wang,*[b] Zhen Xi[c] and Long Yi*[a]

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

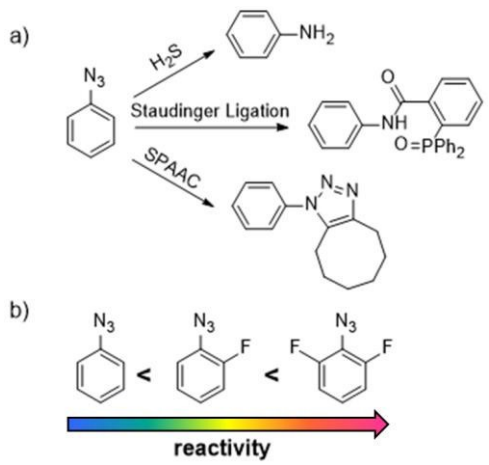
DOI: 10.1039/b000000x

Development of efficient bioorthogonal reactions for sensing of endogenous biomolecules and for bioconjugation should be paramount importance in the field of chemical biology. In this work, the *o,o'*-difluorinated aromatic azide was firstly employed to develop a new fast-response fluorescent probe 1 for H_2S detection and for bioorthogonal reactions. Compared with non- and mono-fluorinated probes, 1 showed faster reaction toward H_2S, the third gasotransmitter, in buffer (pH 7.4), implying that the reaction rate could be enhanced by the dual-fluorine groups. Furthermore, such enhanced reaction rates of 1 were also observed in Staudinger reaction and strain-promoted azide-alkyne cycloaddition (SPAAC) based on comparison studies of the non-fluorinated probe. Our results firstly highlight the *o,o'*-difluorinated aromatic azide group should be useful for fast bioorthogonal reactions and H_2S detection.

Introduction

Hydrogen sulfide (H_2S) is an important endogenous signalling molecule with multi-biological functions.[1,2] Fluorescence-based methods have recently been emerged as a highly desirable and sensitive approach for *in situ* and real-time visualization of H_2S in living biological systems because of its non-destructive sensing of bio-targets with readily available detection.[3] Azide-reduction is a commonly used strategy for the design of H_2S fluorescent probes.[3e,4] However, most of H_2S probes based on reduction of aromatic azide showed a delayed response (typically > 20 min) toward H_2S.[3e,4] Therefore, we developed *o*-fluorinated-azido-based probes for fast detection of H_2S,[5] where the electronic-drawing fluorine group on the *o*-position of the aromatic azide could help improve the reduction rate by H_2S. We hypothesized that addition of dual-fluorine groups on the aromatic azide should further enhance the reactivity of azide toward H_2S. Herein we employed the *o,o'*-difluorinated aromatic azide for development of new fluorescent H_2S probe for the first time.

On the other hand, azide is also an important group in bioorthogonal chemistry, including the famous Staudinger ligation, click reaction and copper-free click reaction (SPAAC).[6] Staudinger ligation was firstly introduced as a mild reaction for the metabolic engineering of cell surfaces by Bertozzi and co-workers in 2000.[7] Shortly after this, they reported a more attractive ligation named traceless Staudinger ligation, in which the triaryl phosphine oxide moiety is cleaved by hydrolysis and phosphorus-containing moieties are not appended to the final product.[8] Their further works on the mechanistic study of the Staudinger ligation demonstrated the overall rate of the reaction is dependent on the electronic properties of both the phosphine and the azide.[9] Electron-donating substituent on the phosphine could accelerate the overall reaction. While the *o,o'*-difluorinated aromatic azide in Staudinger ligation was not clear. Alternatively, the first example of SPAAC was also reported by Bertozzi and co-workers in 2004 when they used cyclooctyne as an active reaction partner with azide.[10] Numerous cyclooctyne derivatives displaying improved rates were developed in the following years, including difluorocyclooctyne,[11] dibenzocyclooctyne,[12] aza-dibenzocyclooctyne[13], bicyclo[6.1.0]nonyne,[14] biarylazacyclooctynone,[15] difluorobenzocyclooctyne,[16] et, al. However, little research about rate enhancement by tuning the structure of azide was carried out for the SPAAC ligation. Therefore, it should be deserved to explore the *o,o'*-difluorinated aromatic azide group in these bioorthogonal reactions.



Scheme 1. a) The azido-involved reaction types. b) The reactivity of fluorinated aromatic azides toward the reactions in a).

In this work, we rationally designed and synthesized a new fluorescence probe **1** based on the *o,o'*-difluorinated aromatic azide group as sensing moiety for proof-of-concept studies of

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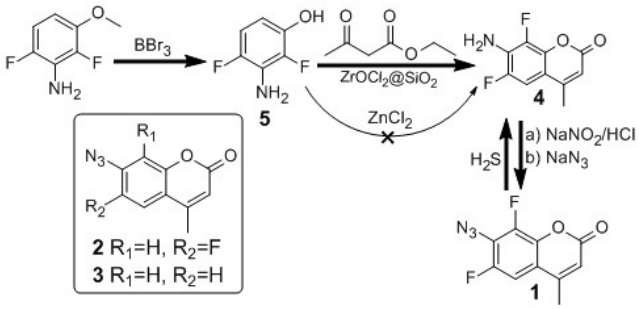
H_2S detection and bioorthogonal reactions (Scheme 1a). Comparing with the control nonfluorinated probe, **1** showed faster reaction rate in H_2S-mediated reduction, SPAAC and Staudinger reactions. For the first time, these results highlight the using of *o,o'*-difluorinated aromatic azide group in fast bioorthogonal reactions and H_2S detection.

Results and discussion

The synthesis route for probe **1** was shown in Scheme 2. Compound **5** was obtained from demethylation of commerically available 2,6-difluoro-3-methoxyaniline under BBr_3. We could not obtain aminocoumarin **4** by using transitional $ZnCl_2$ catalysis as descript in our previous paper.[5] The synthsis of **4** was achieved by using a new catalysis $ZrOCl_2/SiO_2$ under neat condition.[17] Then **4** was transformed into azidocoumarin **1** by means of a Sandmeyer reaction. The structure of probe **1** was confirmed by ^1H, ^{19}F, and ^{13}C NMR spectra and high-resolution mass spectrometry (HRMS). The non- and single-fluorinated azidocoumarins **2** and **3** were employed from our previous work.[5]



Scheme 2. Synthesis of difluorinated fluorescent probe **1** and the structure of the reference probes **2** and **3**. Yeilds: **5**, 77%; **4**, 52%; **1**, 61%.

Firstly, we investigated the absorbance spectra of **1** and its reaction with H_2S (using Na_2S as an equivalent) in PBS buffer (50 mM, pH 7.4). The absorbance of probe **1** at 327 nm exhibited a wide linear range in PBS (Fig. S1), indicating that the probe had good water-solubility up to over 20 μM. The probe **1** exhibited maximal absorbance at 325 nm, which was shift to 350 nm after treatment with H_2S (Fig. S2). We next investigated the fluorescent spectra of probe **1** and its reaction with H_2S (Fig. 1). Probe **1** (1 μM) was non-fluorescence in PBS buffer (pH 7.4). After treatment with Na_2S (100 μM), more than 160-fold fluorescent enhancement was observed at 436 nm emission, implying that the azide groups in **1** were reduced by H_2S to generate strongly turn-on fluorescence. Such fluorescence change from colorless to blue could be clearly observed by naked eye under UV lamp (Fig. 1a).

The time-dependent emission of probes **1**, **2** and **3** in the presence of H_2S could be recorded for kinetic studies. The results could be obtained through building a correlation between the emission signal and the corresponding time (Fig. 1b). The pseudo-first-order rate, k_{obs}, was found to be 20.5×10^{-4}, 9.03×10^{-4} and 4.60×10^{-4} s^{-1} for **1**, **2** and **3**, respectively, by fitting the data with single exponential function. This results revealed that the reactivity of **1** was the highest among these probes and indicated the reaction rate could be enhanced by increasing fluorine groups. The addition of a fluorine group on the *o*-position of the aromatic azide can improve about 2-fold reaction rate for H_2S-mediated reduction.

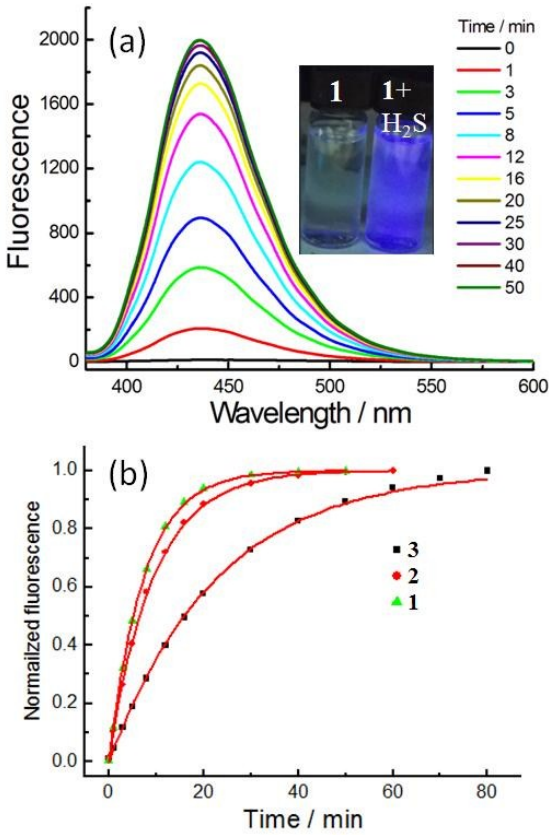


Fig. 1 Probe **1** could react with H_2S fastest among **1**-**3**. (a) Emission spectra of probe **1** (1 μM) in the presence of H_2S (100 μM). The inset photographs show fluorescence changes of **1** upon treatment with H_2S under a UV lamp (excitation = 365 nm). (b) Reaction kinetics of **1**, **2** and **3** (1 μM) toward H_2S (100 μM) in PBS (50 mM, pH 7.4) containing 10% CH_3CN at room temperature.

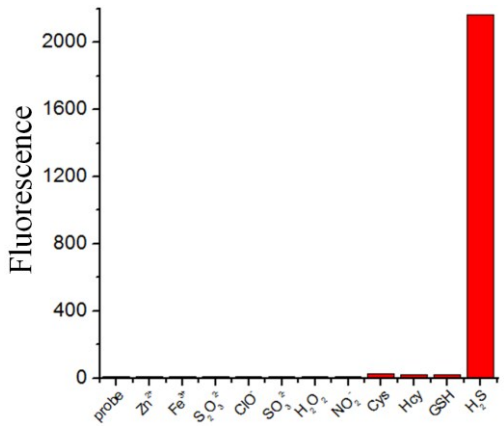


Fig. 2 Selective test of probe **1** (1 μM) to various species (Cys, Hcy, 1 mM; GSH, 5 mM; others, 100 μM) in PBS (50 mM, pH 7.4) containing 10% CH_3CN at room temperature for 60 min.

Inspiring with the above results, we further examined the

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selectivity of probe **1**, because the fast-response molecular probe should also keep good selectivity. Various biological relevant species were used to incubate with probe **1** in PBS buffer and their fluorescence response were measured seperately (Fig. 2). These biological relevant species included reactive sulfur species (glutathione (GSH, 5 mM), cysteine (Cys, 1 mM), homocysteine (Hcy, 1 mM)), reactive oxygen species, anions and cations. The fluorescence increases of the tested molecules were far lower than that of H_2S. Thus, probe **1** is highly selective toward H_2S over other biologically relevant species including millimolar biothiols. The results indicated that probe **1** maintains high selectivity with fast reaction rate toward H_2S.

Encouraged by the above results in H_2S detection, we further studied the using of difluorinated probe **1** for bioorthongal reactions in aqueous buffer (pH 7.4). As shown in Fig. 3, we firstly tested the SPAAC reaction based on **1** and **6**. The measurements were performed under pseudo first-order conditions using an excess of cycloalkyne **6**. We monitored the reaction by HPLC, HRMS, absorbance and fluorescence spectra. As shown in Fig. 3b and 3c, probe **1** could react with alkyne within 5 h, and the reaction for difluorinated probe **1** is faster than that for non-fluorinated **3**. The formation of cycloaddition **7** was identified by HRMS (see ESI). A well defined isosbestic point at 280 nm in the absorbance spectra implied the clean SPAAC reaction (Fig. S3).

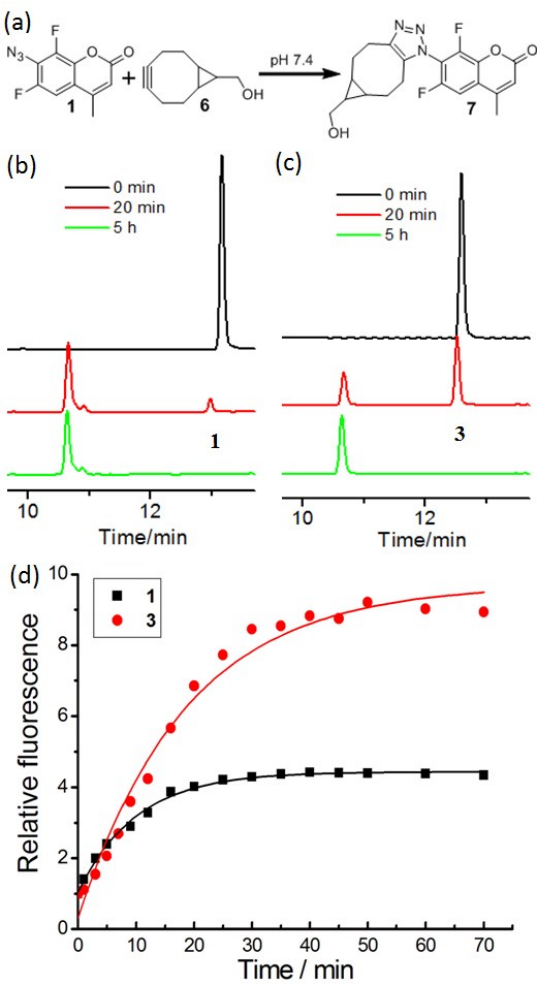

Fig. 3 (a) The reaction of probe **1** and **6** to give **7**. (b,c) HPLC analysis of the reaction of b) **1** (100 μM) or c) **3** (100 μM) and **6** (1 mM) in PBS (50 mM, pH 7.4) containing 70% CH_3CN at room temperature. (d) Reaction kinetics of **1** or **3** (10 μM) toward **6** (1 mM) in PBS (50 mM, pH 7.4) containing 50% CH_3CN at room temperature. The solid lines represent the best first-order fitting. The k_{obs} for the SPAAC of **1** and **3** were 0.10 min^{-1} and 0.053 min^{-1}, respectively.

To gain more insight into the reaction kinetics, we used fluorescence change in buffer to monitor the progress of SPAAC (Fig. 3d), since the SPAAC product was fluorescence. The pseudo-first-order rate, k_{obs} was determined by fitting the fluorescence intensity data with single exponential function. The results indicated that **1** worked more efficient (about 1.9 fold larger in k_{obs}) than that of **3**, and therefore showed fluorination of azide in enhanced SPAAC reaction. To obtain the reaction rate of **1** in SPAAC, the time-dependent fluorescence signal at 430 nm was recorded for data analysis, and the linear fitting between k_{obs} and **6** concentrations gives the reaction rate (k_2) (Fig. 4). The second order rate constant based on **1** was $k_2 = 1.69 \pm 0.02$ M^{-1} s^{-1}. This value is slightly larger than that in the reported SPAAC reactions (0.0024 to 0.96 M^{-1} s^{-1}) that was explored for protein labelling.[6g]

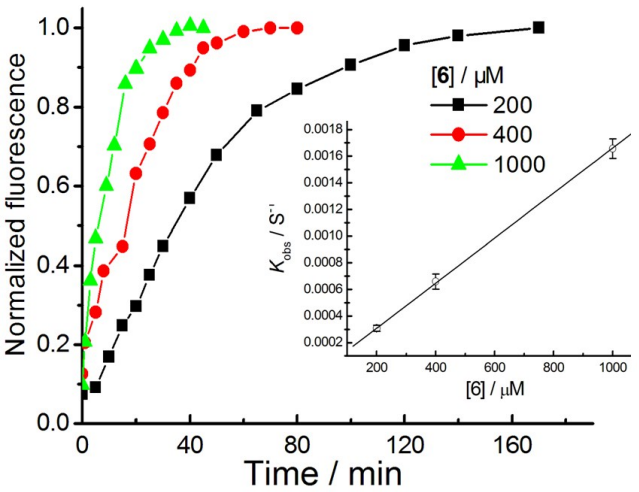

Fig. 4 The fluorescence signal of probe **1** (10 μM) at 430 nm in the presence of different concentrations of **6** in PBS (50 mM, pH 7.4) containing 50% CH_3CN at room temperature. The inset shows the linear-ship plot of the concentration of **6** versus k_{obs}. The k_2 was calculated as 1.69 ± 0.02 $M^{-1}S^{-1}$.

We further studied the using of fluorinated probe in Staudinger reaction, and found that **1** also should improved reaction performance than that of **3**. As shown in Fig. 5, the reaction of **1** and PPh_3 led to strong fluorescence enhancement (larger than 100-fold). Such significant fluorescent turn-on could be obviously observed by naked eye under 365 nm UV lamp, implying that the probe was also sensor for the bioorthongal reactions.[18] To gain insight into the reaction kinetics, the time-dependent emission at 450 nm was recorded and fitted with single exponential function. As expected, the difluorinated probe **1** showed 2.9-fold faster Staudinger reaction than that of probe **3** (Fig. 5b, S4), further supporting fluoride in accelerating the bioorthogonal reactions. It is noted that emission of the Staudinger reaction was around 450 nm, which is about 20 nm

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red-shifted comparing with the H_2S reduction. These results imply that the Staudinger reaction of **1** gave product with larger conjugate system.

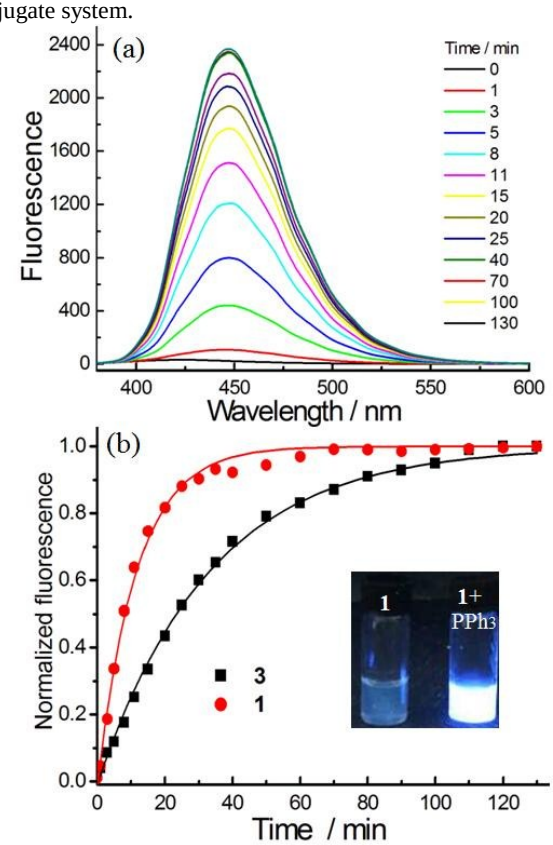

Fig. 5 a) Emission spectra of probe **1** (10 μM) in the presence of PPh_3 (200 μM). b) Reaction kinetics of **1** and **3** (10 μM) toward PPh_3 (200 μM) in PBS (50 mM, pH 7.4) containing 50% CH_3CN at room temperature. The k_{obs} for the Staudinger reaction of **1** and **3** were 0.086 min^{-1} and 0.030 min^{-1}, respectively. The inset photographs show fluorescence changes of **1** upon treatment with PPh_3 under a UV lamp (excitation = 365 nm).

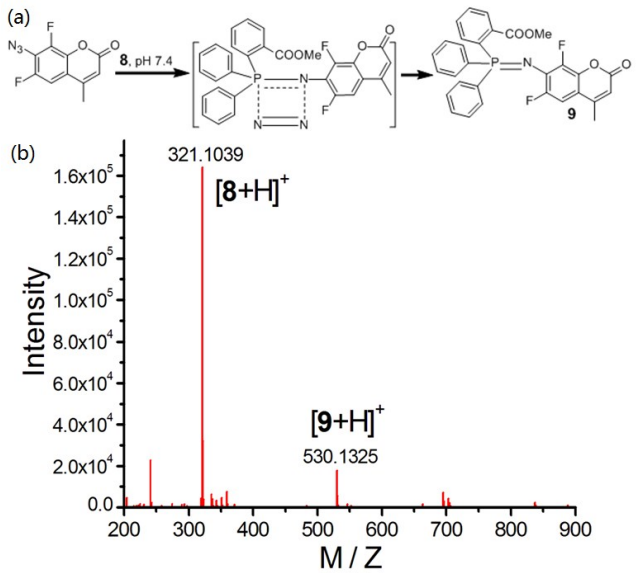

Fig. 6 a) The reaction of probe **1** and **8**. b) The HRMS spectrum of the product of probe **1** (1 mM) treated with **8** (10 mM) in PBS (50 mM, pH 7.4) containing 70% CH_3CN at room temperature for 2 days. The reaction mixture was submitted into ESI-MS without purification.

To further investigate the Staudinger reaction, we also used $PPh_2PhCOOMe$ **8**, and found that the reaction rate was similar with that of PPh_3 (Fig. S5). Such reaction was further monitored by absorbance spectra and time-dependent HRMS tests. The HRMS results indicated the quantitative formation of coupling production **9** was stable for at least 48 h in buffer, and not Staudinger-Bertozzi ligation product was observed in HRMS and spectra (Fig. 6). The time-dependent absorption spectra of the Staudinger reaction showed a new peak at 370 nm, which is larger than that of H_2S-mediated reduction and SPAAC reaction (Fig. S6). **9** had larger conjugate system than **4** and **7** to give longer wavelength in absorbance and emission.

Conclusions

In summary, a new *o,o'*-difluorinated azido-based probe **1** was rationally designed and synthesized for proof-of-concept studies of H_2S detection and bioorthogonal reactions. Comparing with non-fluorinated reference probe, **1** has four-fold faster reaction rate towrad H_2S, 1.9-fold and 2.9-fold faster ligation rate in SPAAC and Staudinger reactions, respectively. Therefore, the introduction of fluoride should be a useful strategy for faster H_2S probe and for enhanced bioorthogonal reactions. Our results firstly highlight the *o,o'*-difluorinated aromatic azide group for fast bioorthogonal reactions and H_2S detection. We propose that efficient bioorthogonal reactions based on the difluorinated and even multifluorinated aromatic azides could be explored in future for better bioconjugation.

Experimental section

General

The coumarin-based probes **2** and **3** were synthesized based on our previous work.[5] All other chemicals and solvents used for synthesis were purchased from commercial suppliers and applied directly in the experiment without further purification. The progress of the organic synthesis reaction was monitored by TLC on pre-coated silica plates (Merck 60F-254, 250 μm in thickness), and spots were visualized by UV light. Merck silica gel 60 (70-200 mesh) was used for general column chromatography purification. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 spectrometer. Chemical shifts are reported in parts per million relative to internal standard tetramethylsilane ($Si(CH_3)_4$ = 0.00 ppm) or residual solvent peaks. ^1H NMR coupling constants (*J*) are reported in Hertz (Hz), and multiplicity is indicated as the following: s (singlet), d (doublet), dd (double doublet), m (multi). High-resolution mass spectra (HRMS) were obtained on a Varian 7.0 T FTICR-MS. Analytical HPLC was performed using an Agilent HPLC system equipped with a ZORBAX SB-C18 column (5 μm, 4.6 × 150 mm) with a flow rate of 1.0 mL/min. The buffer system for the analyses was buffer A H_2O (containing 0.1% TFA) and buffer B MeOH (containing 0.1% TFA). The UV-visible spectra were recorded on a UV-3600 UV-VIS-NIR spectrophotometer (SHIMADZU, Japan). Fluorescence study was carried out using a F-280 spectrophotometer (Tianjin Gangdong Sci & Tech., Development. Co., Ltd).

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Synthesis of 5

2,6-Difluoro-3-methoxyaniline (2 g, 12.6 mmol) was dissolved in CH_2Cl_2. The solution was cooled to 0 ℃ and protected by Ar gas. Then BBr_3 was added dropwisely within 30 min. After stirred at 0 ℃ for 1 h and at room temperature for another 2.5 h, the resulting mixture was poured into ice water slowly and was extracted by CH_2Cl_2 twice. The water layer was collected, adjusted the pH to 8-9 by concentrated ammonia water and extracted by EtOAc twice. The organic layer was dried by anhydrous Na_2SO_4 and the solvent was removed under reduced pressure to give light yellow solid **5** (1.4 g, 77%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.31 (bs, 1H), 6.67-6.62 (m, 1H), 6.11-6.06 (m, 1H), 5.02 (bs, 2H).

Synthesis of 4

5 (50 mg, 0.35 mmol), ethyl acetoacetate (50 μL, 0.35 mmol) and catalyst $ZrOCl_2.8H_2O.SiO_2$ (95 mg) were mixed in 2 mL eppendorf tube. The mixture was uniformly mixed and heated to 90 ℃ in thermostat metal bath overnight. Column chromatography (petroleum ether: EtOAc = 4:1) of the crude product over silica gel gave **4** (38 mg, 52%). R_f = 0.3 (petroleum ether:EtOAc = 2:1). ^{1}H NMR (400 MHz, MeOD-d_4) δ 7.26 (dd, J = 11.3, 2.0 Hz, 1H), 6.13 (s, 1H), 2.40 (d, J = 1.1 Hz, 3H). ^{19}F NMR (376 MHz, MeOD-d_4) δ -137.75, -137.78. HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{10}H_7F_2NO_2$: 212.0518; found: 212.0521.

Synthesis of probe 1

4 (38 mg，0.18 mmol) was dissolved in 5 mL water and 10 mL concentrated HCl and cooled down to 0 ℃. Then 2 mL $NaNO_2$ (100 mg, 1.4 mmol) aqueous was slowly added to the mixture at 0 ℃. After 0.5 h reaction, 2 mL NaN_3 (100 mg, 1.5 mmol) aqueous was slowly added at 0 ℃ and stirred for another 1 h. The mixture was extracted by EtOAc for three times and the organic layer was dried by anhydrous Na_2SO_4. After removed the solvent under reduced pressure, column chromatography (petroleum ether: EtOAc = 15:1) of the crude product over silica gel gave **1** (26 mg, 61%). R_f = 0.6 (petroleum ether:EtOAc = 2:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12 (dd, J = 10.8, 2.2 Hz, 1H), 6.32 (s, 1H), 2.39 (d, J = 1.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.58, 152.56, 152.52, 151.12, 151.09, 151.06, 150.09, 150.06, 115.92, 115.67, 109.33, 109.10, 106.12, 106.08, 105.90, 105.86, 18.82. ^{19}F NMR (376 MHz, $CDCl_3$) δ -127.02, -127.27. HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{10}H_5F_2N_3O_2$: 238.0423; found: 238.0418.

Synthesis of 8

2-(diphenylphosphino)benzoic acid (612.6 mg, 2 mmol), DMAP (4-dimethylaminopyridine, 244.3 mg, 2 mmol), and EDCI (1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide, 1151.6 mg, 6 mmol) were dissolved in MeOH. The mixture was stirred for 22 h at room temperature under N_2 gas. After removed the solvent under reduced pressure, column chromatography (pure CH_2Cl_2) of the crude product over silica gel gave **8** (526 mg, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09-8.00 (m, 1H), 7.41-7.36 (m, 2H), 7.36-7.31 (m, 6H), 7.31-7.26 (m, 4H), 6.97-6.89 (m, 1H), 3.74 (s, 3H). HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{20}H_{18}O_2P$: 321.1039; found: 321.1037.

General Procedure for Spectroscopic Studies

Measurements were performed in phosphate-buffered saline buffer (PBS, 50 mM, pH 7.4, containing 10%, 50% or 70% CH_3CN). Compounds **1**, **2**, **3** and **6** were dissolved into DMSO and PPh_3 and **8** were dissolved into CH_3CN to prepare their stock solutions with a concentration of 10 mM for probes and 100 mM for the other molecules. Stock solutions of Na_2S in degassed PBS buffer were used as H_2S source. Probes were diluted in PBS buffer to afford the final concentration of 1-10 μM. For the selectivity experiment, different biologically relevant molecules (100 mM) were prepared as stock solutions in PBS buffer. Appropriate amount of biologically relevant species were added to separate portions of the probe solution and mixed thoroughly. All measurements were performed in a 3 ml corvette with 2 ml solution. The reaction mixture was shaken uniformly before emission spectra were measured. If it is not stated specially, the excitation wavelength is 350 nm and the emission 360-600 nm was recorded.

Acknowledgements

This work was supported by NSFC (21302010, 21402007, 21572019), 111 project (B14004).

Notes and references

[a] *State Key Laboratory of Organic-Inorganic Composites and Beijing Key Laboratory of Energy Environmental Catalysis, Beijing University of Chemical Technology, Beijing 100029, China. E-mail: yilong@mail.buct.edu.cn*

[b] *Public Hatching Platform for Recruited Talents, College of Science, Beijing University of Chemical Technology, China. E-mail: wangzhq@mail.buct.edu.cn*

[c] *State Key Laboratory of Elemento-Organic Chemistry and Department of Chemical Biology, National Pesticide Engineering Research Center (Tianjin), Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Nankai University, Tianjin, 300071, China.*

† Electronic Supplementary Information (ESI) available: spectra data and additional figures. See DOI: 10.1039/b000000x/

1 (a) C. Szabó, *Nat. Rev. Drug Discov.*, 2007, **6**, 917; (b) L. Li, P. Rose and P. K. Moore, *Annu. Rev. Pharmacol. Toxicol.*, 2011, **51**, 169; (c) B. Predmore, D. Lefer and G. Gojon, *Antioxid. Redox Signal.*, 2012, **17**, 119; (d) M. Whiteman and P. K. Moore, *J. Cell. Mol. Med.*, 2009, **13**, 488; (e) H. Kimura, *Exp. Physiol.*, 2011, **96**, 833; (f) L. Yi, L. Wei, R. Wang, C. Zhang, J. Zhang, T. Tan and Z. Xi, *Chem. Eur. J.*, **2015**, *21*, 15167; (g) K. Módis, Y. Ju, A. Ahmad, A. A. Untereiner, Z. Altaany, L. Wu, C. Szabó and R. Wang, *Pharmacol. Res.*, 2016, **113**, 116.

2 (a) G. D. Yang, L. Y. Wu, B. Jiang, W. Yang, J. S. Qi, K. Cao, Q. H. Meng, A. K. Mustafa, W. T. Mu, S. M. Zhang, S. H. Snyder and R. Wang, *Science*, 2008, **322**, 587; (b) L. F. Hu, M. Lu, Z. Y. Wu, P. T. Wong and J. S. Bian, *Mol. Pharmacol.*, 2009, **75**, 27; (c) K. H. Kulkarni, E. M. Monjok, R. Zeyssig, G. Kouamou, O. N. Bongmba, C. A. Opere, Y. F. Njie and S. E. Ohia, *Neurochem. Res.*, 2009, **34**, 400; (d) H. Kimura, *Amino Acids*, 2011, **41**, 113.

3 For reviews: (a) V. S. Lin, W. Chen, M. Xian and C. J. Chang, *Chem. Soc. Rev.*, 2015, **44**, 4596; (b) F. B. Yu, X. Y. Han and L. X. Chen, *Chem. Commun.*, 2014, **50**, 12234; (c) J. Li, C. Yin and F. Huo, *RSC Adv.*, 2015, **5**, 2191; (d) W. Xuan, C. Sheng, Y. Cao, W. He and W. Wang, *Angew. Chem. Int. Ed.*, 2012, **51**, 2282; (e) M. D. Hartle and M. D. Pluth, *Chem. Soc. Rev.*, 2016, **45**, 6108; (f) L. Yi and Z. Xi, *Org. Biomol. Chem.*, 2017, DOI: 10.1039/c7ob00332c.

View Article Online
DOI: 10.1039/C7OB00830A

4 (a) A. R. Lippert, E. J. New and C. J. Chang, *J. Am. Chem. Soc.*, 2011, **133**, 10078; (b) Y. F. Zhang, H. Y. Chen, D. Chen, D. Wu, X. Q. Chen, S. H. Liu and J. Yin, *Org. Biomol. Chem.*, 2015, **13**, 9760; (c) L. L. Zhang, H. K. Zhu, C. C. Zhao and X. F. Gu, *Chin. Chem. Lett,*. 2017, **28**, 218.

5 (a) C. Wei, R. Y. Wang, L. Wei, L. H. Cheng, Z. F. Li, Z. Xi and L. Yi, *Chem. Asian J.*, 2014, **9**, 3586; (b) L. Wei, Z. Zhu, Y. Li, L. Yi and Z. Xi, *Chem. Commun.*, 2015, **51**, 10463; (c) Z. T. Zhu, Y. Y. Li, C. Wei, X. Wen, Z. Xi and L. Yi, *Chem. Asian J.*, 2016, **11**, 68.

6 (a) E. M. Sletten and C. R. Bertozzi, *Angew. Chem. Int. Ed.*, 2009, **48**, 6974; (b) C. P. Ramil and Q. Lin, *Chem. Commun.*, 2013, **49**, 11007; (c) J. C. Jewett and C. R. Bertozzi, *Chem. Soc. Rev.*, 2010, **39**, 1272; (d) G. Zhang, S. Zheng, H. Liu and P. R. Chen, *Chem. Soc. Rev.*, 2015, **44**, 3405; (e) Z. Hao, S. Hong, X. Chen and P. R. Chen, *Acc. Chem. Res.*, 2011, **44**, 742; (f) D. M. Patterson, L. A. Nazarova and J. A. Prescher, *ACS Chem. Biol.*, 2014, **9**, 592; (g) C. S. McKay and M. G. Finn, *Chem. Biol.*, 2014, **21**, 1075.

7 E. Saxon and C. R. Bertozzi, *Science*, 2000, **287**, 2007.

8 E. Saxon, J. I. Armstrong and C. R. Bertozzi, *Org. Lett.*, 2000, **2**, 2141.

9 F. L. Lin, H. M. Hoyt, H. van Halbeek, R. G. Bergman and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2005, **127**, 2686.

10 N. J. Agard, J. A. Prescher and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2004, **126**, 15046.

11 J. A. Codelli, J. M. Baskin, N. J. Agard and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2008, **130**, 11486.

12 X. Ning, J. Guo, M. A. Wolfert and G. J. Boons, *Angew. Chem., Int. Ed.*, 2008, **47**, 2253.

13 M. F. Debets, S. S. van Berkel, S. Schoffelen, F. P. Rutjes, J. C. van Hest and F. L. van Delft, *Chem. Commun.*, 2010, **46**, 97.

14 J. Dommerholt, S. Schmidt, R. Temming, L. J. Hendrik, F. P. Rutjes, J. C. van Hest, D. J. Lefeber, P. Friedl and F. L. Van Delft, *Angew. Chem., Int. Ed.*, 2010, **49**, 9422.

15 J. C. Jewett, E. M. Sletten and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2010, **132**, 3688.

16 E. M. Sletten, H. Nakamura, J. C. Jewett and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2010, **132**, 11799.

17 G. R. Bardajee, F. Jafarpour and H. S. Afsari, *Cen. Eur. J. Chem.*, 2010, **8**, 370.

18 P. Shieh and C. R. Bertozzi, *Org. Biomol. Chem.*, 2014, **12**, 9307.

View Article Online
DOI: 10.1039/C7OB00830A

Graphic abstract

Based on the *o,o'*-difluorinated aromatic azide, a new fluorescent probe was developed for fast detection of H_2S and for improved copper-free click and Staudinger reactions.