RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Journal Name

COMMUNICATION

Selective and Sensitive Detection of Hydrogen Sulfide in Live Cells†

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Juan Cheng, Baihao Shao, Shuai Zhang, Yongzhou Hu and Xin Li*

We report herein a fluorescent switch-on probe suitable for the detection of hydrogen sulfide (H_2S) in complex biological context. The probe features straightforward synthesis, high sensitivity and good selectivity. As a proof of concept, its capability for detecting biological H_2S has been illustrated by imaging H_2S in live cells.

The wide distribution of hydrogen sulfide (H_2S) in mammals presages its potential biological functions. Actually, numerous researches have demonstrated that the old toxic H_2S is indeed playing pivotal roles for the controllable regulation of various intracellular signaling pathways, and the modulation of various pathological and physiological processes in human biology.[1] H_2S is primarily synthesized from cysteine (Cys), thiocysteine, 3-mercaptopyruvate or other sulfur derivatives, under the enzymatic catalysis of cystathionine β synthetase (CBS), cystathionine γ lyase (CSE), and so on.[2] As a gasotransmitter, the imbalance of its homeostasis is involved in the progress of diverse diseases such as atherosclerosis,[3] osteoarthritis,[4] Alzheimer's disease,[5] Parkinson's disease,[6] and *etc.*

Due to its elusive nature, the local concentration of H_2S in the biological sample is hard to analyze. However, precise detecting of the concentration of H_2S at the site of action will lay foundation on revealing its exact biological roles in human body as well as furthering its biomedical applications. Traditional gas-chromatography and sulfide precipitation methods are limited by their incompatibility with live samples.[7,8] Small-molecular fluorescent probes, on the other hand, have obtained significant development in the last decade attributing to their high sensitivity, good selectivity and more importantly, being applicable for live organisms.[9] In fact, numerous fluorescent probes have been devised based on specific probe-analyte reactions for the *in situ* detection of various biological molecules, among which some are even holding promise for live animal imaging.[10]

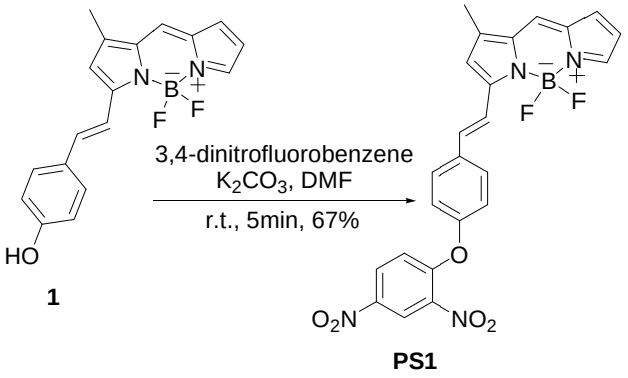
It is commonsense to chemists that H_2S is readily oxidized, favoring electrophilic reagents, and binding copper (II) with a high affinity. These chemical properties have been taken full advanta by researchers to develop reaction-based fluorescent probes for its sensitive detection in the complex biological environmen Pioneered by Chang's group who used an azide group that is reducible by H_2S to cage the fluorescence of rhodamine,[11] the azide group has been widely employed as a reaction trigger for sensing H_2S.[12] Inspired by the azide-H_2S chemistry, azo-, nitro-, or hydroxyamino-based probes were also developed.[13-15] Besides its reducibility, the nucleophilic reactivity of H_2S was also fully exploited for probe design.[16,17] Though there widely present other biothiols, the capability of H_2S being ready for two sequential nucleophilic reactions simplifies the challenge of H_2S-selective probes which can be constructed by installing two adjacent electrophilic functionalities, usually moderately reactive ones such as benzene aldehyde and Micheal receptors, to the photo-property-adjusting part of a fluorophore.[16] H_2S can also be fluorogenically detected relying on a Cu^{2+}-binded and thereby quenche fluorophore,[18] attributing to the especially low solubility of CuS. Actually, there have been several elegant review papers focusing on recent research efforts towards reaction-based fluorescent sensing of H_2S in biological samples.[19] Among the various reaction triggers responding to H_2S, the azide group, though being widely used, is sometimes challenged by the potential hazard of photoactivation due to its photosensitivity.[20] While the nucleophilicity-based two adjacent electrophiles should also be used with caution because they are also susceptible to strong oxidants such as reactive oxygen or nitrogen species widely existing in the biological context.[21] CuS precipitation-based probes, though being able to sense S^{2-} with rapid kinetics, sometimes don't show ideal cell permeability due to its ionic structure. Therefore, new probes employing proper sensing groups that are both orthogonal to the biological milieu and can react specifically and sensitively with H_2S are still highly desirable.

To devise new probes, it is primarily important to understand H_2S chemistry. H_2S, along with its reactivity of undergoing two sequential nucleophilic reactions, differs from other biothiols with its small size. These structural features render it much more nucleophilic than other biothiols. Actually, the nucleophilicity of H_2S is strong enough to thiolyze dinitrophenyl ether,[22] a formidable mission for other biothiols. Since the dinitrophenyl group is photostable and insensitive to oxidants, along with its excellent fluorescence quenching capacity, its thiolysis by H_2S therefore offers a valuable opportunity for the design of H_2S-specific fluorescent switch-on probes.

, China

Lin's group first reported a 2,4-dinitrophenyl ether-based H_2S probe in 2012 and the probe was developed by installing this functionality into the *meso* position of BODIPY (Fig. 1).[23] To bathochromic shift the emission of the probe, the π system of the fluorophore was extended by condensation of the 3-methyl group with Fisher aldehyde. Though this probe was spectroscopically silent to reactive oxygen/nitrogen species examined in their study, the electron-rich alkene attached to the indoline moiety may be susceptible to highly reactive oxidative species such as peroxynitrite, *etc*.[24] Alternative to the incorporation of fisher aldehyde, another strategy to red shift the emission of the probe is to fix the 2, 4-dinitrophenyl group directly to the 3-position of BODIPY *via* a phenol linker. It has been well documented that 3-methyl group of BODIPY is readily undergoing Knoevenagel-type reaction with electron-rich benzaldehydes to furnish red-shifted fluorophores with high yields.[25] Employing this reaction, we have successfully fused some specific sensing warheads to the 3-position of BODIPY and developed several highly sensitive fluorescent probes for the detection of H_2S or other biothiols.[26] With these considerations, we designed probe **PS1**. We envisioned that the 2, 4-dinitrophenyl group, with its strong electron-withdrawing effect, could effectively quench the emission of the BODIPY core *via* photo-induced electron transfer (PeT).[27] While nucleophilic cleavage of the ether bond by H_2S would eliminate this quenching effect and thereby restore the strong emission, signaling the distribution, even concentration of H_2S.

Probe **PS1** was facilely synthesized *via* nucleophilic substitution of 3, 4-dinitrofluorobenzene by the BODIPY fluorophore **1** (Scheme 1), and the later was prepared according to literature procedures.[28] Its structure was carefully confirmed by NMR and HRMS analysis, and purity monitored by fluorescence analysis.



Scheme 1. Structure of **PS1** and its synthesis.

With **PS1** in hand, we first tested its fluorescent response towards H_2S under pseudo biological conditions with the presence of cetyltrimethyl ammonium bromide (CTAB) as a cationic surfactant.[23] As shown in Fig. 1, **PS1** alone (5 μM) in PBS (10 mM, pH 7.4, with 5% ethanol, 37°C) was only weakly emissive. While treating the probe with NaHS (500 μM) as a H_2S source triggered a time-dependent increase of the fluorescence intensity. And the time-lapsed increase of the fluorescence intensity at emission maximum (570 nm) reached a plateau in about 10 minutes, indicating the fast kinetic of the detection reaction.

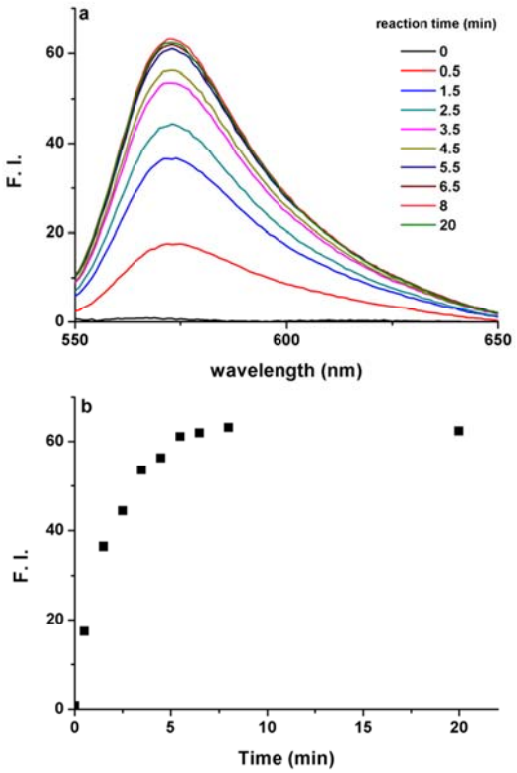


Fig. 1 The fluorescence switch-on response of **PS1** towards NaHS. a) Fluorescence spectra of **PS1** (5 μM) before and after treated with NaHS (500 μM) for various time. b) Time-dependent fluorescence intensity enhancement of **PS1** at 570 nm after treated with NaHS (500 μM). Measurements were conducted in PBS (10 mM, PH=7.4, 5% ethanol, 37°C) with CTAB (100 μM). λ_{ex}=535 nm. Slit widths: 10 nm for excitation and 5 nm for emission.

Then, we evaluated the feasibility of probe **PS1** to quantify H_2S. For this purpose, the fluorescence responses of **PS1** (5 μM) in PBS (10 mM, pH 7.4, with 5% ethanol, 37°C) towards NaHS at various concentrations were recorded (Fig. 2a, Fig. S1). Much to our delight, the logarithm of the fluorescent intensity difference between the maximum intensity and that recorded at given NaHS concentration was linear to the concentration of NaHS ranging from 0-80 μM with a correction coefficient of 0.991 (Fig. 2b), implying the potential of **PS1** to quantify H_2S. The detection limit was determined to be 500 nM (Fig. S2).

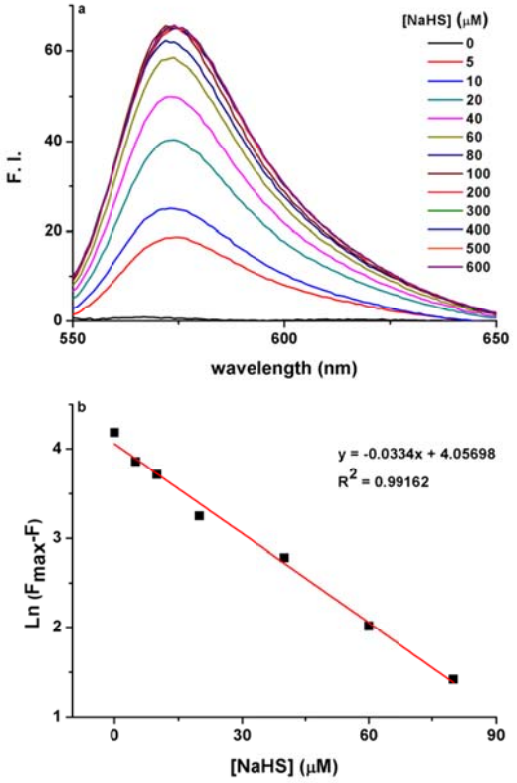


Fig. 2 Fluorescent response of **PS1** (5 μM) towards various concentrations of NaHS. a) Fluorescent spectra of **PS1** after treated with various concentrations of NaHS for 20 min. b) Plot of Ln(F_{max}-F) *versus* the concentration of NaHS where F_{max} is the maximum fluorescence intensity of **PS1** at 570 nm after treated with excessive NaHS for 20 min, and F is the intensity at the same wavelength of **PS1** in the presence of NaHS at given concentration. Data were collected in PBS (10 mM, PH=7.4, 5% ethanol, 37°C) with CTAB (100 μM) as a cationic surfactant. λ_{ex}=535 nm. Slit widths: 10 nm for excitation and 5 nm for emission.

Later on, selectivity of **PS1** was examined to determine if the fluorescent switch-on response of this probe was specific towards NaHS. Since biothiols are often regarded as the main interference for the detection of H_2S, as has been reported that the selectivity of some reduction-based H_2S probes do suffer from the interference from Cys and GSH,[29,30] we were especially concerned about the selectivity of **PS1** towards various biothiols. It turned out that among the various biothiols tested, only NaHS could trigger the fluorescent intensity enhancement of **PS1**. Moreover, to further confirm the specificity of **PS1** towards H_2S over other amino acids, cations or anions widely present in the complex biological system, its fluorescent responses towards these analytes were also studied and it turned out that none could switch on the fluorescence of **PS1**, indicating the high sensitivity of this probe towards H_2S. What is more, **PS1** could still respond to NaHS with dramatic fluorescence increase in the coexistence of other analytes, implying the potential of **PS1** to detect H_2S in the complex biological environment (Fig. 3).

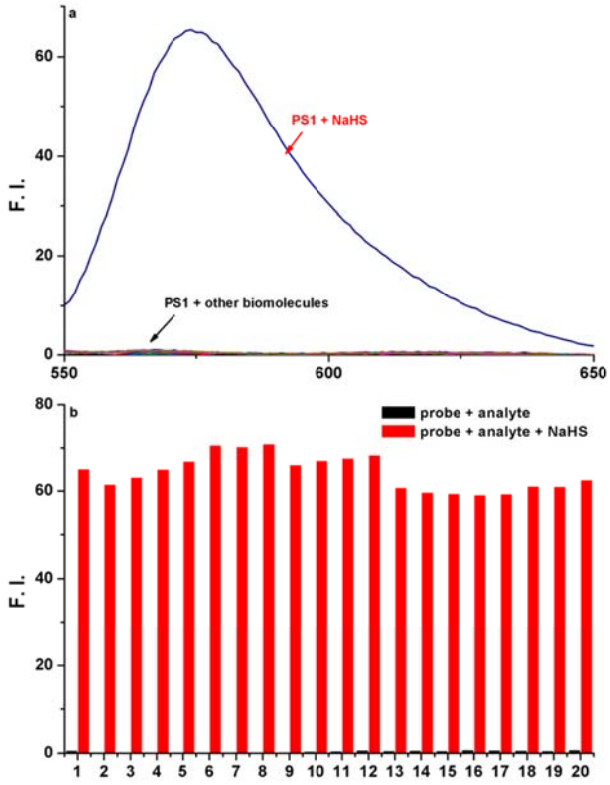


Fig. 3 PS1 is highly selective towards H_2S over other bio-relevant molecules. a) Fluorescent spectra of **PS1** (5 μM) after treated with various analyte (500 μM) for 20min. b) The fluorescent intensity of **PS1** (5 μM) at 570 nm after treated with with NaHS (1), Ala (2), Cys (3), 2-Mercaptoethanol (4), Gly (5), GSH (6), Hcy (7), Cu^{2+} (8), Mg^{2+} (9), Fe^{2+} (10), Ca^{2+} (11), Na^{+} (12), K^{+} (13), Zn^{2+} (14), Fe^{3+} (15), CN^{-} (16), F^{-} (17), SO_3^{2-} (18), Ethanethiol (19), H_2O_2 (20) for 20 min with or without the co-existence of NaHS (500 μM). Data were obtained in PBS (10 mM, PH=7.4, 5% ethanol, 37°C) with CTAB (100 μM) as a cationic surfactant. λ_{ex}=535nm. Slit widths: 10 nm for excitation and 5 nm for emission.

With the fluorescent response of **PS1** towards NaHS carefully characterized, we finally tested its feasibility to image H_2S in live cells. For this purpose, Hela cells were chosen as the model cell line. After loading the cells with **PS1** for 15 min, cells were washed with fresh PBS twice and then various concentrations of NaHS (0, 100, 200, 300 μM) were added to different wells. After one hour of further incubation, the cells were washed quickly with fresh PBS twice and imaged with fluorescence microscopy. As shown in Fig. 4, intact Hela cells showed only negligible fluorescence. While those cells treated with exogenous NaHS showed obvious fluorescence enhancement and the increase tendency was positively related to the concentration of NaHS in the culture medium. These results, on the one hand indicate the good cell-permeability of **PS1** and its capacity to image H_2S in live cells, on the other hand suggest that endogenous H_2S in Hela cells, if there is any, is expressed at levels below the detection limit of **PS1**.

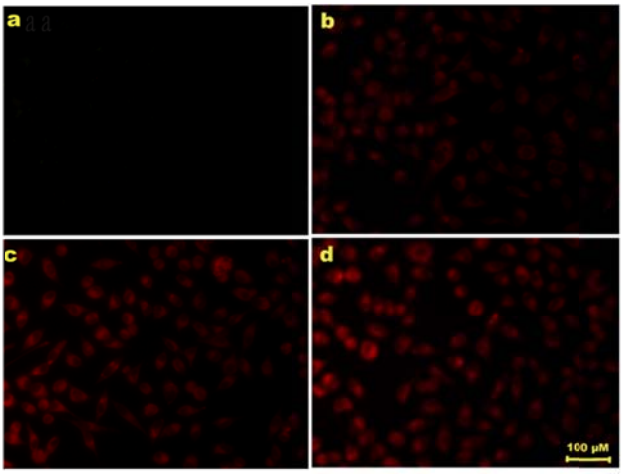


Fig. 4 Imaging H_2S in Hela cells with **PS1**. Intact Hela cells were treated with **PS1** (5 μM) for 30 mins. After twice of quick wash, cells were then treated with 0 μM (a), 100 μM (b), 200 μM (c), 300 μM (d) of NaHS. After further incubation of one hour, the cells were quickly washed with fresh PBS and observed under a LEICA DMI 4000B fluorescence microscopy equipped with a Cy3 filter set.

Conclusions

Taken together, by employing dinitrophenyl ether functionality as both a fluorescence quencher and a H_2S-reaction trigger, we have developed a probe for the fluorescent switch-on detection of H_2S. The probe features fast response and good selectivity towards H_2S. It is also easily available *via* one-step synthesis. Its capability to image H_2S in live cells has been demonstrated.

Acknowledgments

This work was supported by Zhejaing Provincial Natural Science Foundation of China (LY15H300003).

Notes and references

1 (a) H. Kimura, N. Shibuya, Y. Kimura, *Antioxid. Redox Signal.* 2012, **17**, 45. (b) H. Kimura, *Antioxid. Redox Signal.* 2015, **22**, 362.
2 H. Kimura, *Antioxid. Redox Signal.* 2014, **20**, 783.
3 S. H. Cheung, W. K. Kwok, K. F. To, J. Y. Lau, *PloS one* 2014, **9**, e113038.
4 D. Sieghart, M. Liszt, A. Wanivenhaus, H. Broll, H. Kiener, B. Klosch, G. Steiner, *J. Cell Mol. Med.* 2015, **19**, 187.
5 D. Giuliani, A. Ottani, D. Zaffe, M. Galantucci, F. Strinati, R. Lodi, S. Guarini, *Neurobiol. Learn Mem.* 2013, **104**, 82.
6 M. Wang, J. Zhu, Y. Pan, J. Dong, L. Zhang, X. Zhang, L. Zhang, *J. Neurosci. Res.* 2015, **93**, 487.
7 C. J. Richardson, E. A. Magee, J. H. Cummings, *Clin. Chim. Acta.* 2000, **293**, 115.
8 M. Ishigami, K. Hiraki, K. Umemura, Y. Ogasawara, K. Ishii, H. Kimura, *Antioxid. Redox Signal.* 2009, **11**, 205.
9 (a) A. Nadler, C. Schultz, *Angew. Chem. Int. Ed. Engl.* 2013, **52**, 2408. (b) T. Ueno, T. Nagano, *Nat. Methods* 2011, **8**, 642.
10 (a) H. Kobayashi, M. Ogawa, R. Alford, P. L. Choyke, Y. Urano, *Chem. Rev.* 2010, **110**, 2620. (b) M. Schäferling, *Angew. Chem. Int. Ed. Engl.* 2012, **51**, 3532. (c) Z. Guo, S. Park, J. Yoon, I. Shin, *Chem. Soc. Rev.* 2014, **43**, 16.
11 A. R. Lippert, E. J. New, C. J. Chang, *J. Am. Chem. Soc.* 2011, **133**, 10078.
12 (a) K. Wang, H. Peng, N. Ni, C. Dai, B. Wang, *J. Fluoresc.* 2014, **24**, 1. (b) B. Chen, W. Li, C. Lv, M. Zhao, H. Jin, H. Jin, J. Du, L. Zhang, X. Tang, *Analyst* 2013, **138**, 946. (c) Q. Qiu, X. Deng, L. Jiao, T. Zhao, F. Meng, W. Huang, H. Qian, *Chem. Biol. Drug Des.* 2014, doi: 10.1111/cbdd.12483. (d) W. Li, W. Sun, X. Yu, L. Du, M. Li, *J. Fluoresc.* 2013, **23**, 181. (e) T. Saha, D. Kand, P. Talukdar, *Org. Biomol. Chem.* 2013, **11**, 8166. (f) T. Ozdemir, F. Sozmen, S. Mamur, T. Tekinay, E. U. Akkaya, *Chem. Commun.* 2014, **50**, 5455. (g) K. Zheng, Lin, W. L. Tan, *Org. Biomol. Chem.* 2012, **10**, 9683. (h) F. J. Huo, J. Kang, C. Yin, J. Chao, Y. Zhang, *Sci. Rep.* 2015, 5, 8969. (I) M. D. Hammers, M. J. Taormina, M. M. Cerda, L. A. Montoya, D. T. Seidenkranz, R. Parthasarathy, M. D. Pluth, *J. Am. Chem. Soc.* 2015, DOI: 10.1021/jacs.5b04196
13 X. Li, J. Cheng, Y. Gong, B. Yang, Y. Hu, *Biosens. Bioelectron.* 2015, **65**, 302.
14 (a) C. Wei, Q. Zhu, W. Liu, W. Chen, Z. Xi, L. Yi, *Org. Biomol. Chem.*, 2014, **12**, 479. (b) L. Zhang, S. Li, M. Hong, Y. Xu, S. Wang, Y. Liu, Y. Qian, J. Zhao, *Org. Biomol. Chem.* 2014, **12,** 5115. (c) R. Wang, F. Yu, L. Chen, H. Chen, L. Wang, W. Zhang, *Chem. Commun.* 2012, **48**, 11757.
15 W. Xuan, R. Pan, Y. Cao, K. Liu, W. Wang, *Chem. Commun.* 2012, **48**, 10669.
16 (a) Y. Qian, J. Karpus, O. Kabil, S. Y. Zhang, H. L. Zhu, R. Banerjee, J. Zhao, C. He, *Nature Comm.* 2011, **2**, 495. (b) Y. Qian, L. Zhang, S. Ding, X. Deng, C. He, X. E. Zheng, H. Zhu, J. Zhao, *Chem. Sci.* 2012, **3**, 2920. (c) X. Wang, J. Sun, W. Zhang, X. Ma, J. Lv, B. Tang, *Chem. Sci.* 2013, **4**, 2551. (d) C. Liu, J. Pan, S. Li, Y. Zhao, L. Y. Wu, C. E. Berkman, A. R. Whorton, M. Xian, *Angew. Chem. Int. Ed. Engl.* 2011, **50**, 10327.
17 (a) X. F. Yang, L. Wang, H. Xu, M. Zhao, *Anal. Chim. Acta* 2009, **631**, 91. (b) Y. Yang, C. Yin, F. Huo, Y. Zhang, J. Chao, *Sensor. Actuat. B-Chem.* 2014, 203, 596. (c) L. Fu, F. F. Tian, L. Lai, Y. Liu, P. D. Harvey, F. L. Jiang, *Sensor. Actuat. B-Chem.* 2014, **193**, 701.
18 (a) K. Sasakura, K. Hanaoka, N. Shibuya, Y. Mikami, Y. Kimura, T. Komatsu, T. Ueno, T. Terai, H. Kimura, T. Nagano, *J. Am. Chem. Soc.* 2011, **133**, 18003. (b) X. Li, Y. Gong, K. Wu. S. H. Liang, J. Cao, B. Yang, Y. Hu, Y. Han, *RSC Adv.* 2014, **4**, 36106. (c) X. Li, C. Yang, K. Wu, Y. Hu, Y. Han, S. H. Liang, *Theranostics* 2014, **4**, 1233.
19 (a) V. S. Lin, W. Chen, M. Xian, C. J. Chang, *Chem. Soc. Rev.* 2015, **44**, 4596. (b) F. Yu, X. Han, L. Chen, *Chem. Commun.* 2014, **50**, 12234. (c) J. Li, C. Yin, F. Huo, *RSC Adv.* 2015, 5, 2191.
20 T. S. Bailey, M. D. Pluth, *J. Am. Chem. Soc.* 2013, **135**, 16697.
21 A. Bindoli, M. P. Rigobello, *Antioxid. Redox Signal.* 2013, **18**, 1557.
22 R. L. Letsinger, J. Fontaine, V. Malhadevan, D. A. Schexnayder, R. E. Leone, *J. Org. Chem.* 1964, **29**, 2615.
23 X. Cao, W. Lin, K. Zheng, L. He, *Chem. Comm.* 2012, **48**, 10529.
24 D. Oushiki, H. Kojima, T. Terai, M. Arita, K. Hanaoka, Y. Urano, T. Nagano, *J. Am. Chem. Soc.* 2010, **132**, 2795.
25 Z. Dost, S. Atilgan, E. U. Akkaya, *Tetrahedron* 2006, **62**, 8484.
26 (a) X. Li, S. Qian, Q. He, B. Yang, J. Li, Y. Hu, *Org. Biomol. Chem.* 2010, **8**, 3627. (b) X. Li, S. Zhang, J. Cao, N. Xie, T. Liu, B. Yang, Q. He, Y. Hu, *Chem. Commun.* 2013, **49**, 8656.
27 J. R. Lakowicz, *Principles of Fluorescence Spectroscopy (Third Edition)*, Springer: New York, 2006.
28 J. S. Lee, N. Y. Kang, Y. K. Kim, A. Samanta, S. Feng, H. K. Kim, M. Vendrell, J. H. Park, Y. T. Chang, *J. Am. Chem. Soc.* 2009, **131**, 10077.
29 W. Xuan, R. Pan, Y. Cao, K. Liu, W. Wang, *Chem. Comm.* 2012, **48**, 10669.
30 C. Wei, Q. Zhu, W. Liu, W. Chen, Z. Xi, L. Yi, *Org. Biomol. Chem.* 2014, **12**, 479.