Organic LETTERS

Letter

pubs.acs.org/OrgLett

Selective and Sensitive Superoxide Detection with a New *Diselenide*-Based Molecular Probe in Living Breast Cancer Cells

Sudesh T. Manjare,[†,‡] Sungsoo Kim,[§] Won Do Heo,[§] and David G. Churchill*[,‡]

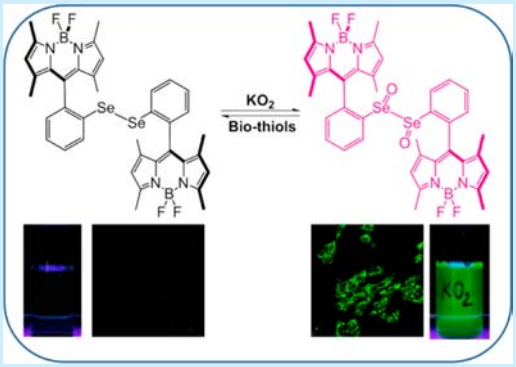
[†]Center for Catalytic Hydrocarbon Functionalizations, Institute for Basic Science (IBS), 373-1 Guseong-dong, Yuseong-gu, Daejeon, 305-701, Republic of Korea

[‡]Molecular Logic Gate Laboratory, Department of Chemistry, Korea Advanced Institute of Science and Technology (KAIST), 373-1 Guseongdong, Yuseong-gu, Daejeon, 305701, Republic of Korea

[§]Bio-Imaging & Cell Signaling Lab, Department of Biological Sciences, Korea Advanced Institute of Science and Technology (KAIST), 373-1 Guseong-dong, Yuseong-gu, Daejeon, 305-701, Republic of Korea

Supporting Information

ABSTRACT: A diselenide-based BODIPY probe was prepared; it was found to be sensitive and selective for superoxide in giving [-Se(O)Se(O)-] oxidation. Probing was reversible through the use of biothiols; ^{77}Se NMR and other types of spectroscopy were employed. Practical medicinal utility was demonstrated in MCF-7/ADR cancer cells.

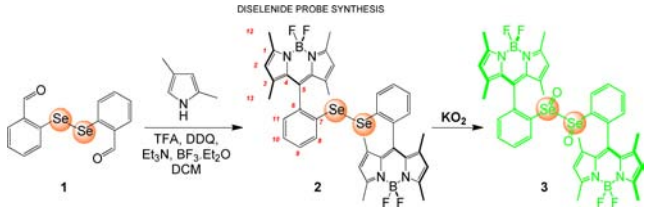


Reactive oxygen species (ROS) exist in and are utilized by biology and neurobiology, yet they constitute the so-called oxidative stress in unwanted excess. This may be a major contributing factor for diseases such as diabetes, cancer, and molecular neurodegenerative disorders such as Alzheimer's and Parkinson's disease.[1] Reactive oxygen species (ROS) are often mentioned in conjunction with nitrogen-based analogues (RNS), under various species such as HOCl, $OH^{\bullet}$, H_2O_2, NO, $ONOO^-$, $O_2^{\bullet-}$, ^{t}BuOOH, and ^{t}BuO$^{\bullet}$. Sensitive and selective detection of such species with fluorescence microscopy requires discrete synthetic molecular designs and facile syntheses. These designs of potential probes and their facile syntheses make this field important and challenging.[2]

Superoxide ($O_2^{\bullet-}$) is a particularly important ROS and has a short half-life; therefore, its real-time detection has become an important yet complex issue. It is often discussed that superoxide has elaborate chemistry such as being able to combine with NO to form peroxynitrite.[3] Various fluorescent probes for the detection of superoxide have been designed and developed.[4] Selenium is known to play an important antioxidant role in the active site of certain enzymes; this action converts peroxide species (vide supra) to water.[5] Several organoselenium compounds have been synthesized and used for mimicking these redox transformations.[6] However, in recent years, sulfur-[7] and selenium-based compounds[8] have emerged as a potential sensors for the detection ROS. As per our knowledge, until now, only a fluorescein-based diselenide probe for the detection of hydrogen peroxide has been reported in the literature.[9] Thus, in order to study the nature of the BODIPY-based diselenide probe in ROS sensing, we have designed a novel BODIPY-based probe featuring a diselenide unit that is studied in selective and sensitive fluorescent probing of superoxide.

Synthesis of the selenium-containing compound **2** is outlined in Scheme 1. Bis(*O*-formyl-phenyl)diselenide (**1**)[10] was treated

Scheme 1. Synthesis of Bis(BODIPY)diselenide 2 and 3



with 4.5 equiv of 2,4-dimethylpyrrole in the presence of a catalytic amount of trifluoroacetic acid (TFA) under nitrogen, followed by DDQ, triethylamine, and $BF_3{\cdot}Et_2O$ in dichloromethane. The reaction progress was monitored by TLC. The compound was characterized by common spectroscopy techniques (^{1}H, ^{13}C, ^{77}Se NMR spectroscopy and MS spectrometry). The ^{77}Se signal for bis(BODIPY)diselenide

Received: November 15, 2013
Published: December 26, 2013

dx.doi.org/10.1021/ol4033013 | *Org. Lett.* 2014, 16, 410–412

(**2**) was observed at δ 398 ppm, significantly upfield-shifted compared to bis(*O*-formyl-phenyl)diselenide (468 ppm)[10] signifying notable electronic differences. Compound **2** is the first example of bis(BODIPY)diselenide for ROS detection.

The diselenide-bearing **2** led us to thoroughly analyze ROS responses arising from the anticipated chalcogen oxidation. Spectroscopic properties of **2** were determined in acetonitrile and water (70:30). When the sensing ability of the probe was tested with various ROS (HOCl, $OH^{\bullet}$, H_2O_2, $O_2^{\bullet-}$, ^{t}BuOOH, and ^{t}BuO$^{\bullet}$) in acetonitrile and water (70:30), bis(BODIPY)-diselenide **2** showed a strong green fluorescence for superoxide ($O_2^{\bullet-}$) in a selective fashion over other ROS through oxidation at selenium (Figure 1).

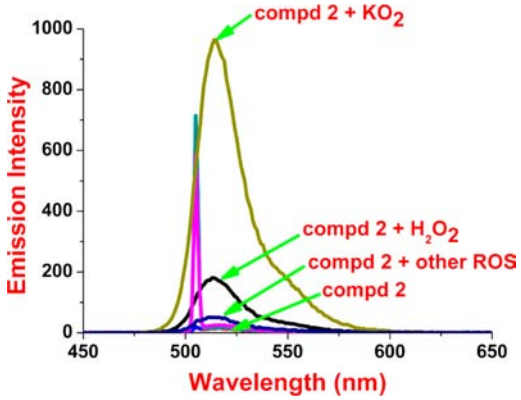


Figure 1. Emission spectra of compound **2** (45×10^{-6} M, acetonitrile/water 70:30) with ROS ($O_2^{\bullet-}$, H_2O_2, OCl^-, ^{t}BuOOH, $OH^{\bullet}$, and ^{t}BuO$^{\bullet}$) (~ 7 equiv in water) incubated for 30 min at rt (λ_{ex} = 504 nm and λ_{em} = 514 nm).

The quantitative emission spectra were obtained for bis(BODIPY)diselenide **2** with various ROS; however, superoxide ($O_2^{\bullet-}$) was the only species to show potential increase in fluorescence. The λ_{max} of absorption and emission for probe **2** was observed at 504 and 514 nm, respectively. In this assay, 3 mL of probe (**2**) solution (45×10^{-6} M, CH_3CN/H_2O 70:30) with 7 equiv of superoxide was used (water). Probe **2** gives a 29-fold increase in fluorescence intensity for superoxide.

A steady increase in fluorescence intensity was detected when the concentration of KO_2 was increased (Figure 2a), and the detection limit was found to be 12.9 μM. The interference study of superoxide with other ROS was performed. This suggested that the hydrogen peroxide and hypochlorite act as oxidants in an additive fashion (Figure S31, Supporting Information). Also, a time-dependent study of probe **2** with

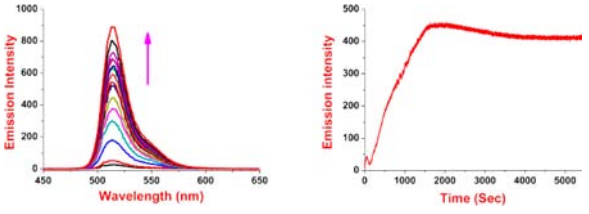


Figure 2. (a) Emission spectra (left) of **2** (45×10^{-6} M, acetonitrile/water 70:30) with increasing concentration of KO_2 (6.67−100 μM in water) incubated for 30 min at rt after each addition. (b) Time-dependent emission spectrum (right) of **2** (45×10^{-6} M, acetonitrile/water 70:30) with KO_2 (~3 equiv in water) (λ_{exc} = 504 nm and λ_{em} = 514 nm).

superoxide over 1.5 h showed a steady increase in fluorescence intensity during the first 30 min before saturation was observed (Figure 2b). The fluorescence quantum yield for probe **2** and oxidized compound **3** were found to be 2 and 58%, respectively (fluorescein in 0.1 M was used as a standard). In order to understand the redox cycling capacity of diselenide in **2**, the solution of **2**(O_{Se},O_{Se}) preoxidized with superoxide was treated with a reducing reagent. Biothiols (glutathione, *N*-acetyl-L-cysteine, D-L-homocysteine, and L-cysteine) were used to determine whether the selenium oxide could revert to its original reduced state (**2**). The obtained results showed a significant decrease in fluorescence intensity after biothiol addition, strongly supporting the regeneration of probe **2**. This may be useful to monitor the dynamic variations of superoxide in living organisms (Figure S33, Supporting Information).

To verify the chemical and photomechanisms involved in the fluorescence "turn on" event, probe **2** was treated with 3 equiv of KO_2. The oxidized compound was isolated and characterized with NMR spectroscopy (^{1}H, ^{13}C, ^{11}B, ^{19}F, and ^{77}Se) and mass spectrometry. The ^{77}Se NMR signal in the spectrum was significantly downfield-shifted (δ 1008 ppm) compared to that for **2** (δ 398 ppm), reflecting mono-oxidation of each selenium (Figure S29, Supporting Information). Also, the mass spectrum of the product confirmed the oxidized dimeric compound **2**(O_{Se},O_{Se}), not **2**$_{0.5}$(O_{Se}) (Figure S26, Supporting Information). This suggested that the ability for the photoinduced electron transfer (PET) from the diphenyldiselenide group to the BODIPY moiety is removed and the probe gives a BODIPY-based fluorescence response. The detailed PET mechanism has been discussed previously in the literature.[8]

Finally, to show the utility of probe **2** to detect superoxide in living systems, we performed an assay of probe **2** with a living breast cancer cell line (MCF-7/ADR) (Figure 3). It has been

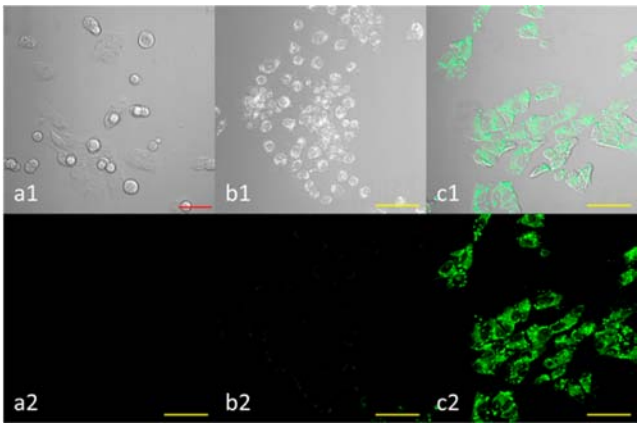


Figure 3. Fluorescence microscopic images of living breast cancer MCF-7/ADR cells. Bright-field and fluorescence images of (a) MCF-7/ADR cells, (b) MCF-7/ADR cells with probe **2**, (c) MCF-7/ADR cells pretreated with PMA (generates superoxide in the cell) and probe **2**. Scale bar length = 10 μm.

known that during normal respiration 1−4% of O_2 consumption results in hydrogen peroxide and superoxide production.[11] It has been reported in the literature that cells contain receptors for PMA (phorbol 12-myristate 13-acetate), a complex lipid which accelerates the production of intracellular superoxide concentration.[12] Therefore, MCF-7/ADR cells were co-incubated with 1 μg/mL of PMA and probe **2** (10 μM) for 30 min at 37 °C under 5% CO_2 incubation; the samples were

dx.doi.org/10.1021/ol4033013 | *Org. Lett.* 2014, 16, 410−412

then washed 3 times with PBS solution (pH = 7.5). Cell imaging data was acquired on a confocal microscope (Nikon A1R). The nonfluorescent cells with PMA gave good fluorescence imaging after incubation with probe **2**. As a result of these MCF-7/ADR cell assays, probe **2** was found to be cell-permeable and selective for the detection of intracellular superoxide.

In conclusion, novel bis(BODIPY)diselenide probe **2** was synthesized, characterized, and studied as a potential molecular ROS probe. The bis(BODIPY)diselenide was found to be sensitive and selective for superoxide detection (quantum yield of the oxidized product = 58% and detection limit = 12.9 μM). Also, the utility of probe **2** for the detection of superoxide in living breast cancer cells has been explored. Reversible generation of the oxidized probe by biothiols was also achieved.

■ ASSOCIATED CONTENT

Supporting Information

Methods, experimental procedures, additional spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: dchurchill@kaist.ac.kr.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Prof. D. G. Churchill (D.G.C.) acknowledges support from the NRF (National Research Foundation) of Korea (2011-0017280). Dr. S. T. Manjare acknowledges the support from Institute of Basic Science (IBS), Korea.

■ REFERENCES

(1) (a) Yap, Y. W.; Whiteman, M.; Cheung, N. S. *Cell. Signal.* **2007**, *19*, 219. (b) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515. (c) Gunnlaugsson, T.; Glynn, M.; Tocci, G. M.; Kruger, P. E.; Pfeffer, F. M. *Coord. Chem. Rev.* **2006**, *250*, 3094. (d) Que, E. L.; Domaille, D. W.; Chang, C. J. *Chem. Rev.* **2008**, *108*, 1517.

(2) (a) Dickinson, B. C.; Chang, C. J. *Nat. Chem. Biol.* **2011**, *7*, 504. (b) Chang, C. J. *Curr. Opin. Chem. Biol.* **2010**, *14*, 50. (c) Winterbourn, C. C. *Nat. Chem. Biol.* **2008**, *4*, 278. (d) Wardman, P. *Free Radical Biol. Med.* **2007**, *43*, 995. (e) Soh, N. *Anal. Bioanal. Chem.* **2006**, *386*, 532.

(3) (a) Chaudhury, S.; Sarkar, P. K. *Biochim. Biophys. Acta* **1983**, *763*, 93. (b) Kozumbo, W. J.; Trush, M. A.; Kensler, T. W. *Chem. Biol. Interact.* **1985**, *54*, 199.

(4) (a) Murale, D. P.; Kim, H.; Choi, W. S.; Churchill, D. G. *Org. Lett.* **2013**, *15*, 3630. (b) Singh, A. P.; Murale, D. P.; Ha, Y.; Liew, H.; Lee, K. M.; Segev, A.; Suh, Y.-H.; Churchill, D. G. *Dalton Trans.* **2013**, *42*, 3285. (c) Hideg, É.; Barta, C.; Kálai, T.; Vass, I.; Hideg, K.; Asada, K. *Plant Cell Physiol.* **2002**, *43*, 1154.

(5) (a) Mukherjee, A. J.; Zade, S. S.; Singh, H. B.; Sunoj, R. B. *Chem. Rev.* **2010**, *110*, 4357. (b) Mugesh, G.; du Mont, W.-W.; Sies, H. *Chem. Rev.* **2001**, *101*, 2125.

(6) (a) Mugesh, G.; Panda, A.; Singh, H. B.; Punekar, N. S.; Butcher, R. J. *J. Am. Chem. Soc.* **2001**, *123*, 839. (b) Iwaoka, M.; Tomoda, S. *J. Am. Chem. Soc.* **1994**, *116*, 2557. (c) Selvakumar, K.; Shah, P.; Singh, H. B.; Butcher, R. J. *Chem.—Eur. J.* **2011**, *17*, 12741. (d) Sarma, B. K.; Mugesh, G. *J. Am. Chem. Soc.* **2005**, *127*, 11477. (e) Nascimento, V.; Alberto, E. E.; Tondo, D. W.; Dambrowski, D.; Detty, M. R.; Nome, F.; Braga, A. L. *J. Am. Chem. Soc.* **2012**, *134*, 138.

(7) (a) Ahrens, J.; Böker, B.; Brandhorst, K.; Funk, M.; Bröring, M. *Chem.—Eur. J.* **2013**, *19*, 11382. (b) Singh, A. P.; Lee, K. M.; Murale, D. P.; Jun, T.; Liew, H.; Suh, Y.-H.; Churchill, D. G. *Chem. Commun.* **2012**, *48*, 7298.

(8) (a) Liu, S.-R.; Wu, S.-P. *Org. Lett.* **2013**, *15*, 878. (b) Wang, B.; Li, P.; Yu, F.; Song, P.; Sun, X.; Yang, S.; Lou, Z.; Han, K. *Chem. Commun.* **2013**, *49*, 1014. (c) Lou, Z.; Li, P.; Pan, Q.; Han, K. *Chem. Commun.* **2013**, *49*, 2445. (d) Wang, B.; Yu, F.; Li, P.; Sun, X.; Han, K. *Dyes Pigm.* **2013**, *96*, 383. (e) Sun, C.; Shi, W.; Song, Y.; Chen, W.; Ma, H. *Chem. Commun.* **2011**, *47*, 8638.

(9) Lou, Z.; Li, P.; Sun, X.; Yang, S.; Wang, B.; Han, K. *Chem. Commun.* **2013**, *49*, 391.

(10) Panda, S.; Zade, S. S.; Singh, H. B.; Wolmershäuser, G. *J. Organomet. Chem.* **2005**, *690*, 3142.

(11) Spitz, D. R.; Sim, J. E.; Ridnour, L. A.; Galoforo, S. S.; Lee, Y. J. *Ann. N.Y. Acad. Sci.* **2000**, *899*, 349.

(12) (a) Lehrer, R. I.; Cohen, L. *J. Clin. Invest.* **1981**, *68*, 1314. (b) Ichinose, Y.; Hara, N.; Ohta, M.; Motohiro, A.; Kuda, T.; Aso, H.; Yagawa, K. *Infect. Immun.* **1989**, *57*, 2529.