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ARTICLE

A Probe for ratiometric near-infrared fluorescence and colorimetric hydrogen sulfide detection and imaging in live cells

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A ratiometric, near-infrared (NIR), fluorescence and colorimetric probe **DNPOCy** for hydrogen sulfide (H₂S) has been developed. The chemical basis for the operation of the probe is thiolysis of a dinitrophenyl ether, which liberates a cyanine dye chromophore. The probe exhibits a rapid response and high sensitivity to H₂S in pure aqueous media, in the near infrared optical window. **DNPOCy** is highly selective for H₂S over other biologically relevant species including biothiols. This probe can be conveniently used for monitoring H₂S without the interference from pH dependent effects of physiological media. The practical utility of probe was demonstrated by its application to the detection of H₂S in live cells.

Introduction

Along with nitric oxide and carbon monoxide, hydrogen sulfide (H₂S) is an important endogenous gasotransmitter.¹ Although being toxic, H₂S at very low concentrations plays an essential role in many biological processes. Three enzymes, namely cystathionine-β-synthase, cystathionine-γ-lyase, and 3-mercaptopyruvate sulfur transferase, catalyze the production of H₂S from cysteine or its derivatives in various organs and tissues.^{1,2} In humans, altered levels of H₂S have been correlated with Down syndrome, Alzheimer's and chronic kidney disease, liver cirrhosis, and diabetes.³⁻⁷ Therefore, it is crucial to understand the chemistry and to be able to monitor concentrations of H₂S in biological systems.

In recent years, several fluorescence probes have been devised for *in vitro* as well as *in vivo* detection of H₂S.⁸⁻¹⁸ Although functional, these probes suffer from several important drawbacks. For example, most of the reported probes detect only high concentrations of H₂S. However, the concentrations of this analyte in blood is in the 10–100 μM range and even lower (submicromolar range) in living cells. Furthermore, nearly all of the current probes detect H₂S in fluorescence off-on manner, which is not ideal for quantitative analysis as compared to those that use ratiometric based approaches. In addition, some of the reported probes detect H₂S only in aqueous-organic mixtures, and many are plagued by serious

limitations associated with high energy absorption and emission, and long response times (20 min to 2 h).

NIR dyes have a highly unique advantage in tracking molecular events *in vivo* because light in this wavelength region more deeply penetrates into tissues than does that in the UV and visible regions. Therefore, a need exists for highly selective probes that are able to detect low concentrations of H₂S in a purely aqueous medium and that utilize ratiometric fluorescence in the NIR wavelength region.

In earlier efforts, we and others designed cyanine based probes in which a latent electron donating phenolate moiety is conjugated with two electron acceptor quaternary ammonium moieties.¹⁹⁻²⁴ In our previous report, **DNBSCy** was designed to react with thiol, by protecting the phenolate donor moiety in the probe with a trigger group (2,4-dinitrobenzenesulfonyl: DNBS).²⁰ Thiols such as glutathione (GSH) promoted the removal of trigger group to generate **Cy-quinone** chromophore in which donor (phenolate) and two acceptors (quaternary ammonium groups) form a conjugated π-system backbone that is isoelectronic with the well-known cyanine dyes such as Cy7.

A similar approach has been employed in the current investigation to construct a H₂S selective NIR probe. Although we realized that selection of a trigger group that would be selective for H₂S in the presence of other biological thiols would not be straightforward, we envisaged that the lower pK_a (6.8) and smaller size of H₂S would make it more a more potent

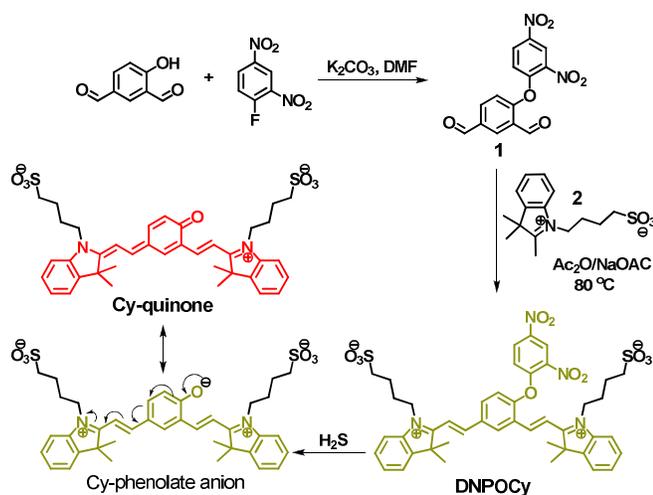
nucleophile than thiols such as cysteine (Cys) and GSH. This nucleophilicity difference in conjunction with thiolysis of dinitrophenyl (DNP) ethers has been used advantageously in the past to design probes for H₂S.²⁵⁻³⁰

Result and discussion

We prepared the reaction-based, masked heptamethine cyanine type probe **DNPOCy** (Scheme 1), which contains a DNP protected phenol as a H₂S responsive trigger (Scheme 1). The latent phenolate moiety in **DNPOCy** is conjugated with two quaternary ammonium groups, which together form a cyanine type probe. We anticipated that HS⁻ species would participate in a nucleophilic aromatic substitution reaction with **DNPOCy** leading to the generation of phenolate ion. This anion will reversibly form the NIR emitting **Cy-quinone** dye (Scheme 1).

DNPOCy was synthesized by using the straightforward two-step route outlined in Scheme 1, involving a key aromatic substitution reaction of 1-fluoro-2,4-dinitrobenzene with 4-hydroxy-1,3-benzenedicarboxaldehyde under basic conditions to install the 2,4-dinitrophenyl phenyl ether moiety in **1**. **DNPOCy** was then produced through condensation of the bisaldehyde functionalized ether **1** with 2 equivalents of indolium-3-butylsulfonate (**2**). All compounds in this synthetic route were characterized by using NMR spectroscopy, mass spectrometry and elemental analysis.

The UV-visible spectrum of **DNPOCy** in PBS buffer (pH = 7.4) contains an absorption band located at *ca.* 400 nm with an extinction coefficient (ϵ) of $3.9 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ (Fig. S1, ESI†). The high energy of this transition is a consequence of the reduced π -electron conjugation caused by the presence of the protected phenolate group. As predicted, the absorption spectrum undergoes a dramatic change when **DNPOCy** is incubated with 20 μM of NaSH (common source of H₂S).



Scheme 1 Synthesis of **DNPOCy** and its reaction with H₂S. **DNPOCy**: greenish-yellow fluorescence ($\lambda_{em} = 555 \text{ nm}$); **Cy-Quinone**: NIR red fluorescence ($\lambda_{em} = 695 \text{ nm}$). $\lambda_{ex} = 510 \text{ nm}$.

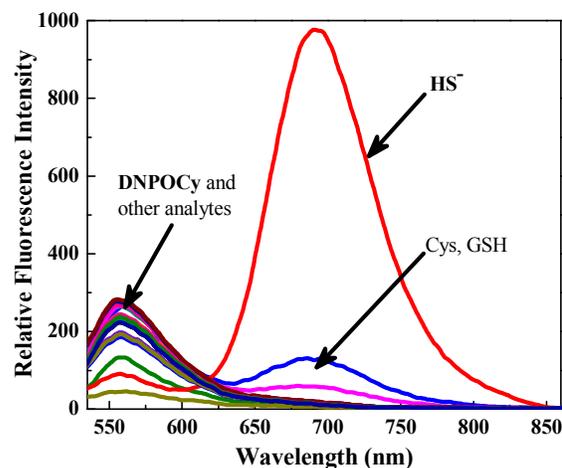


Fig. 1 Fluorescence responses ($\lambda_{ex} = 510 \text{ nm}$) of **DNPOCy** (10 μM , in PBS buffer, pH = 7.4) 2 min following mixing with different analytes.

The absorbance at 400 nm decreases while two new absorption bands appear at 475 nm ($\epsilon = 3.8 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$) and 583 nm ($\epsilon = 4.3 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$), which exactly match those in the spectrum of **Cy-quinone**. Concurrently, the color of the solution changes from greenish yellow to blue. These results indicate that **DNPOCy** serves as a “naked-eye” probe for H₂S. Concentration dependent titration study showed that in the presence of 10 μM **DNPOCy**, a decrease in absorbance at 400 nm and an increase in absorbance at 475 and 583 nm take place simultaneously and reach respective minimum and maximum values at a NaSH concentration of 20.0 μM (Fig. S2, ESI†).

The results of fluorescence spectroscopy studies show that **DNPOCy** in PBS buffer (pH = 7.4) emits with a maximum at *ca.* 555 nm upon excitation at 510 nm (Fig. 1). Addition of 20 μM NaSH to a solution of **DNPOCy** in this buffer causes a simultaneous decrease in the intensity of the fluorescence band at 555 nm and an increase in an emission band at *ca.* 695 nm. This remarkably large change (*ca.* 140 nm) is a highly desirable feature of a ratiometric type fluorescence probe because it assists in increasing the signal-to-noise ratio. Indeed, the ratio of fluorescence intensities at 695 and 555 nm (I_{695}/I_{555}) was observed to increase linearly with increasing HS⁻ concentrations in the range of 0-30 μM (Fig. 2). The results of this varying concentration investigation reveal that minimum 1 μM concentration of H₂S can be readily detected using the new probe.

It is significant that similar color and fluorescence changes do not occur when 1 mM concentrations of various anions (Cl⁻, Br⁻, I⁻, AcO⁻, N₃⁻, CN⁻, CO₃²⁻, NO₂⁻) and metal cations (K⁺, Mg²⁺, Ca²⁺, Zn²⁺), and 5 equivalents of the reactive oxygen species H₂O₂, OCl⁻ and reducing agents ascorbic acid, S₂O₃²⁻, SO₃²⁻ are added to solutions of **DNPOCy** in PBS buffer (Fig. 1 and S1, ESI†). Moreover, addition of 1 mM GSH or Cys to **DNPOCy** promotes only minimal color and fluorescence

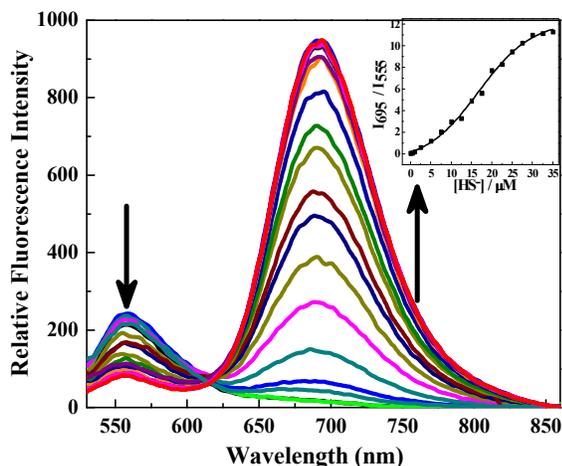


Fig. 2 Ratiometric fluorescence responses ($\lambda_{\text{ex}} = 510 \text{ nm}$) of **DNPOCy** ($10 \mu\text{M}$, in PBS buffer, $\text{pH} = 7.4$) to increasing concentrations of HS^- from 0 to $30 \mu\text{M}$ after 2 min of mixing.

changes. Importantly, solutions containing **DNPOCy** along with either GSH or Cys undergo significant ratiometric responses upon addition of NaSH (Fig. S3, ESI[†]). The combined results demonstrate that **DNPOCy** displays a high selectivity for H_2S over other biologically relevant species including biothiols and that its response to NaSH is not interfered with by other thiols. Because **DNPOCy** reacts efficiently with H_2S in the biologically relevant pH range of 6.5–8.5 to form the NIR emitting **Cy-quinone** dye (Fig. S4, ESI[†]), this probe is very convenient for monitoring H_2S without interference from the pH dependent effects of physiological media.

The HS^- induced transformation of **DNPOCy** to **Cy-quinone** was confirmed by using mass spectrometry (Fig. S6, ESI[†]). Analysis of the mass spectrum of the product mixture shows that peaks at $m/z = 705$ ($\text{C}_{38}\text{H}_{43}\text{N}_2\text{O}_7\text{S}_2 + \text{H}^+$) corresponding to **Cy-quinone** and at $m/z = 224.0$ ($\text{C}_6\text{H}_4\text{N}_2\text{O}_4\text{S} + \text{Na}^+ + \text{H}^+$) corresponding to the 2,4-dinitrothiophenol are generated when HS^- is added to **DNPOCy**.

Theoretical study

A theoretical study was carried out for the microscopic understanding of the expected excitation and emission spectra as described above. In our calculation the structure of **DNPOCy** and **Cy-quinone** were simplified with the model compound **A** and **B** in which alkylsulfonate side chains were replaced by methyl groups. Optimized ground state structures of **A** and **B** (Fig. 3) were determined using ab initio density functional theory (DFT) calculations and their excitation and emission properties were calculated using time-dependent density functional theory (TDDFT) as implemented in Gaussian 09 package. (for reference see the ESI[†]) For DFT and TDDFT calculations, a B3LYP³¹⁻³³ exchange and correlation functional with 6-31g(d) basis set was used. By using optimized ground

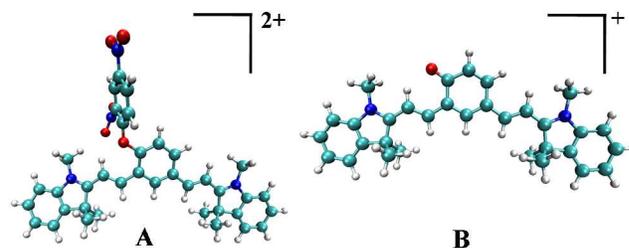


Fig. 3 The optimized structures of the model compounds of **DNPOCy** (**A**) and **Cy-quinone** (**B**).

state (S_0) geometries and TDDFT calculations, the excitation energies and oscillator strengths corresponding to lowest singlet excited states of **A** and **B** states were determined (Table 1). Maxima in the emission spectrum of **A**, calculated using optimized first excited state (S_1) geometries and TDDFT calculations, were found to be 458 nm and 503 nm, respectively. In addition, the excitation and emission maxima for **B** were determined to be the same as those reported earlier.²¹ In agreement with the experimental results, **A** has higher energy absorption and emission transitions than does **B** owing to a larger HOMO-LUMO gap in the former (2.96 eV) compared to the latter (2.30 eV) (Fig. S10, ESI[†]). Importantly, the results of calculations with **A** and **B** match the experimental finding in that they show that **DNPOCy** should emit in the visible region while **Cy-quinone** should fluoresce in the NIR region.

Table 1 Calculated excitation/emission transitions and corresponding oscillator strengths for **DNPOCy** (**A**) and **Cy-quinone** (**B**)

Compound	Excitation Energy (nm) $S_0 \rightarrow S_1$	Oscillator Strength (excitation)	Emission Energy (nm) $S_1 \rightarrow S_0$	Oscillator Strength (emission)
A	458.55	0.71	503.72	1.53
B	562.81	1.53	629.08	1.02

Live cell imaging

Finally, the use of **DNPOCy** in conjunction with fluorescence microscopy to track H_2S ratiometrically inside living cells was explored (Fig. 4). For this purpose, HEK293T cells were incubated with $10 \mu\text{M}$ of **DNPOCy**. The cytoplasm of the cells was observed to display green fluorescence. However, treatment of the **DNPOCy** loaded cells with HS^- ($20 \mu\text{M}$) followed by incubation for 30 min resulted in a change in color of the light emitted from the cytoplasm from green to red. These results indicate that **DNPOCy** is cell membrane permeable and that it can be employed as a ratiometric fluorescence imaging agent for the detection of H_2S in live cells.

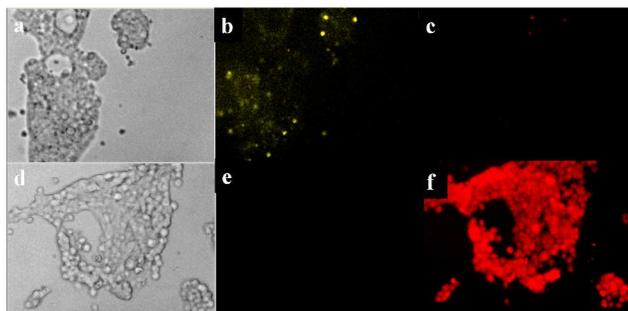


Fig. 4 Top panel represents the HEK293T cells pre-incubated with **DNPOCy** (10 μM) for 2 h and the bottom panel represents the same after treatment of **DNPOCy** internalized cells with NaSH (20 μM) for 30 min. (a) and (d) The bright field images, (b), (c) and (e), (f) images are captured in the yellow/CFP/540 nm and red/RFP/630 nm filter respectively.

Conclusion

The investigation described above has led to the development of a highly sensitive ratiometric NIR fluorescence and colorimetric probe, **DNPOCy**, for H_2S . **DNPOCy** reacts rapidly with H_2S in an aqueous medium to release the blue cyanine dye, **Cy-quinone**, which displays NIR emission. The results of theoretical calculations corroborate the experimentally observed changes in the emission properties associated with the H_2S promoted reaction. Furthermore, this effort demonstrates that **DNPOCy** serves as a non-invasive probe for ratiometric fluorescence imaging of H_2S , in living cells. These findings should motivate the development of new ratiometric NIR fluorescence probes for other biologically important small molecules.

Experimental section

Materials and Instruments.

All the solvents and reagents were obtained from Sigma-Aldrich and used as received unless otherwise mentioned. ^1H and ^{13}C NMR were recorded on a Bruker AV-400 spectrometer with chemical shifts reported as ppm (in DMSO-d_6 , tetramethylsilane as internal standard). Mass spectra were obtained on Shimadzu 2020 LC-MS. Elemental analysis was carried out on Thermo Scientific FLASH 2000 Organic Element Analyzer. UV/Vis spectra were recorded on a Perkin Elmer Lambda 900 spectrophotometer and fluorescence spectra were recorded on a Perkin Elmer LS 55 spectrophotometer. High-resolution mass spectra were obtained on UHD Accurate-Mass Q-TOF LC/MS.

Synthesis 2,4-dinitrophenyl 2,4-diformylphenyl ether (1)

A mixture of 4-hydroxy-1,3-benzenedicarboxaldehyde (100 mg, 0.67 mmol), 1-fluoro-2,4-dinitrobenzene (619 mg, 3.3 mmol) and K_2CO_3 (920 mg, 6.66 mmol) in DMF (4 mL) was

stirred at room temperature for overnight under inert atmosphere. After completion, the reaction mixture was concentrated by evaporation under reduced pressure. The reaction mixture was dissolved in dichloromethane. The organic phase was washed with water. Finally it was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by flash column chromatography (hexane/ethyl acetate: 7/3) as eluent to obtain ether **1** as light yellow solid (148 mg, 70%). ^1H NMR (400 MHz, CDCl_3) δ_{ppm} 7.09 (1H, d, $J = 8.4$ Hz), 7.29 (1H, d, $J = 8.8$ Hz), 8.16 (1H, dd, $J = 2$ Hz, 6.4 Hz), 8.50 (2H, m), 8.98 (1H, d, $J = 2.8$ Hz), 10.08 (1H, s), 10.45 (1H, s). ^{13}C NMR (100 MHz, CDCl_3) δ_{ppm} 119.1, 121.8, 122.6, 127.3, 129.4, 132.8, 133.7, 135.5, 140.9, 143.7, 153.0, 160.2, 186.7, 189.4. LCMS: $m/z = 316.3$ $[\text{M}]^+$ for $\text{C}_{14}\text{H}_8\text{N}_2\text{O}_7$. Elemental analysis: Found: C, 53.15; H, 2.56; N, 8.87, Calcd: C, 53.17; H, 2.55; N, 8.86 for $\text{C}_{14}\text{H}_8\text{N}_2\text{O}_7$.

Synthesis of Dinitrophenyl-ether-cyanine (DNPOCy) probe

A mixture of 2,4-dinitrophenyl 2,4-diformylphenyl ether (1) (50 mg, 0.16 mmol), indolium-3-butyl-sulfonate (2) (84 mg, 0.28 mmol) and NaOAc (23 mg, 0.28 mmol) was dissolved in Ac_2O (3 mL). The reaction mixture was stirred for 30 min at 80 $^\circ\text{C}$ under an argon atmosphere. After completion, the reaction mixture was concentrated by evaporation under reduced pressure. The crude product was diluted with 3.0 mL H_2O , 3.0 mL acetonitrile, 300 μL acetic acid, and purified by preparative RP-HPLC (grad. 10-90% acetonitrile in water, 20 min) to obtain the probe **DNPOCy** (115 mg, 82%) as yellow powder. ^1H NMR (400 MHz, DMSO-d_6) δ_{ppm} 1.72 (6H, s), 1.86-1.89 (10H, m), 2.07-2.10 (4H, m), 2.62-2.70 (4H, m), 4.80-4.85 (4H, m), 7.49 (1H, d, $J = 8.8$ Hz), 7.64-7.67 (5H, m), 7.87-7.91 (2H, m), 8.01-8.21 (4H, m), 8.39-8.43 (1H, m), 8.53-8.60 (3H, m), 8.99 (1H, d, $J = 2.4$ Hz), 9.34 (1H, m). ^{13}C NMR (100 MHz, DMSO-d_6) δ_{ppm} 22.1, 25.5, 25.6, 26.5, 26.6, 46.9, 47.2, 49.3, 49.5, 52.4, 52.5, 114.9, 115.5, 115.8, 117.1, 119.6, 121.6, 122.1, 123.0, 126.6, 129.1, 129.2, 129.7, 129.9, 132.0, 132.6, 136.4, 140.1, 140.6, 140.7, 142.8, 143.3, 143.9, 144.1, 150.7, 152.6, 156.1, 158.0, 158.4, 181.5, 181.9. LCMS: $m/z = 870.3$ $[\text{M}]^+$ for $\text{C}_{44}\text{H}_{46}\text{N}_4\text{O}_{11}\text{S}_2$. Elemental analysis: Found: C, 60.65; H, 5.33; N, 6.44, Calcd: C, 60.67; H, 5.32; N, 6.43 for $\text{C}_{44}\text{H}_{46}\text{N}_4\text{O}_{11}\text{S}_2$. HRMS: $m/z = 871.2663$ $[\text{M} + \text{H}]^+$ and calculated $m/z = 871.2683$ for $\text{C}_{44}\text{H}_{47}\text{N}_4\text{O}_{11}\text{S}_2$.

Live cell imaging experiments

HEK293 cells were seeded in coverslip bottom dishes and allowed to grow till 60% confluency. Subsequently, the monolayers were treated with 10 μM of **DNPOCy** for 2 h. Post treatment the monolayers were washed twice with 1X PBS and then imaging was performed with Axioscope (Carl Zeiss) Meta fluorescent microscope in the yellow/CFP/540 nm and red/RFP/630 nm filter. The same culture dishes were further incubated in the presence of 20 μM of NaSH for 30 min and then the images were captured after washing with 1X PBS using fluorescent microscope as mentioned earlier.

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Notes and references

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†Electronic Supplementary Information (ESI) available: Characterization, additional absorption, fluorescence pH and theoretical data, HPLC and mass analysis, and spectroscopic data. See DOI: 10.1039/b000000x/

- 1 E. Blackstone, M. Morrison and M. B. Roth, *Science*, 2005, **308**, 518.
- 2 V. S. Lin and C. J. Chang, *Curr. Opin. Chem. Biol.*, 2012, **16**, 595.
- 3 K. Eto, T. Asada, K. Arima, T. Makifuchi and H. Kimura, *Biochem. Biophys. Res. Commun.*, 2002, **293**, 1485.
- 4 P. Kamoun, M.-C. Belardinelli, A. Chabli, K. Lallouchi and B. Chadeaux-Vekemans, *Am. J. Med. Genet.*, 2003, **116A**, 310.
- 5 W. Yang, G. Yang, X. Jia, L. Wu and R. Wang, *J. Physiol.*, 2005, **569**, 519.
- 6 S. Fiorucci, E. Antonelli, A. Mencarelli, S. Orlandi, B. Renga, G. Rizzo, E. Distrutti, V. Shah and A. Morelli, *Hepatology*, 2005, **42**, 539.
- 7 Y. Kaneko, Y. Kimura, H. Kimura and I. Niki, *Diabetes*, 2006, **55**, 1391.
- 8 A. R. Lippert, E. J. New and C. J. Chang, *J. Am. Chem. Soc.*, 2011, **133**, 10078.
- 9 H. Peng, Y. Cheng, C. Dai, A. L. King, B. L. Predmore, D. J. Lefer and B. Wang, *Angew. Chem. Int. Ed.*, 2011, **50**, 9672.
- 10 C. Liu, J. Pan, S. Li, Y. Zhao, L. Y. Wu, C. E. Berkman, A. R. Whorton and M. Xian, *Angew. Chem. Int. Ed.*, 2011, **50**, 10327.
- 11 K. Sasakura, K. Hanaoka, N. Shibuya, Y. Mikami, Y. Kimura, T. Komatsu, T. Ueno, T. Terai, H. Kimura and Nagano, T. *J. Am. Chem. Soc.*, 2011, **133**, 18003.
- 12 Y. Qian, J. Karpus, O. Kabil, S.-Y. Zhang, H.-L. Zhu, R. Banerjee, J. Zhao and C. He, *Nat. Commun.*, 2011, **11**, 495.
- 13 F. Yu, P. Li, P. Song, B. Wang, J. Zhao and K. Han, *Chem. Commun.*, 2012, **48**, 2852.
- 14 C. Liu, B. Peng, S. Li, C.-M. Park, A. R. Whorton and M. Xian *Org. Lett.*, 2012, **14**, 2184.
- 15 L. A. Montoya and M. D. Pluth, *Chem. Commun.*, 2012, **48**, 4767.
- 16 S. Chen, Z.-J. Chen, W. Ren and H.-W. Ai, *J. Am. Chem. Soc.*, 2012, **134**, 9589.
- 17 W. Xuan, R. Pan, Y. Cao, K. Liu and W. Wang, *Chem. Commun.*, 2012, **48**, 10669.
- 18 K. Zheng, W. Lin and L. Tan, *Org. Biomol. Chem.*, 2012, **10**, 9683.
- 19 N. Karton-Lifshin, E. Segal, L. Omer, M. Portnoy, R. Satchi-Fainaro and D. Shabat, *J. Am. Chem. Soc.*, 2011, **133**, 10960.
- 20 D. Maity and T. Govindaraju, *Org. Biomol. Chem.*, 2013, **11**, 2098.
- 21 N. Karton-Lifshin, L. Albertazzi, M. Bendikov, P. S. Baran and D. Shabat, *J. Am. Chem. Soc.*, 2012, **134**, 20412.
- 22 J. Han, M. S. Han and C.-H. Tung, *Mol. BioSyst.*, 2013, **9**, 3001.
- 23 Y. Shi, S. Zhang and X. Zhang, *Analyst*, 2013, **138**, 1952.
- 24 O. Redy-Keisar, E. Kisin-Finifer, S. Ferber, R. Satchi-Fainaro and D. Shabat, *Nature Protocols*, 2014, **1**, 27.
- 25 Y. Chen, C. Zhu, Z. Yang, J. Chen, Y. He, Y. Jiao, W. He, L. Qiu, J. Cen and Z. Guo, *Angew. Chem. Int. Ed.*, 2013, **52**, 1688.
- 26 S. K. Bae, C. H. Heo, D. J. Choi, D. Sen, E.-H. Joe, B. R. Cho and H. M. Kim, *J. Am. Chem. Soc.*, 2013, **135**, 9915.
- 27 L. A. Montoya, T. F. Pearce, R. J. Hansen, L. N. Zakharov and M. D. Pluth, *J. Org. Chem.*, 2013, **78**, 6550.
- 28 J. Liu, Y.-Q. Sun, J. Zhang, T. Yang, J. Cao, L. Zhang and W. Guo, *Chem. Eur. J.*, 2013, **19**, 4717.
- 29 X. Cao, W. Lin, K. Zheng and L. He, *Chem. Commun.*, 2012, **48**, 10529.
- 30 T. Liu, Z. Xu, D. Spring and R. J. Cui, *Org. Lett.*, 2013, **15**, 2310.
- 31 C. T. Lee, W. T. Yang and R. G. Parr, *Phys. Rev. B*, 1988, **37**, 785.
- 32 B. Miehlisch, A. Savin, H. Stoll and H. Preuss, *Chem. Phys. Lett.*, 1989, **157**, 200.
- 33 A. D. Becke, *J. Chem. Phys.*, 1993, **98**, 5648.

Graphical Abstract

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