A highly selective ratiometric fluorescent probe for 1,4-dithiothreitol (DTT) detection†

Baocun Zhu,[a] Xiaoling Zhang,*[a] Hongying Jia,[b] Yamin Li,[a] Haipeng Liu[c] and Weihong Tan*[c]

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A highly selective ratiometric fluorescent probe, which contains an aminonaphthalimide fluorophore and a self-immolative spacer for 1,4-dithiothreitol (DTT) detection was designed and synthesized. The probe displays a 66 nm red-shift of fluorescence emission and the color changes from colorless to jade-green upon reaction with DTT. These properties are mechanistically ascribed to the strong reducing capability of DTT.

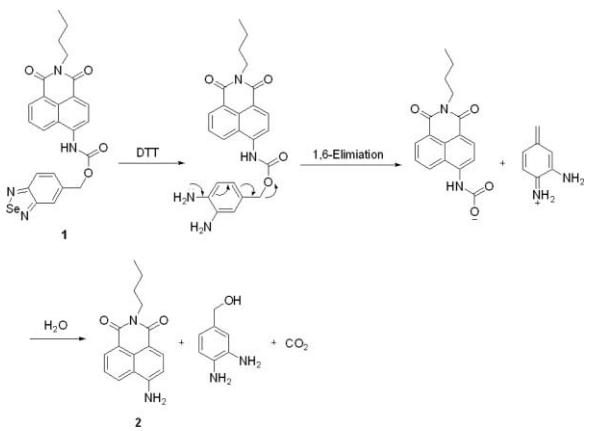
Introduction

1,4-Dithiothreitol (DTT),[1] a synthetic thiol, has been widely used in cell biology, biochemistry, and biomedical application. For example, DTT has been used as a powerful reducing agent,[2] an antidote protecting cells and tissues,[3] and a radioprotector,[4] to name a few. However, it is also noted that DTT is a highly toxic substance.[5] It can cause oxidative damage of biomolecules in the presence of transition metals[6] and enhance the toxic action of some arsenic- and mercury-containing compounds.[7] Therefore, developing an appropriate analytical probe for selective detection of DTT is central for the safe use of this important substance.

Among the various methods used to detect thiols, fluorescent probes are commonly developed due to their operational simplicity and high sensitivity.[8] However, current probes show that most of them have poor selectivity between biothiols and DTT.[8d,e,g,j,k] Accordingly, fluorescent indicators that can effectively discriminate DTT from biothiols are needed. Although some probes[9] for the detection and discrimination of specific thiols have been reported, to the best of our knowledge, there has been no specific fluorescent indicator reported for DTT. Additionally, most of current probes detect thiols only by intensity-responsive fluorescence signal, which is known to be disturbed in quantitative detection by many factors, such as variabilities in excitation and emission efficiency, sample environments, and probe distribution. Ratiometric fluorescent probes can eliminate most or all ambiguities by self-calibration of two emission bands.[10] In this paper, we describe a novel, highly selective, colorimetric and ratiometric fluorescent DTT probe **1** containing self-immolative spacer based on the internal charge transfer (ICT) mechanism.

Results and discussion

Very recently, Chang *et al.*[11] designed a novel ratiometric fluorescent probe PL1 for H_2O_2 and PL1 features a chemospecific H_2O_2 switch to modulate ICT with a marked blue-to-green emission color change. This strategy inspired us to design ratiometric fluorescent probes for other analytes. As illustrated in Scheme 1, our probe can be divided into three distinct parts. The first part is a fluorophore, 4-aminonaphthalimide[12] was chosen due to its desirable spectroscopic properties and outstanding ICT structure. The second part consisted of an organoselenium[8j,k,13] as DTT receptor. Finally, the third part, 3,4-diaminophenyl methanol, a self-immolatvie spacer was chosen to connect the fluorophore and receptor. We hypothesize that reaction of **1** with DTT triggers the cleavage of piazselenole-based carbamate protecting group, as a result, restores the green fluorescence of 4-aminonaphthalimide **2**. The reaction mechanism of **1** with DTT is shown in Scheme 1. Scheme 2 outlines the synthesis of probe **1**. The detailed synthetic procedures and characterizations are described in the Experimental Section.

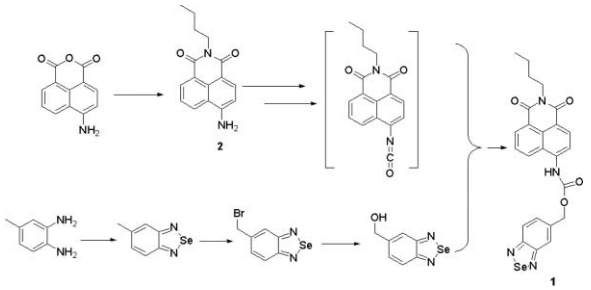


Scheme 1 Reaction mechanism of **1** with DTT.

The spectral properties of **1** were measured under a mixture of ethanol and water (1 : 1, v/v) solution containing phosphate buffered saline (PBS) (20 mM, pH 7.4). **1** exhibits hunchbacked absorption band (absorption peak around 340 nm from piazselenole and around 370 nm from carbamate-derived naphthalimide)

[a]*Department of Chemistry, School of Science, Beijing Institute of Technology, Beijing 100081, P. R. China. E-mail: Zhangxl@bit.edu.cn*
[b]*Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, P. R. China*
[c]*Center for Research at Bio/nano Interface, Department of Chemistry and Department of Physiology and Functional Genomics, Shands Cancer Center, UF Genetics Institute and McKnight Brain Institute, University of Florida, Gainesville, Florida 32611-7200, USA. E-mail: tan@chem.ufl.edu*

† Electronic supplementary information (ESI) available: The determination of quantum yield, additional spectroscopic data, ^{1}H-NMR, ^{13}C-NMR and MS spectra of probe **1**. See DOI: 10.1039/b923754b



Scheme 2 The synthesis of probe **1**.

(Fig. 1). When DTT was added to the solution of **1**, the maximum absorption peak showed a 60 nm red shift (Fig. 1) and the color of the solution turned from colorless to jade-green (Fig. S1, ESI†). In the fluorescence emission spectrum, **1** displays the maximum emission peak at 461 nm with a quantum yield of 0.69 (ESI†). Upon addition of DTT (final concentration: 100 mM), the maximum emission peak undergoes a red shift to 527 nm with a quantum yield of 0.48, and the ratio of fluorescence intensities (F_{527}/F_{461}) changes from 0.2 to 19 (R = 95-fold). Moreover, a ratiometric response was observed when adding different concentrations of DTT to the solution of **1**. A well-defined isoemission point at 507 nm can be clearly seen (Fig. 2a). Also, there was a good linearity between the ratio of fluorescence intensities (F_{461}/F_{527}) and concentrations of DTT in the range of 5 to 60 mM with a linear coefficient of 0.9916 (Fig. 2b). This data demonstrated that probe **1** could detect DTT qualitatively and quantitatively by ratiometric fluorescence method. Therefore, **1** is a very promising probe for the detection of DTT in environmental and life science because DTT become toxic at a higher concentration (10 mM).[14]

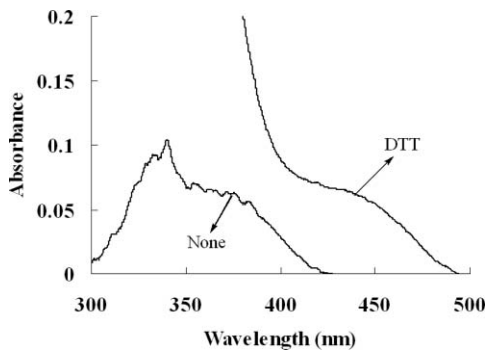


Fig. 1 Absorption response of **1** (5 μM) toward DTT (final concentration: 100 mM) in PBS (20 mM) solution (ethanol/water = 1 : 1, v/v, pH 7.4). Each spectrum was acquired 10 h after DTT addition at 25 °C.

The selectivity of probe **1** toward DTT was evaluated by testing the response of the assay to physiologically important reducing agent ascorbic acid (Vc) and other environmentally relevant thiols, including L-cysteine (L-Cys), β-mercaptoethylamine (MEA), thioglycerol (TG), thioglycolic acid (TA), and glutathione (GSH) at a concentration of 200 mM. As showed in Fig. 3a, compared with DTT, other thiols and Vc showed very little in the change of the fluorescence intensities ratio (F_{527}/F_{461}). This result is inconsistent with the previously reported conclusion by Tang *et al.*[8j,k] and Zhang *et al.*[13] We suspect that the reaction of **1** with DTT is mainly triggered by the strong reducing capability of thiols rather than the reported nucleophilic substitution of sulfhydryl.

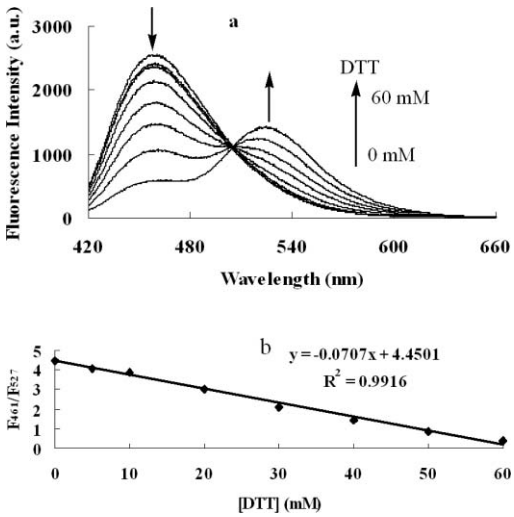


Fig. 2 Fluorescence response of **1** (5 μM) toward different concentration of DTT (final concentration: 0, 5, 10, 20, 30, 40, 50, 60 mM) in PBS (20 mM) solution (ethanol/water = 1 : 1, v/v, pH 7.4). Excitation wavelength was 410 nm, excitation and emission slit widths were 2.5 nm and 5.0 nm. (a) Fluorescence spectra of **1** in the presence of increasing concentrations of DTT; (b) Fluorescence intensity ratio F_{461}/F_{527} of **1** *versus* increasing concentrations of DTT. Each spectrum was acquired 10 h after DTT addition at 25 °C.

To confirm this hypothesis, 1,2-dimercaptoethane (200 mM), a reducing compound similar to DTT, was added to the solution of **1**, and a relative large change of the intensity ratio (R = 26-fold) was observed (Fig. 4). According to the previous reports,[15] this result favors the reducing ability of thiols as a key element in the initiation of the fluorescence response. Therefore, in this regard, we believe that **1** will have great potential for studying the redox state in living systems because the normal reducing environment in the cytosol equalled to a solution containing 10 mM DTT.[16] We next studied the effect of interference of above-mentioned analytes on monitoring DTT. The results show that the probe **1** possesses high selectivity toward DTT when present with other analytes (Fig. 3b).

To confirm the reaction mechanism of **1** with DTT, the reaction of **1** with DTT was conducted under the same conditions as described above. The unique fluorescent reaction product was obtained and characterized by ^{1}H NMR, ^{13}C NMR and HRMS[17] to be compound **2**. Additionally, to further demonstrate the reaction of **1** with DTT by thiols, we added *N*-ethylmaleimide (NEM, a known thiol-blocking agent) to the system. The addition of thiol-blocking agent, however, showed no obvious fluorescence emission peak at 527 nm (Fig. 5). The above data indicate that the probe most likely undergoes a designed mechanism as showed in Scheme 1.

Next, to further demonstrate the practical application of the probe, we carried out experiments in living cells. HeLa cells incubated with the probe **1** (10 μM) for 1 h showed an intense intracellular fluorescence in the cytosol (Fig. 6a, b). The results reveal that the probe **1** can penetrate the cell membrane and labeled designedly the cytosol. Furthermore, HeLa cells were pretreated with 50 μM *N*-ethylmaleimide (as a thiol-blocking reagent, NEM) for 2 h to decrease the reducing capability because reduced thiols maintain the reducing environment in living cells, and then they were incubated with the probe **1** (10 μM) for another 1 h. Distinct

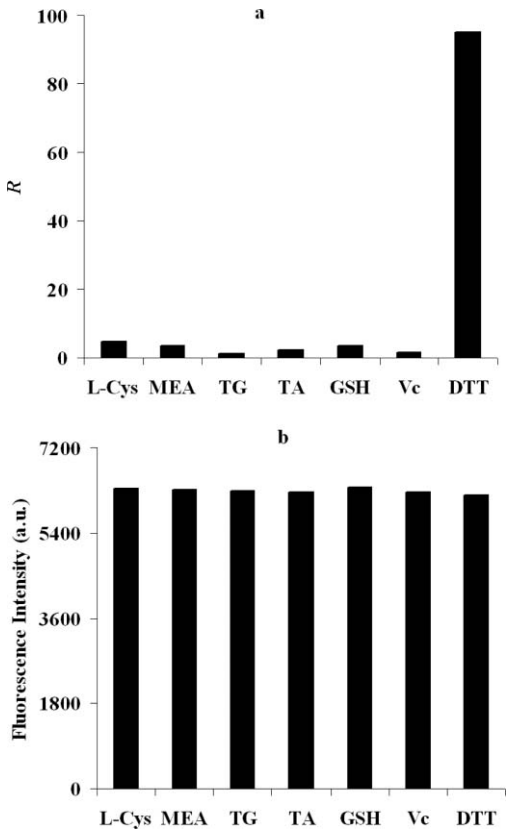


Fig. 3 (a) Fluorescence response of **1** (5 μM) to different thiols and Vc (200 mM). Thiols: L-Cys (200 mM), MEA (200 mM), TG (200 mM), TA (200 mM), GSH (200 mM) and dithiothreitol (DTT) (100 mM). Bars represent the change of fluorescence intensities ratio (F_{527}/F_{461}). Excitation wavelength was 410 nm. (b) Fluorescence response of **1** (5 μM) to DTT (100 mM) in the absence and presence of other analytes (200 mM), including L-Cys, MEA, TG, TA, GSH, and Vc. Bars represent the fluorescence intensity at 527 nm. Excitation wavelength was 430 nm. Each spectrum was acquired 10 h after various analytes addition at 25 °C.

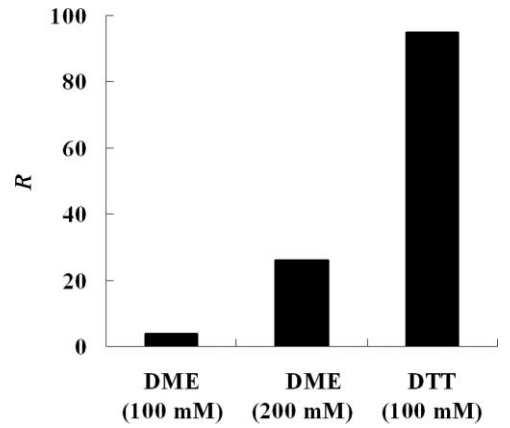


Fig. 4 Fluorescence response of **1** (5 μM) to 1,2-dimercaptoethane (DME) and DTT in PBS (20 mM) solution (ethanol/water = 1 : 1, v/v, pH 7.4). Bars represent the change of fluorescence intensities ratio (F_{527}/F_{461}). Excitation wavelength was 410 nm and excitation and emission slit widths were 2.5 nm. Each spectrum was acquired 10 h after various analytes addition at 25 °C.

decrease of green fluorescence in HeLa cells was observed (Fig. 6e). In addition, HeLa cells pretreated with DTT (100 mM) for 30 min

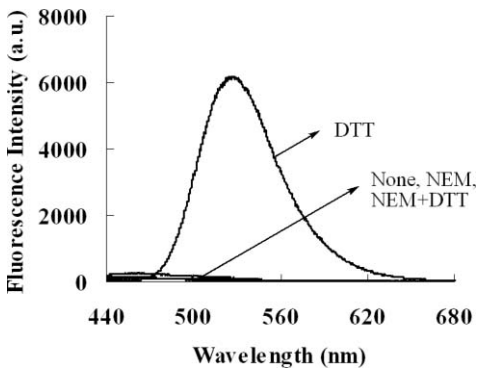


Fig. 5 Fluorescence response of **1** (5 μM) toward NEM, a mixture of NEM and DTT, DTT in PBS (20 mM) solution (ethanol/water = 1 : 1, v/v, pH 7.4). Excitation wavelength was 430 nm, and excitation and emission slit widths were 2.5 nm. Each spectrum was acquired 10 h after various analytes addition at 25 °C.

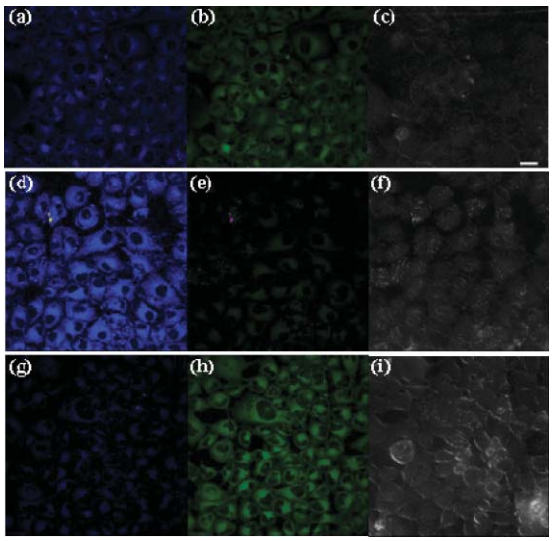


Fig. 6 Confocal fluorescence images of living HeLa cells: HeLa cells incubated with the probe **1** (10 μM) for 1 h (a) blue channel, (b) green channel, and (c) bright-field image; HeLa cells incubated with the probe **1** (10 μM) for 1 h after preincubation with 50 μM NEM for 2 h (d) blue channel, (e) green channel, and (f) bright-field image; HeLa cells incubated with the probe **1** (10 μM) for 1 h after preincubation with 100 mM DTT for 30 min (g) blue channel, (h) green channel, and (i) bright-field image. Incubation was performed at 37 °C under a humidified atmosphere containing 5% CO_2. Scale bar = 20 μm.

were incubated with the probe **1** (10 μM) for another 1 h. The enhancement of green fluorescence was observed (Fig. 6h), which demonstrates the probe **1** can be used for imaging of DTT in living cells. These results also suggest that the fluorescence changes were really due to the changes in the intracellular redox state.

Conclusions

In conclusion, we have presented the synthesis and properties of a new, ICT and naphthalimide fluorescent probe with 3,4-diaminophenyl methanol as self-immolative spacer and organoselenium as receptor. The probe exhibits high DTT-selectivity over biothiols, including cysteine and glutathione, which was ascribed

to the strong reducing capability of DTT. Additionally, the probe displays a 66 nm red-shift of fluorescence emission and the color changes from colorless to jade-green upon addition of DTT, and thus can serve as a "naked-eye" probe for DTT. Importantly, probe **1** can detect DTT quantitatively by ratiometric fluorescence method. The living cell image experiments further demonstrate its value in detecting DTT and studying the changes of redox environment in living systems.

Experimental section

Materials and general methods

All chemicals used in this paper were commercial products of analytical grade. ^{1}H-NMR and ^{13}C-NMR were recorded on a Bruker AV-400 spectrometer with chemical shifts reported as ppm (in $CDCl_3$ or DMSO-d_6, TMS as internal standard). Mass spectral analyses were carried out on a MALDI-TOF spectrometer. IR spectrum was recorded on Nicolet AVATAR FT-IR 360 infrared spectrophotometer using KBr pellet sample. High-resolution mass data were measured with Fourier transform ion cyclotron resonance mass spectrometer (APEX IV). Absorption spectra were recorded on TU-1901 UV-vis spectrophotometer. Fluorescence emission and excitation spectra were measured on Hitachi F-7000. All pH measurements were made with a Sartorius basic pH-meter PB-10.

Cell culture

HeLa cells (gifted from the center of cells, Peking Union Medical College) were cultured in culture media (DMEM/F12 supplemented with 10% FBS, 50 unit/mL penicillin, and 50 μg mL^{-1} of streptomycin) at 37 °C under a humidified atmosphere containing 5% CO_2. HeLa cells were seeded in a 6-well plate at a density of 104 cells per well in culture media. After 24 h, the cells were incubated with 10 μM **1** in culture media for 1 h at 37 °C. HeLa cells were pretreated with 50 μM NEM for 2 h to decrease the reducing capability, and then they were incubated with the probe **1** (10 μM) for another 1 h. In addition, HeLa cells were pretreated with 100 mM DTT for 30 min, and then they were incubated with the probe **1** (10 μM) for another 1 h. After the medium was removed and the cells were carefully washed with PBS for twice, fluorescence imaging of living HeLa cells was observed under confocal fluorescence microscope (excitation light source: Blue; Olympus IX 71 S 8F-2).

Synthesis of probe 1

5-Methyl-2,1,3-benzoselenadiazol. 4-Methylbenzene-1,2-diamine (1.2217 g, 10 mmol) and selenium dioxide (1.1096 g, 10 mmol) were ground respectively, and then mixed in a mortar at room temperature. After 30 min of grinding, the crude products were obtained and dissolved in n-hexane, and then filtered. The solvent was removed under reduced pressure to give the desired product (1.8133 g, 9.2 mmol, 92% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (*10^{-6}): 2.46 (s, 3H), 7.30 (d, J = 9.2 Hz, 1H), 7.57 (s, 1H), 7.70 (d, J = 9.2 Hz, 1H).

5-Bromomethyl-2,1,3-benzoselenadiazol. 5-Methyl-2,1,3-benzoselenadiazol (1.3797 g, 7 mmol), *N*-bromsuccinimide (2.4918 g, 14 mmol) and dibenzoylperoxide (as a catalyzer) were dissolved in 15 mL CCl_4 and 7 mL $CHCl_3$. The resulting solution was heated to reflux for 5 h. The hot reaction mixture was filtrated and the portion of solvent was removed under reduced pressure. After cooling to room temperature, the crystal product (1.0487 g, 3.8 mmol, 54% yield) was obtained after filtration. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (*10^{-6}): 4.58 (s, 2H), 7.51 (d, J = 9.2 Hz, 1H), 7.81–7.84 (m, 2H).

2,1,3-Benzoselenadiazol-5-methanol. 5-Bromomethyl-2,1,3-benzoselenadiazol (552.0 mg, 2 mmol) was suspended in 100 mL H_2O containing K_2CO_3 (621.9 mg, 4.5 mmol). The resulting solution was heated to reflux for 30 min under nitrogen atmosphere. After cooling to room temperature, the crystal product (277.6 mg, 1.3 mmol, 65% yield) was obtained after filtration. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (*10^{-6}): 2.37 (s, 1H), 4.81 (s, 2H), 7.43 (d, J = 9.2 Hz, 1H), 7.76–7.78 (m, 2H).

Probe 1. To a mixture of **2**[18] (268.3 mg, 1 mmol) and DIPEA (439.4 mg, 3.4 mmol) in 20 mL toluene was added a solution of triphosgene (305.9 mg, 1 mmol) in 5 mL toluene dropwise. The resulting solution was heated to reflux for 3 h. After cooling to room temperature, the reaction mixture was diluted with 20 mL THF and filtered. After removal of the solvents, to the residues was added the 2,1,3-benzoselenadiazol-5-methanol (213.1 mg, 1 mmol) in 20 mL THF. The solution was stirred at room temperature for an additional 12 h. After removal of THF, the residues were purified by silica gel column chromatography using chloroform as eluent to afford 1 (264.9 mg, 0.52 mmol, 52% yield). ^{1}H-NMR (400 MHz, DMSO-d_6) δ (*10^{-6}): 0.93 (t, J = 7.4 Hz, 3H), 1.32–1.38 (m, 2H), 1.60–1.63 (m, 2H), 4.04 (t, J = 7.4 Hz, 2H), 5.43 (s, 2H), 7.64 (d, J = 8.6 Hz, 1H), 7.84–7.91 (m, 2H), 7.98 (s, 1H), 8.23 (d, J = 8.0 Hz, 1H), 8.48–8.52 (m, 2H), 8.75 (d, J = 8.8 Hz, 1H), 10.47 (s, 1H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ (*10^{-6}): 14.1, 20.2, 30.1, 66.2, 117.7, 118.7, 121.4, 122.7, 123.7, 124.4, 126.9, 128.8, 129.7, 129.8, 131.4, 132.1, 138.4, 141.0, 154.3, 159.8, 160.1, 163.4, 163.9. IR (KBr, cm^{-1}): 3267, 2956, 2865, 1697, 1652, 1544, 1358, 1236, 1062, 783, 658. MALDI-TOF calcd for $C_{24}H_{21}N_4O_4Se$ [M+H]$^+$ 509.1, found 509.1. HRMS (ESI positive) calcd for $C_{24}H_{21}N_4O_4Se$ [M+H]$^+$ 509.07239, found 509.07176.

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Notes and references

1 (*a*) R. M. Evans, J. B. Fraser and L. N. Owen, *J. Chem. Soc.*, 1949, 248–258; (*b*) W. W. Cleland, *Biochemistry*, 1964, **3**, 480–483.
2 (*a*) M. C. Alliegro, *Anal. Biochem.*, 2000, **282**, 102–106; (*b*) B. Ates, B. C. Ercal, K. Manda, L. Abraham and N. Ercal, *Biomed. Chromatogr.*, 2009, **23**, 119–123.
3 (*a*) K. I. Ahammadsahib, R. R. Jinna and D. Desaiah, *Cell Biochem. Funct.*, 1989, **7**, 185–192; (*b*) M. M. de Mecca, G. D. Castro and J. A. Castro, *Arch. Toxicol.*, 1993, **67**, 547–551; (*c*) L. E. Rikans and Y. Cai, *Toxicology*, 1994, **86**, 147–161; (*d*) H. Hu, G. Moller and M. Abedi-Valugerdi, *Clin. Exp. Immunol.*, 1997, **107**, 68–75.
4 I. G. Kim, S. Y. Park and T. J. Oh, *Arch. Biochem. Biophys.*, 2001, **388**, 1–6.
5 (*a*) H. P. Misra, *J. Biol. Chem.*, 1974, **249**, 2151–2155; (*b*) A. V. Kachur, K. D. Held, C. J. Koch and J. E. Biaglow, *Radiat. Res.*, 1997, **147**, 409–415.

6 (*a*) N. Spear and S. D. Aust, *J. Biochem. Mol. Toxicol.*, 1998, **12**, 125–132; (*b*) D. J. Taatjes, G. Gaudiano and T. H. Koch, *Chem. Res. Toxicol.*, 1997, **10**, 953–961.

7 (*a*) J. R. Gurr, D. T. Bau, F. Liu, S. Lynn and K. Y. Jan, *Mol. Pharmacol.*, 1999, **56**, 102–109; (*b*) B. Hultberg, A. Andersson and A. Isaksson, *Toxicology*, 2001, **156**, 93–100.

8 (*a*) S. Sreejith, K. P. Divya and A. Ajayaghosh, *Angew. Chem., Int. Ed.*, 2008, **47**, 7883–7887; (*b*) L. Duan, Y. Xu, X. Qian, F. Wang, J. Liu and T. Cheng, *Tetrahedron Lett.*, 2008, **49**, 6624–6627; (*c*) T. Kim, D. Lee and H. Kim, *Tetrahedron Lett.*, 2008, **49**, 4879–4881; (*d*) J. Bouffard, Y. Kim, T. M. Swager, R. Weissleder and S. A. Hilderbrand, *Org. Lett.*, 2008, **10**, 37–40; (*e*) M. M. Pires and J. Chmielewski, *Org. Lett.*, 2008, **10**, 837–840; (*f*) W. Lin, L. Long, L. Yuan, Z. Cao, B. Chen and W. Tan, *Org. Lett.*, 2008, **10**, 5577–5580; (*g*) S. Banerjee, S. Kar, J. M. Perez and S. Santra, *J. Phys. Chem. C*, 2009, **113**, 9659–9663; (*h*) Y. Zhang, Y. Li and X. Yan, *Anal. Chem.*, 2009, **81**, 5001–5007; (*i*) B. Han, J. Yuan and E. Wang, *Anal. Chem.*, 2009, **81**, 5569–5573; (*j*) B. Tang, Y. Xing, P. Li, N. Zhang, F. Yu and G. Yang, *J. Am. Chem. Soc.*, 2007, **129**, 11666–11667; (*k*) B. Tang, L. Yin, X. Wang, Z. Chen, L. Tong and K. Xu, *Chem. Commun.*, 2009, 5293–5295.

9 (*a*) W. Wang, O. Rusin, X. Xu, K. K. Kim, J. O. Escobedo, S. O. Fakayode, K. A. Flectcher, M. Lowry, C. M. Schowalter, C. M. Lawrence, F. R. Fronczek, I. M. Warner and R. M. Strongin, *J. Am. Chem. Soc.*, 2005, **127**, 15949–15958; (*b*) H. Chen, Q. Zhao, Y. Wu, F. Li, H. Yang, T. Yi and C. Huang, *Inorg. Chem.*, 2007, **46**, 11075–11081; (*c*) H. Li, J. Fan, J. Wang, M. Tian, J. Du, S. Sun, P. Sun and X. Peng, *Chem. Commun.*, 2009, 5904–5906.

10 (*a*) Y. Zhang, X. Guo, W. Si, L. Jia and X. Qian, *Org. Lett.*, 2008, **10**, 473–476; (*b*) X. Zhang, Y. Xiao and X. Qian, *Angew. Chem., Int. Ed.*, 2008, **47**, 8025–8029.

11 D. Srikun, E. W. Miller, D. W. Domaille and C. J. Chang, *J. Am. Chem. Soc.*, 2008, **130**, 4596–4597.

12 (*a*) A. P. de Silva, H. Q. N. Gunaratne, J. Habib-Jiwan, C. P. McCoy, T. E. Rice and J. Soumillion, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 1728–1731; (*b*) S. R. Greenfield, W. A. Svec, D. Gosztola and M. R. J. Wasielewski, *J. Am. Chem. Soc.*, 1996, **118**, 6767–6777; (*c*) Y. Q. Gao and R. A. Marcus, *J. Phys. Chem. A*, 2002, **106**, 1956–1960; (*d*) H. He, M. A. Mortellaro, M. J. P. Leiner, R. J. Fraatz and J. K. Tusa, *J. Am. Chem. Soc.*, 2003, **125**, 1468–1469; (*e*) H. He, M. A. Mortellaro, M. J. P. Leiner, S. T. Young, R. J. Fraatz and J. K. Tusa, *Anal. Chem.*, 2003, **75**, 549–555; (*f*) X. Guo, X. Qian and L. Jia, *J. Am. Chem. Soc.*, 2004, **126**, 2272–2273.

13 W. Wang, L. Li, S. Liu, C. Ma and S. Zhang, *J. Am. Chem. Soc.*, 2008, **130**, 10846–10847.

14 (*a*) C. Riondet, R. Cachon, Y. Wache, G. Alcarez and C. Divies, *Eur. J. Biochem.*, 1999, **262**, 595–599; (*b*) G. Kirakosyan, K. Bagramyan and A. Trchounian, *Biochem. Biophys. Res. Commun.*, 2004, **325**, 803–806.

15 (*a*) R. Singh and G. M. Whitesides, *J. Org. Chem.*, 1991, **56**, 2332–2337; (*b*) J. Liu, H. Liu, Z. Jia, V. Bulmus and T. P. Davis, *Chem. Commun.*, 2008, 6582–6584.

16 M. A. Kostiainen and H. Rosilo, *Chem.–Eur. J.*, 2009, **15**, 5656–5660.

17 ^{1}H NMR, ^{13}C NMR and HRMS data of compound **2**: ^{1}H-NMR (400 MHz, DMSO-d_6) δ (*10-6): 0.92(t, J = 7.4 Hz, 3H), 1.27-1.36(m, 2H), 1.55-1.60(m, 2H), 4.01(t, J = 7.4 Hz, 2H), 6.85(d, J = 8.4 Hz, 1H), 7.43(s, 2H), 7.65(t, J = 7.8 Hz, 1H), 8.19(d, J = 8.4 Hz, 1H), 8.42(d, J = 7.2 Hz, 1H), 8.61(d, J = 7.6 Hz, 1H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ (*10-6): 13.96, 20.46, 30.20, 40.85, 121.91, 123.53, 123.74, 125.26, 129.06, 130.23, 131.46, 134.14, 134.94, 149.55, 162.08, 162.87. HRMS (ESI positive) calcd for $C_{16}H_{17}N_2O_2$ $[M+H]^+$ 269.12845, found 269.12817; calcd for $C_{16}H_{16}N_2NaO_2$ $[M+Na]^+$ 291.11040, found 291.11020.

18 H. Tian, T. Xu, Y. Zhao and K. Chen, *J. Chem. Soc., Perkin Trans. 2*, 1999, 545–549.

