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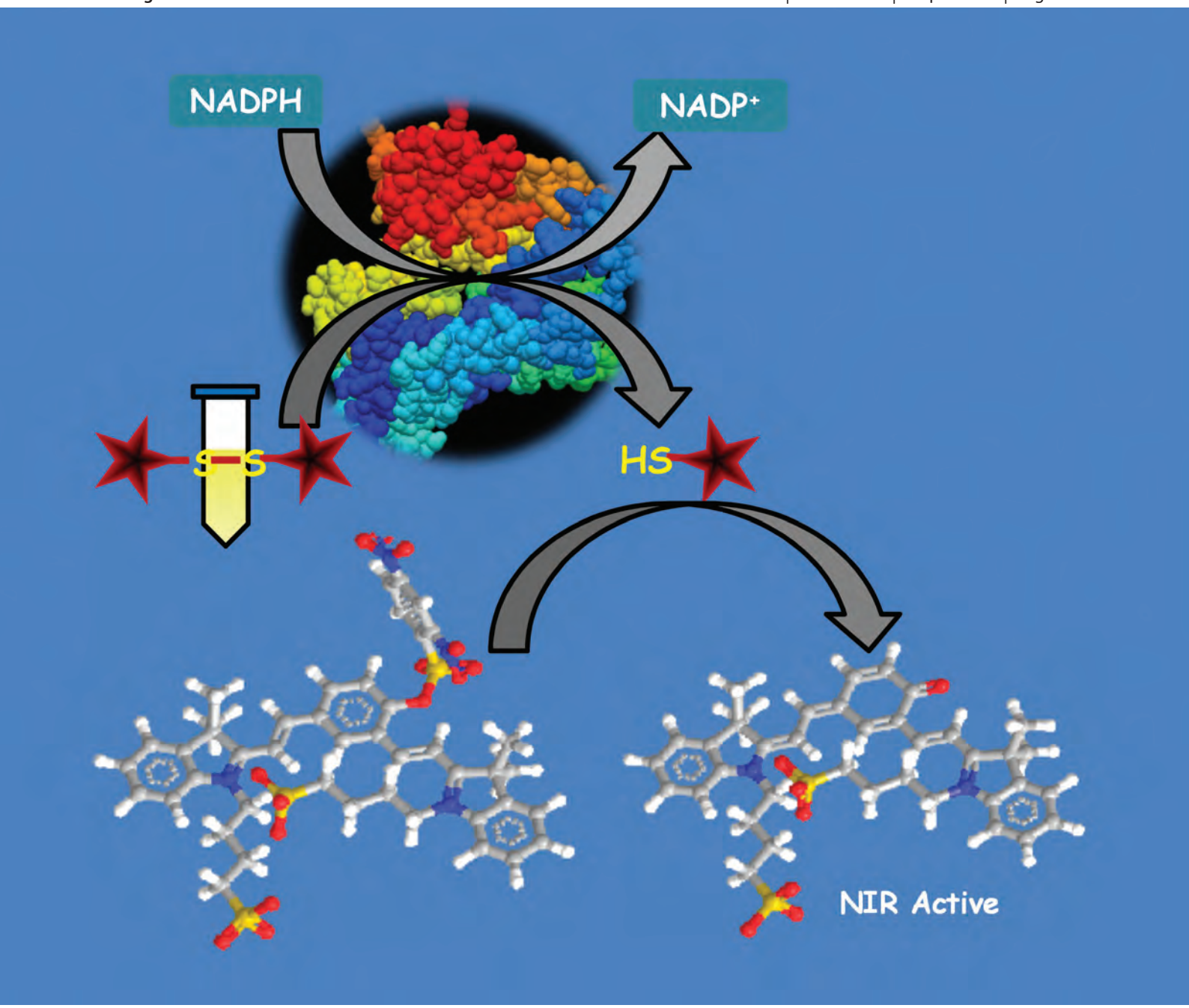


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# A turn-on NIR fluorescence and colourimetric cyanine probe for monitoring the thiol content in serum and the glutathione reductase assisted glutathione redox process†

Debabrata Maity and T. Govindaraju*

We report a novel reaction-based thiol selective turn-on near-infrared (NIR) fluorescence and colourimetric dinitrobenzenesulfonyl-cyanine (**DNBSCy**) probe. In the presence of thiols such as glutathione (GSH), new absorption bands (476 and 581 nm) were observed, with the colour of the solution (10 mM PBS, pH = 7.4) changing from light green to blue. Interestingly, relatively non-fluorescent **DNBSCy** exhibited enhanced fluorescence emission around 700 nm in the NIR region. GSH reacted efficiently with the electron withdrawing sulfonyl ester moiety of **DNBSCy**, releasing the quinone embedded heptamethine cyanine (**Cy-quinone**) with extended π-electron conjugation responsible for the turn-on NIR fluorescence. **Cy-quinone** also displayed a conjugated π-electron push–pull character under physiological conditions. The **DNBSCy** probe was effectively employed to monitor the thiols in fetal bovine serum (FBS). The probe was capable of monitoring the oxidized glutathione (GSSG)/GSH redox process in the presence of glutathione reductase and NADPH with NIR fluorescence and colourimetric optical response. Thus, **DNBSCy** has the potential to measure the activity of glutathione reductase as a measure of oxidative stress.

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## Introduction

The optical monitoring of thiols such as glutathione (GSH), cysteine (Cys) and homocysteine (Hcy) is of significant interest due to their crucial roles in maintaining the biological redox homeostasis through the equilibrium of free thiols and oxidized disulfides in biological systems.[1] These low molecular weight thiols (LMWTs) are essential for the growth of cells and tissues in living organisms.[2] Dramatic changes in the intracellular thiol concentrations in biological fluids are implicated in a number of diseases including liver damage, heart disease, cancer, AIDS, inflammatory bowel disease, osteoporosis, Alzheimer's and cardiovascular diseases.[3] Among these LMWTs, GSH is a major endogenous antioxidant produced in the cell (0.5–10 mM).[4] GSH protects the cells from oxidative stress by trapping free radicals and reactive oxygen species (ROS); it also maintains exogenous antioxidants in their reduced forms.[5] The abnormal ratio of reduced GSH to oxidized glutathione is one of the potential reasons for oxidative stress.[6] In cells, the enzyme glutathione reductase catalyses the reduction of GSSG to free GSH, thus preventing the occurrence of oxidative stress. Hence, it is very crucial to monitor the concentration of GSH and the activity of glutathione reductase in physiological media. Although several strategies have been developed to detect these mercapto-biomolecules, fluorescence detection has been recognized as the most convenient method due to its simplicity and low detection limit.[7] In the past few years, researchers have developed thiol-selective fluorescence probes exploiting the strong nucleophilicity of the thiol group and their cation binding affinity.[7a] However, the existing fluorescence probes for thiols are plagued by certain serious limitations like excitation and emission in the UV-visible region, relatively time consuming procedures, and the use of organic–water mixed media.

NIR molecular probes have gained special interest in recent years due to several inherent advantages over the UV-visible probes.[8] Deeper penetration of the NIR radiation (650–900 nm) enables the assessment of molecular and physiological events several layers deep inside the analyte samples and tissues. Moreover, optical response in the NIR region is not affected by the autofluorescence generated from chromophores and macromolecules present in the analyte or tissue

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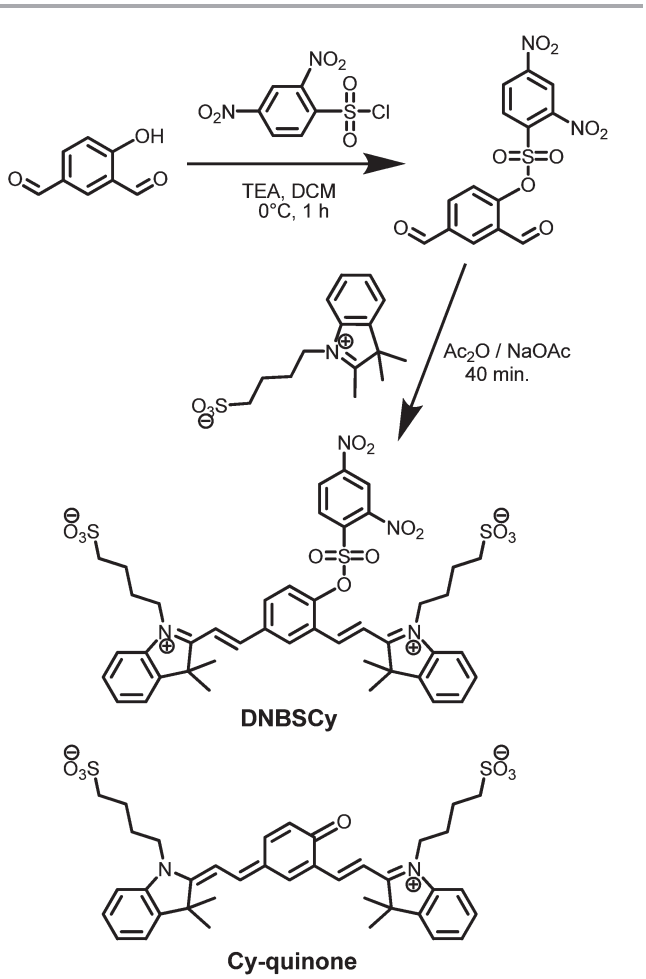
samples.[9] A few research groups have taken the initiative in designing thiol-selective NIR sensors.[10] However, many of these thiol sensors work by means of reduction in NIR fluorescence. Therefore, a turn-on NIR fluorescence probe based on the extended π-electron conjugated system that could instantly monitor thiols in complete physiological media is necessary.

Our design strategy for the development of a turn-on NIR fluorescence probe relied on a reaction-based approach in which a thiol mediated bond-cleaving reaction converted a weak or non-fluorescence probe into a robust NIR fluorescent dye.[11] In this full paper we report a highly sensitive turn-on NIR fluorescence and colourimetric probe for monitoring thiols, especially GSH, over GSSG in physiological media. The thiol assisted removal of 2,4-dinitrobenzenesulfonyl (DNBS) from the hydroxyl group moiety of a fluorophore has been found to have excellent selectivity towards thiol detection with off–on fluorescence signaling.[12] Strong electron withdrawing DNBS functionality would significantly disturb the internal charge transfer (ICT) process, maximising the fluorescence switching on–off process. Heptamethine cyanine (Cy), a NIR fluorescent dye with high extinction coefficient and quantum yield, has been extensively used in NIR bioimaging.[13] We devised an interesting approach by incorporating the DNBS protected phenolic moiety onto the conjugated cyanine backbone.[14] The DNBS-phenolic moiety incorporated heptamethine cyanine (**DNBSCy**) was designed, however, with a completely altered π-conjugation pattern and positive charges localised on both the nitrogen atoms (Scheme 1). The thiol mediated nucleophilic reaction on the **DNBSCy** probe releases the thiol-dinitrobenzene (DNB) by-product, generating the Cy-phenolate which rearranges itself by neutralising the positive charge on a nitrogen atom to form stable **Cy-quinone**. This **Cy-quinone** resumes the extended π-electron conjugation pattern of the cyanine dyes. Similar to parent heptamethine cyanine (Cy), the **Cy-quinone** has an odd number of carbon atoms between two nitrogen atoms to facilitate conjugation along the polymethine chain. It was thus expected to exhibit turn-on NIR fluorescence besides showing unique visible colour changes due to the conjugated π-electron push–pull character.




**Scheme 1** Synthesis of **DNBSCy** and molecular structure of **Cy-quinone**.

## Results and discussion

### Synthesis of DNBSCy and its photophysical studies in the presence of thiol

A two-step straightforward synthetic route was developed for the preparation of the **DNBSCy** probe as outlined in Scheme 1. The arenesulfonate ester moiety was synthesized by sulfonating the hydroxyl group of 4-hydroxy-1,3-benzenedicarboxaldehyde with 2,4-dinitrobenzenesulfonyl chloride. Good yields of **DNBSCy** were obtained by condensing this dialdehyde-arenesulfonate ester intermediate with 2 equiv. of indolium-3-butyl-sulfonate. All the compounds were characterized by NMR, mass spectrometry, and elemental analysis. The arenesulfonate ester moiety in the **DNBSCy** probe is highly reactive towards thiolate, which releases the hydroxyl group of the cyanine fluorophore backbone in the presence of free thiols. The indolium-3-butyl-sulfonate chains in **DNBSCy** are chosen to impart water solubility and to prevent aggregation of the cyanine backbone in solution. **DNBSCy** is a yellow powder and readily dissolves in water to form a light green solution. **DNBSCy** exhibits an absorption band centred around 390 nm with an extinction coefficient ($\varepsilon$) of 2.6 × 104 $M^{-1}$ $cm^{-1}$ in 10 mM PBS buffer (pH = 7.4) (Fig. S1, ESI†). The probe itself is almost non-fluorescent, displaying a very weak emission band around 700 nm (Fig. 1).

In the presence of GSH, the absorbance at 390 nm decreased drastically while two new absorption bands appeared at 476 nm and 581 nm (Fig. S1, ESI†). Concurrently, the colour of the solution changed from light green to blue. As anticipated upon 600 nm excitation ($\lambda_{ex}$), the fluorescence emission spectra displayed a switch-on peak around 700 nm in the NIR region (Fig. 1). This large Stokes shift (~119 nm) between the absorbance and emission peaks is a highly desirable characteristic feature of a fluorescence probe that assists in increasing the signal-to-noise ratio. Other free thiol containing amino acids like Cys and Hcy showed similar absorption and emission spectral changes (Fig. S1, ESI†). However, the presence of amino acids with no thiol functionality or

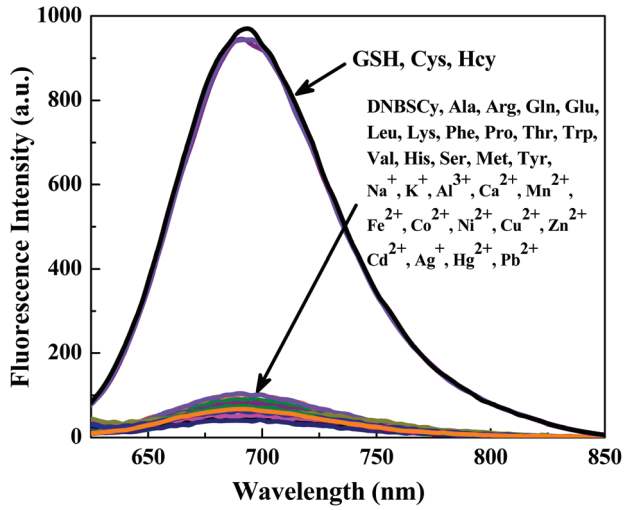



**Fig. 1** Fluorescence response of the **DNBSCy** probe (10.0 μM) towards various amino acids (1.0 mM) and metal ions (1.0 mM). Each spectrum was acquired after 30 min incubation of the probe with an analyte in 10 mM PBS buffer (pH 7.4) with $\lambda_{ex}$ = 600 nm.

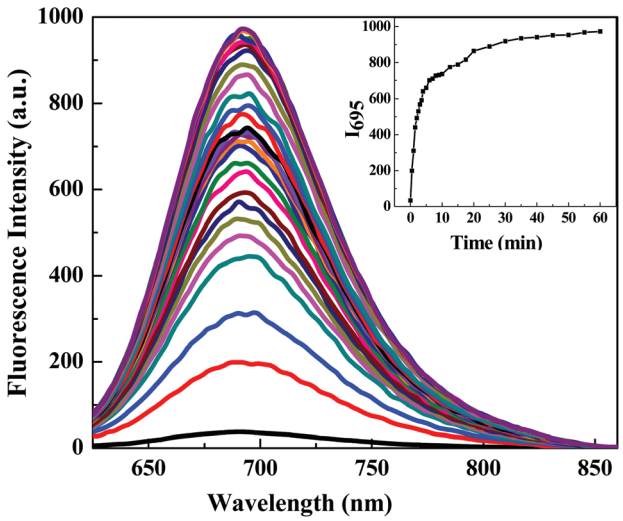



**Fig. 2** Time-dependent NIR fluorescence spectra recorded when the **DNBSCy** probe (10.0 μM) was treated with GSH (5.0 μM) in 10 mM PBS buffer (pH 7.4) with $\lambda_{ex}$ = 600 nm. Inset: Fluorescence intensity changes at 695 nm recorded as a function of time.

biologically relevant metal ions did not show any significant spectral changes with **DNBSCy** (Fig. 1). Though thiols are present in millimolar concentration inside cells, only 5.0 μM GSH was found to be enough to display significant NIR fluorescence response within two minutes after reacting with the arenesulfonate ester moiety of the **DNBSCy** probe (10 μM) (Fig. 2). This clearly emphasised the sensitivity of the probe, as **DNBSCy** responds quickly towards the detection of GSH as low as three orders of magnitude less than the actual concentration present in normal cells. On addition of GSH to the **DNBSCy** solution, NIR fluorescence emission intensity at $E_{max}$ = 695 nm increased rapidly in the initial stage and reached the maximum (>25 fold) over a period of 20 min. The GSH titration study shows that **DNBSCy** could detect submicromolar concentrations of GSH efficiently (Fig. S2, ESI†). The GSH mediated rapid nucleophilic reaction with the electron withdrawing sulfonyl ester moiety of the **DNBSCy** probe releases the Cy-phenolate anion. The negative charge of Cy-phenolate rearranges itself by neutralising the positive charge on one of the nitrogen atoms to form stable **Cy-quinone** (Scheme 1). In the process, **Cy-quinone** resumes a heptamethine cyanine-like extended π-electron conjugation pattern which is responsible for the observed characteristic turn-on NIR fluorescence. The conjugated π-electron push–pull character is also generated in the **Cy-quinone** system that lends a unique blue colour to the solution. Further, **DNBSCy** was also found to be highly selective for GSH over other competing non-thiol amino acids in the buffer solution (Fig. S3, ESI†). Mass spectroscopic analysis confirmed that **DNBSCy** was converted to the **Cy-quinone** dye under the experimental conditions used for GSH detection (Fig. S4, ESI†). The ESI-mass spectra showed peaks at $m/z$ = 705.2 (calculated for $C_{38}H_{43}N_2O_7S_2 + H^+$) corresponding to **Cy-quinone** and at $m/z$ = 474.0 (calculated for $C_{16}H_{19}N_5O_{10}S$) corresponding to the by-product γ-glutamyl-*S*-(2,4-dinitrophenyl)-cysteinylglycine.

## pH dependent study

The effect of pH on the GSH mediated arenesulfonate ester bond cleavage was studied to understand the efficiency of the process. **DNBSCy** alone was stable within the pH range of 5–8 while small changes were observed in the pH range of 8–10 (Fig. 3). However, the **DNBSCy** probe reacted selectively with GSH in the biologically relevant pH range of 6–10 to form the NIR fluorescence emitting **Cy-quinone** dye. The optimum NIR fluorescence emission was observed in the commonly used physiological pH range of 6.5–8.5, which makes the **DNBSCy** probe convenient for the detection and monitoring of thiols

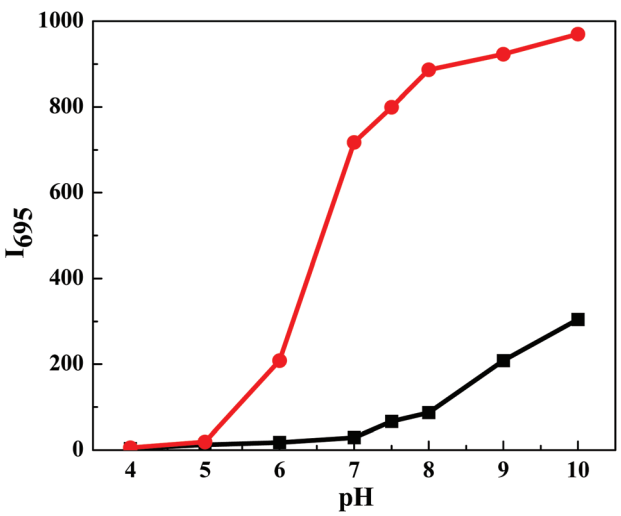



**Fig. 3** Effect of pH on the NIR emission of **DNBSCy** monitored at 695 nm. Black trace: **DNBSCy** (10 μM) and red trace: **DNBSCy** (10.0 μM) with 5.0 μM of GSH in 10 mM PBS buffer.

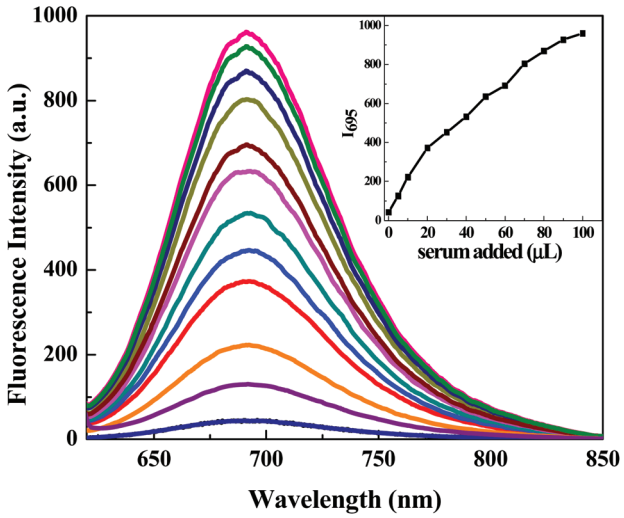
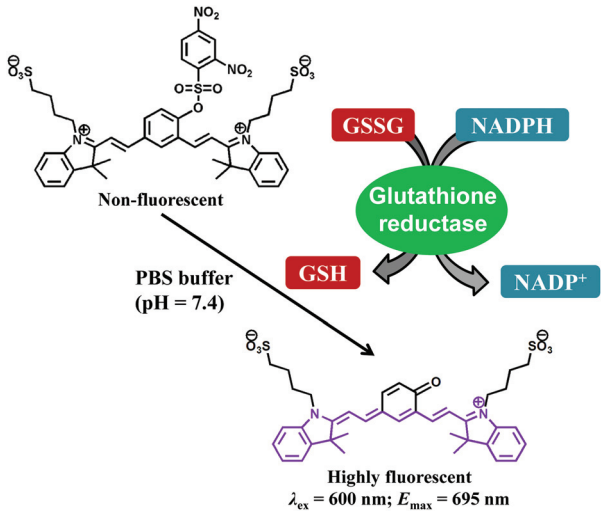



**Fig. 4** NIR fluorescence spectra of **DNBSCy** (10.0 μM) upon addition of aliquots (0, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 μL) of reduced fetal bovine serum ($\lambda_{ex}$ = 600 nm; $E_{max}$ = 695 nm).




**Scheme 2** Efficient monitoring of free thiol generated from glutathione reductase (in the presence of NADPH) catalysed GSSG/GSH conversion by coupling the thiol responsive **DNBSCy**/**Cy-quinone** transformation with unique turn-on NIR fluorescence and colourimetric optical response.

without interference from the pH-dependent effects of the physiological media.

### Reduced thiol detection in fetal bovine serum (FBS)

To ascertain the practical applicability of our turn-on NIR fluorescence probe, thiol content in the fetal bovine serum (FBS) sample was studied. Thiol containing amino acids generally occur in the disulfide form in blood serum. Estimation of the thiol pool in blood serum is crucial for understanding the role of thiols in the pathogenesis of cardiovascular diseases. We first treated the plasma (FBS) with a reducing agent triphenylphosphine to reduce all the oxidized disulfide to free thiols.[15] Different aliquots of the reduced FBS were added to **DNBSCy** in buffer solution (2 mL, 10 mM PBS, pH 7.4) at ambient temperature. Fig. 4 shows the turn-on NIR fluorescence signal and linear increase in the intensity of the signal with the addition of reduced FBS. These data clearly suggest the presence of free thiols that react rapidly with the **DNBSCy** probe to form the NIR fluorescence emitting **Cy-quinone** dye. In addition, spontaneous colourimetric changes were observed in the absorption spectra (Fig. S9, ESI†). The visible light greenish colour of the solution gradually changed to blue as the amount of reduced FBS was increased in the analysis mixture under study. This experiment demonstrates that **DNBSCy** is capable of monitoring thiols in the serum sample by means of characteristic turn-on NIR fluorescence as well as colourimetric optical responses.

### Selective detection of reduced GSH over oxidized GSSG using a combination of glutathione reductase and DNBSCy

Significant imbalance in the GSH/GSSG ratio is responsible for oxidative stress and has direct implications for human health. Therefore, the measure of glutathione reductase activity serves as an indicator for the assessment of oxidative stress. The enzyme glutathione reductase catalyses the reduction of GSSG to GSH in the presence of the reducing agent nicotinamide adenine dinucleotide phosphate (NADPH) under physiological conditions.[16] Thus, we monitored the formation of GSH by reducing the oxidized GSSG in the presence of glutathione reductase by coupling our free thiol responsive **DNBSCY**/**Cy-quinone** transformation with enzyme catalysed GSSG/GSH conversion (Scheme 2). **DNBSCy** reacted instantaneously with the free GSH generated from GSSG under the assay conditions (10 mM PBS buffer, 1 unit per mL glutathione reductase, 0.4 mM NADPH) as indicated by the enhanced NIR emission (>11 fold) within 2 min of adding the enzyme (Fig. S10, ESI†). This preliminary experiment motivated us to study the kinetic effect of the reduction of GSSG to GSH at different concentrations of the enzyme. This experiment revealed that higher concentrations of glutathione reductase lowered the time required for completion of the GSSG/GSH redox process and its subsequent detection (Fig. 5). Maximum NIR emission signal was achieved within 20 min when 10 mU $mL^{-1}$ of glutathione reductase was employed. However, more time (double) was required to reach the maximum intensity when half the concentration of glutathione reductase was used. In the absorption spectra, the **DNBSCy** absorbance band at 385 nm decreased while new bands appeared at 475 and 582 nm with the addition of NADPH and glutathione reductase (Fig. 6). A clear isosbestic point at 440 nm indicated the formation of the **Cy-quinone** dye by the reaction of free GSH on **DNBSCy**. The solution colour changed from light green to blue as a result of formation of the cyanine dye with extended conjugation.

Normally, the glutathione reductase activity is monitored with NADPH consumption by recording the decreased absorbance at 340 nm or the free GSH generated can be visualized from the increased absorbance at 412 nm using Ellman's reagent. Our **DNBSCy** probe, with dual optical response

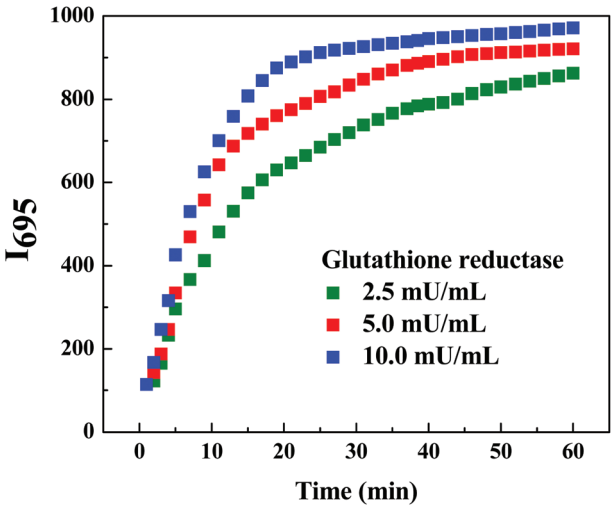



**Fig. 5** Glutathione reductase activity assay. NIR fluorescence response of **DNBSCy** (10.0 μM) at 695 nm ($\lambda_{ex}$ = 600 nm) monitored as a function of time. In the assay mixture glutathione reductase reduces GSSG to reduced free GSH form using a reducing agent NADPH (100.0 μM). The activity of enzyme glutathione reductase was measured at three different concentrations (2.5, 5.0 and 10.0 mU $mL^{-1}$) in 10 mM PBS buffer (pH 7.4).

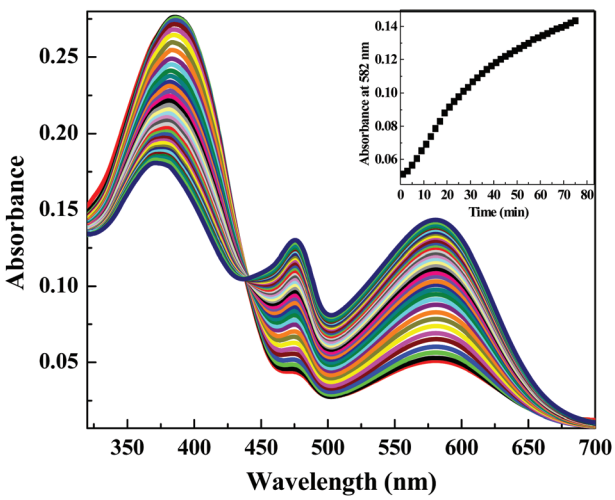



**Fig. 6** Time-dependent UV/Vis absorption spectra of **DNBSCy** (10.0 μM). Assay conditions: 10 mM PBS buffer (pH = 7.4), GSSG, NADPH (10.0 μm), glutathione reductase (10 mU $mL^{-1}$). Inset: Absorbance changes at 582 nm recorded as a function of time.

(turn-on NIR fluorescence and colourimetric response), provides a sophisticated, non-invasive and accurate way of monitoring the activity of glutathione reductase which in turn can be a parameter for measuring oxidative stress. As an added advantage, this probe allows the naked eye detection of GSSG to GSH conversion in the presence of glutathione reductase without the need for expensive instrumentation. To the best of our knowledge, dual responsive **DNBSCy** is the first probe with an excellent combination of turn-on NIR fluorescence and colourimetric optical response to detect the free thiol content in serum and to measure the activity of glutathione reductase in the GSSG/GSH redox process (Scheme 2).

## Conclusions

In conclusion, we have successfully developed a thiol selective water soluble turn-on NIR fluorescence probe **DNBSCy**. This molecular probe readily reacts with thiols, in particular GSH, to release the NIR fluorescence emitting cyanine dye over a wide pH range. The probe was used effectively for the turn-on NIR fluorescence and colourimetric monitoring of thiols in fetal bovine serum (FBS). For the first time, we also demonstrated the ability of the **DNBSCy** probe to be used in monitoring the GSSG/GSH redox process in the presence of glutathione reductase enzyme and the reducing agent NADPH with the unique combination of NIR turn-on fluorescence and colourimetric optical response. Therefore, this probe is capable of monitoring the activity of glutathione reductase which in turn serves as a tool to assess the levels of oxidative stress. In general, **DNBSCy** can be used as a non-invasive tool for the determination of thiol content in biological fluids, measuring the activities of the enzymes involved in thiol coupled redox processes and *in vivo* NIR fluorescence imaging applications.

## Experimental section

### Materials and instruments

All the solvents and reagents were obtained from Sigma-Aldrich and used as received unless otherwise mentioned. $^{1}H$ and $^{13}C$ NMR were recorded on a Bruker AV-400 spectrometer with chemical shifts reported as ppm (in DMSO-$d_6$, tetramethylsilane as an internal standard). Mass spectra were obtained on a Shimadzu 2020 LC-MS. Elemental analysis was carried out on a ThermoScientific 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.

### Synthesis of 2,4-dinitrophenyl 2,4-diformylphenyl sulphate

A mixture of 4-hydroxy-1,3-benzenedicarboxaldehyde (150 mg, 1 mmol), 2,4-dinitrobenzenesulfonyl chloride (292 mg, 1.1 mmol) and triethylamine (0.15 mL, 1.1 mmol) in dichloromethane (10 mL) was stirred at 0 °C for 1 h under an inert atmosphere. The reaction mixture was washed with water. The organic phase was dried over $Na_2SO_4$ and the solvent was evaporated to dryness. The crude product was purified by column chromatography on silica gel using chloroform as an eluent to obtain a yellow solid (68%). $^{1}H$ NMR (400 MHz, [$d_6$]DMSO) $\delta_{ppm}$ 7.50 (1H, d, $J$ = 8.4 Hz), 8.23 (1H, dd, $J$ = 6.4 Hz, 2 Hz), 8.37 (1H, d, $J$ = 8.8 Hz), 8.46 (1H, d, $J$ = 2.0 Hz), 8.65 (1H, dd, $J$ = 6.4 Hz, 2.0 Hz), 9.14 (1H, d, $J$ = 2.4 Hz), 10.11 (1H, s), 10.16 (1H, s). $^{13}C$ NMR (100 MHz, [$d_6$]DMSO) $\delta_{ppm}$ 121.2, 124.2,

127.7, 129.3, 130.3, 131.4, 133.8, 135.6, 135.8, 148.0, 151.7, 152.0, 187.4, 191.5. LCMS: $m/z$ = 381.2 $[M + H]^+$ for $C_{14}H_8N_2O_9S$. Elemental analysis: Found: C, 44.19; H, 2.12; N, 7.38, Calcd: C, 44.22; H, 2.12; N, 7.37 for $C_{14}H_8N_2O_9S$.

### Synthesis of the dinitrobenzenesulfonyl-cyanine (DNBSCy) probe

A mixture of 2,4-dinitrophenyl-2,4-diformylphenyl sulfate (76 mg, 0.2 mmol), indolium-3-butyl-sulfonate (118 mg, 0.4 mmol) and NaOAc (32 mg, 0.4 mmol) was dissolved in 3 mL $Ac_2O$. The reaction mixture was stirred for 30 min at 80 °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 ACN, 300 μL AcOH, and purified by preparative RP-HPLC (grad. 10–90% ACN in water, 20 min) to obtain the probe **DNBSCy** (130 mg, 70%) as a yellow powder. $^1$H NMR (400 MHz, [$d_6$]DMSO) $\delta_{ppm}$ 1.79 (6H, s), 1.84 (6H, s), 1.86–1.91 (4H, m), 2.04–2.11 (4H, m), 2.62 (2H, t, $J$ = 6.8 Hz), 2.69 (2H, t, $J$ = 6.7 Hz), 4.80–4.87 (4H, m), 7.41 (1H, d, $J$ = 8.5 Hz), 7.65–7.69 (4H, m), 7.89–7.93 (2H, m), 8.03–8.12 (3H, m), 8.21–8.35 (3H, m), 8.48–8.53 (2H, m), 8.68 (1H, dd, $J$ = 6.4 Hz, 2.2 Hz), 9.18 (1H, d, $J$ = 2.2 Hz), 9.35 (1H, d, $J$ = 1.5 Hz). $^{13}$C NMR (100 MHz, [$d_6$]DMSO) $\delta_{ppm}$ 22.0, 22.1, 25.3, 26.4, 26.6, 47.1, 47.4, 49.2, 49.4, 52.5, 53.0, 115.7, 115.9, 116.5, 118.1, 121.5, 123.1, 127.9, 129.0, 129.1, 129.3, 129.9, 130.1, 130.7, 131.1, 133.9, 135.1, 136.0, 140.6, 140.7, 142.4, 144.1, 144.2, 148.0, 149.4, 149.6, 151.7, 157.9, 158.3, 181.5, 181.9. LCMS: $m/z$ = 935.1 $[M]^+$ for $C_{44}H_{46}N_4O_{13}S_3$. Elemental analysis: Found: C, 56.51; H, 4.95; N, 6.01, Calcd: C, 56.52; H, 4.96; N, 5.99 for $C_{44}H_{46}N_4O_{13}S_3$.

## Acknowledgements

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## Notes and references

1 (*a*) Z. A. Wood, E. Schröder, J. Robin Harris and L. B. Poole, *Trends Biochem. Sci.*, 2003, **28**, 32; (*b*) M. Zhang, M. Yu, F. Li, M. Zhu, M. Li, Y. Gao, L. Li, Z. Liu, J. Zhang, D. Zhang, T. Yi and C. Huang, *J. Am. Chem. Soc.*, 2007, **129**, 10322; (*c*) S.P. Wang, W.-J. Deng, D. Sun, M. Yan, H. Zheng and J.-G. Xu, *Org. Biomol. Chem.*, 2009, **7**, 4017; (*d*) V. Hong, A. A. Kislukhin and M. G. Finn, *J. Am. Chem. Soc.*, 2009, **131**, 9986; (*e*) X. Yang, Y. Guo and R. M. Strongin, *Angew. Chem., Int. Ed.*, 2011, **50**, 10690.

2 (*a*) A. P. de Silva, H. Q. N. Gunaratne and T. Gunnlaugsson, *Tetrahedron Lett.*, 1998, 5077; (*b*) D. H. Kim and M. S. Han, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 2543; (*c*) T. Matsumoto, Y. Urano, T. Shoda, H. Kojima and T. Nagano, *Org. Lett.*, 2007, **9**, 3375; (*d*) S.-T. Huang, K.-N. Ting and K.-L. Wang, *Anal. Chim. Acta*, 2008, **620**, 120; (*e*) W. Jiang, Y. Cao, Y. Liu and W. Wang, *Chem. Commun.*, 2010, **46**, 1944; (*f*) H. S. Jung, J. H. Han, T. Pradhan, S. Kim, S. W. Lee, J. L. Sessler, T. W. Kim, C. Kang and J. S. Kim, *Biomaterials*, 2012, **33**, 945; (*g*) P. Wang, J. Liu, X. Lv, Y. Liu, Y. Zhao and W. Guo, *Org. Lett.*, 2012, **14**, 520.

3 (*a*) L. A. Herzenberg, S. C. De Rosa, J. G. Dubs, M. Roederer, M. T. Anderson, S. W. Ela, S. C. Deresinski and L. A. Herzenberg, *Proc. Natl. Acad. Sci. U. S. A.*, 1997, **94**, 1967; (*b*) S. Shahrokhian, *Anal. Chem.*, 2001, **73**, 5972; (*c*) D. M. Townsend, K. D. Tew and H. Tapiero, *Biomed. Pharmacother.*, 2003, **57**, 145; (*d*) R. Njälsson and S. Norgren, *Acta Paediatr.*, 2005, **94**, 132; (*e*) H. P. Wu, C. C. Huang, T. L. Cheng and W. L. Tseng, *Talanta*, 2008, **76**, 347; (*f*) H. S. Jung, J. H. Han, Y. Habata, C. Kang and J. S. Kim, *Chem. Commun.*, 2011, **47**, 5142.

4 A. Meister and M. E. Anderson, *Annu. Rev. Biochem.*, 1983, **52**, 711.

5 (*a*) R. H. Schirmer, J. G. Müller and R. L. Krauth-Siegel, *Angew. Chem.*, 1995, **107**, 153; (*b*) T. P. Dalton, H. G. Shertzer and A. Puga, *Annu. Rev. Pharmacol. Toxicol.*, 1999, **39**, 67; (*c*) S. M. Kanzok, R. H. Schirmer, I. Turbachova, R. Iozef and K. Becker, *J. Biol. Chem.*, 2000, **275**, 40180; (*d*) R. L. Krauth-Siegel, H. Bauer and R. H. Schirmer, *Angew. Chem.*, 2005, **117**, 698; (*e*) X. Chen, S.-K. Ko, M. J. Kim, I. Shin and J. Yoon, *Chem. Commun.*, 2010, **46**, 2751.

6 (*a*) T. P. Akerboom, M. Bilzer and H. Sies, *J. Biol. Chem.*, 1982, **257**, 4248; (*b*) O. W. Griffith, *Free Radical Biol. Med.*, 1999, **27**, 922; (*c*) D. M. Townsend, K. D. Tew and H. Tapiero, *Biomed. Pharmacother.*, 2003, **57**, 145; (*d*) D. Han, N. Hanawa, B. Saberi and N. Kaplowitz, *Am. J. Physiol.*, 2006, **291**, G1; (*e*) D. Ścibior, M. Skrzycki, M. Podsiad and H. Czeczot, *Clin. Biochem.*, 2008, **41**, 852; (*f*) H. S. Jung, K. C. Ko, G.-H. Kim, A.-R. Lee, Y.-C. Na, C. Kang, J. Y. Lee and J. S. Kim, *Org. Lett.*, 2011, **13**, 1498; (*g*) B. K. McMahon and T. Gunnlaugsson, *J. Am. Chem. Soc.*, 2012, **134**, 10725.

7 (*a*) X. Chen, Y. Zhou, X. Peng and J. Yoon, *Chem. Soc. Rev.*, 2010, **39**, 2120; (*b*) D. Maity and T. Govindaraju, *Eur. J. Inorg. Chem.*, 2011, 5479; (*c*) D. Maity and T. Govindaraju, *Chem. Commun.*, 2012, **48**, 1039.

8 (*a*) G. Patonay and M. D. Antoine, *Anal. Chem.*, 1991, **63**, 321A; (*b*) R. B. Thompson, *Top. Fluoresc. Spectrosc.*, 1994, **4**, 151; (*c*) S. Stoyanov, *Pract. Spectrosc.*, 2001, **25**, 35; (*d*) W. M. Leevy, S. T. Gammon, J. R. Johnson, A. J. Lampkins, H. Jiang, M. Marquez, D. Piwinica-Worms and B. D. Smith, *Bioconjugate Chem.*, 2008, **19**, 686; (*e*) D. Maity and T. Govindaraju, *Chem.–Eur. J.*, 2011, **17**, 1410; (*f*) D. Maity, A. K. Manna, D. Karthigeyan, T. K. Kundu, S. K. Pati and T. Govindaraju, *Chem.–Eur. J.*, 2011, **17**, 11152.

9 (*a*) J. Fabian, H. Nakazumi and M. Matsuoka, *Chem. Rev.*, 1992, **92**, 1197; (*b*) J. V. Frangioni, *Curr. Opin. Chem. Biol.*, 2003, **7**, 626; (*c*) B. A. Smith, W. J. Akers, W. M. Leevy,

A. J. Lampkins, S. Z. Xiao, W. Wolter, M. A. Suckow, S. Achilefu and B. D. Smith, *J. Am. Chem. Soc.*, 2010, **132**, 67; (*d*) J. M. Baumes, J. J. Gassensmith, J. Giblin, J.-J. Lee, A. G. White, W. J. Culligan, W. M. Leevy, M. Kuno and B. D. Smith, *Nature Chem.*, 2010, **2**, 1025.

10 (*a*) S. Sreejith, K. P. Divya and A. Ajayaghosh, *Angew. Chem., Int. Ed.*, 2008, **47**, 7883; (*b*) H. S. Hewage and E. V. Anslyn, *J. Am. Chem. Soc.*, 2009, **131**, 13099; (*c*) Z. Guo, S. W. Nam, S. Park and J. Yoon, *Chem. Sci.*, 2012, **3**, 2760; (*d*) R. Wang, L. Chen, P. Liu, Q. Zhang and Y. Wang, *Chem.–Eur. J.*, 2012, **18**, 11343.

11 (*a*) B. Tang, Y. Xing, P. Li, N. Zhang, F. Yu and G. Yang, *J. Am. Chem. Soc.*, 2007, **129**, 11666; (*b*) M. M. Pires and J. Chmielewski, *Org. Lett.*, 2008, **10**, 837; (*c*) J. H. Lee, C. S. Lim, Y. S. Tian, J. H. Han and B. R. Cho, *J. Am. Chem. Soc.*, 2010, **132**, 1216; (*d*) X. Li, S. Qian, Q. He, B. Yang, J. Li and Y. Hu, *Org. Biomol. Chem.*, 2010, **8**, 3627; (*e*) B. Zhu, X. Zhang, Y. Li, P. Wang, H. Zhang and X. Zhuang, *Chem. Commun.*, 2010, **46**, 5710; (*f*) C. Zhao, Y. Zhou, Q. Lin, L. Zhu, P. Feng, Y. Zhang and J. Cao, *J. Phys. Chem. B*, 2011, **115**, 642; (*g*) X. Cao, W. Lin and Q. Yu, *J. Org. Chem.*, 2011, **76**, 7423; (*h*) K. Cui, Z. Chen, Z. Wang, G. Zhang and D. Zhang, *Analyst*, 2011, **136**, 191; (*i*) L. Long, W. Lin, B. Chen, W. Gao and L. Yuan, *Chem. Commun.*, 2011, **47**, 893; (*j*) C. S. Lim, G. Masanta, H. J. Kim, J. H. Han, H. M. Kim and B. R. Cho, *J. Am. Chem. Soc.*, 2011, **133**, 11132; (*k*) M. H. Lee, J. H. Han, P.-S. Kwon, S. Bhuniya, J. Y. Kim, J. L. Sessler, C. Kang and J. S. Kim, *J. Am. Chem. Soc.*, 2012, **134**, 1316; (*l*) Q. Lin, C. Bao, S. Cheng, Y. Yang, W. Ji and L. Zhu, *J. Am. Chem. Soc.*, 2012, **134**, 5052; (*m*) D. Maity, V. Kumar and T. Govindaraju, *Org. Lett*, 2012, **14**, 6008.

12 (*a*) H. Maeda, H. Matsuno, M. Ushida, K. Katayama, K. Saeki and N. Itoh, *Angew. Chem., Int. Ed.*, 2005, **44**, 2922; (*b*) H. Maeda, K. Katayama, H. Matsuno and T. Uno, *Angew. Chem., Int. Ed.*, 2006, **45**, 1810; (*c*) W. Jiang, Q. Fu, H. Fan, J. Ho and W. Wang, *Angew. Chem., Int. Ed.*, 2007, **46**, 8445; (*d*) J. Bouffard, Y. Kim, T. M. Swager, R. Weissleder and S. A. Hilderbrand, *Org. Lett.*, 2008, **10**, 37; (*e*) A. Shibata, K. Furukawa, H. Abe, S. Tsuneda and Y. Ito, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 2246; (*f*) S. Ji, J. Yang, Q. Yang, S. Liu, M. Chen and J. Zhao, *J. Org. Chem.*, 2009, **74**, 4855; (*g*) H. Guo, Y. Jing, X. Yuan, S. Ji, J. Zhao, X. Li and Y. Kan, *Org. Biomol. Chem.*, 2011, **9**, 3844; (*h*) J. Shao, H. Sun, H. Guo, S. Ji, J. Zhao, W. Wu, X. Yuan, C. Zhang and T. D. James, *Chem. Sci.*, 2012, **3**, 1049.

13 (*a*) W. M. Leevy, S. T. Gammon, H. Jiang, J. R. Johnson, D. J. Maxwell, E. N. Jackson, M. Marquez, D. Piwnica-Worms and B. D. Smith, *J. Am. Chem. Soc.*, 2006, **128**, 16476; (*b*) K. Kiyose, H. Kojima and T. Nagano, *Chem.–Asian. J.*, 2008, **3**, 506; (*c*) S. Atilgan, T. Ozdemir and E. U. Akkaya, *Org. Lett.*, 2008, **10**, 4065; (*d*) X. Chen, S. W. Nam, G. H. Kim, N. Song, Y. Jeong, I. Shin, S. K. Kim, J. Kim, S. Park and J. Yoon, *Chem. Commun.*, 2010, **46**, 8953; (*e*) Z. Guo, W. Zhu, M. Zhu, X. Wu and H. Tian, *Chem.–Eur. J.*, 2010, **16**, 14424.

14 N. Karton-Lifshin, E. Segal, L. Omer, M. Portnoy, R. Satchi-Fainaro and D. Shabat, *J. Am. Chem. Soc.*, 2011, **133**, 10960.

15 W. Lin, L. Yuan, Z. Cao, Y. Feng and L. Long, *Chem.–Eur. J.*, 2009, **15**, 5096.

16 L. Yi, H. Li, L. Sun, L. Liu, C. Zhang and Z. Xi, *Angew. Chem., Int. Ed.*, 2009, **121**, 4094.

 