A Metal-Free Turn-On Fluorescent Probe for the Fast and Sensitive Detection of Inorganic Azide

Bioorg. Med. Chem. Lett. **2016**, *26*, 1651–1654

Sodium azide is a colorless, tasteless, odorless and salt-like solid that has been widely used in agricultural, laboratory, and medical applications. For example, azide is used in automobile airbags, for pest control, as a preservative, and in chemical research.[1,2] However, in addition to environmental concerns regarding this substance, it has also recently attracted attention for safety issues. In fact, due to its acute toxicity while being odorless and tasteless, several poisoning cases have been reported in the past 20 years.[3–5] Despite the clear public health concerns related to sodium azide, there is no quick detection method available for environmental, medical, and forensic applications: in one case of deliberate azide poisoning, for example, it was reported that it took the FBI five months to determine that azide was the poison used.[5]

Meanwhile, in the field of click chemistry, Huisgen 1,3-dipolar cycloaddition has been commonly used as a useful synthetic tool.[6–8] Because of this reaction's simple operation, fast reaction rate and biocompatibility, it has become an important step in intermediate synthesis in medicinal chemistry. However, a trace amount of sodium azide would affect the bioactivity and cytotoxicity of synthesized drug candidates. As a result, there is a real need for the development of a simple, rapid and accurate azide detection method. The groups of Professor Binghe Wang at Georgia State University (USA) and Professor Geert-Jan Boons at the University of Georgia (USA) have therefore been investigating a method of detecting inorganic azide, resulting in this paper.

"Current sodium azide detection methods include chromatography[9–11] and electrochemical detection,[12–14] which involves complicated procedures and specialized instruments," said Professor Wang. He continued: "Fluorescence has emerged as a simple and rapid detection tool, and a few fluorescent probes for sodium azide have been reported.[15–17] However, each one of them leaves something to be desired including the ability for quantitatively determining azide concentrations.[16] In some cases, interference from other inorganic anions was an issue too.[17] Therefore, we were interested in developing a method for the simple, sensitive, selective, and quantitative detection of sodium azide."

In click chemistry, organic azido compounds (N_3-R) are known to react easily and selectively with terminal alkynes via copper(I)-catalyzed cycloaddition (CuAAC), and strained alkynes without Cu(I) catalysis via strain-promoted azide–alkyne cycloaddition (SPAAC).[18] Unlike organic azido com-

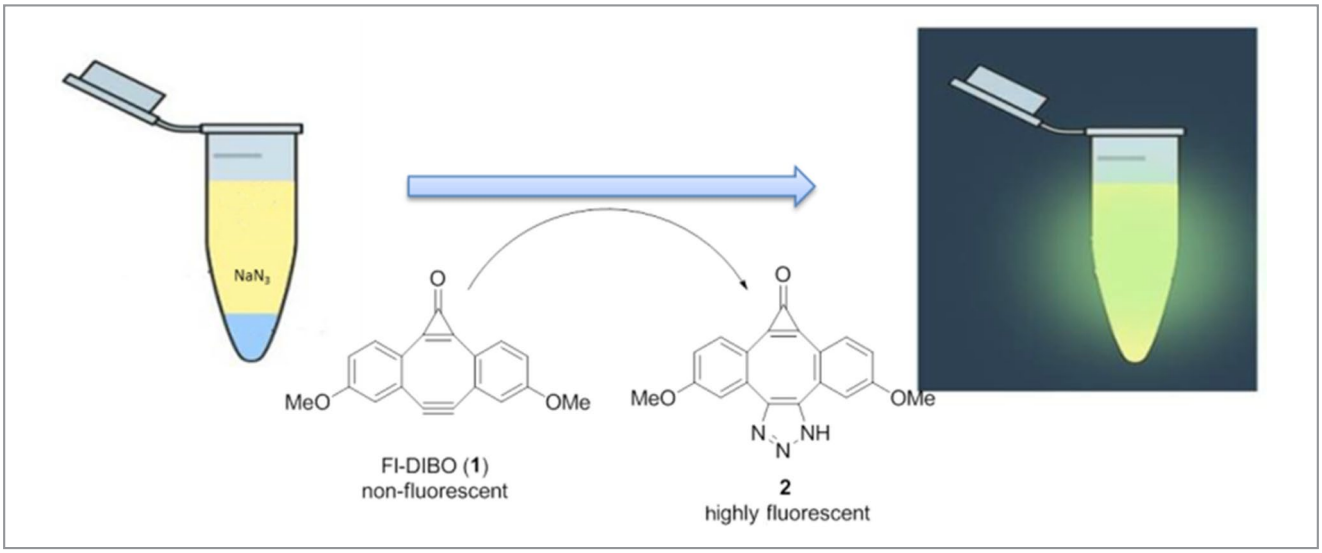


Figure 1 A cartoon representation of the inorganic azide and FI-DIBO reaction

pounds, inorganic azide does not readily undergo the same reaction in most cases. In 2011, the Wang lab reported a liquid chromatography–mass spectrometry (LC-MS) detection method for sodium azide based on the reaction between a strained alkyne, dibenzocycloocta-4a,6a-diene-5,11-diyne (DBA), and inorganic azide.[19]

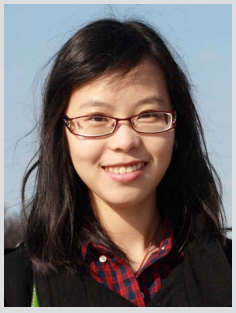
Over the past eight years, the Boons lab has developed many strained dibenzocycloalkyne (DIBO) probes in order to visualize complex glycans in living cells.[20] Interested in modifying the physical properties of these molecules, they also developed a fully water-soluble sulfated analogue S-DIBO[20] and recently a fluorogenic cyclooctyne (Fl-DIBO),[21–23] which only generates fluorescence after a click reaction. Professor Wang said: "Such results triggered our interest in examining whether such a strained alkyne could be used to react with inorganic azide, leading to a fluorescent cycloaddition product for azide detection using fluorescence."

Professor Wang continued: "To demonstrate the design, we used Fl-DIBO to react with sodium azide in a mixture of dioxane and H_2O (1:1). Because of the nonpolar nature of DIBO, 50% of organic solvent was required to fully dissolve the probe. A highly fluorescent product was obtained and characterized (Figure 1). Other chemosensor properties were examined as well, leading to the conclusion that this strained alkyne compound was suitable as a fast, sensitive and selective probe for inorganic azide."

Figure 2 Fluorescence responses of Fl-DIBO to sodium azide in a tea sample; Fl-DIBO 100 μM, NaN_3 (1.12 mg/cup) in a mixture of tea solution and dioxane (1:1) at pH 7.4

To test the utility of this sodium azide probe in real life, tea samples were prepared with azide at various concentrations. This probe showed concentration-dependent fluorescence intensity changes upon addition of sodium azide. "The sensitivity of the detection method is in a pathologically relevant range," said Professor Wang. As seen in Figure 2, the fluorescence emission can be easily observed by the naked eye (λ_{ex} = 363 nm). Professor Wang concluded: "This probe showed excellent potential to be applied in real samples for azide detection. We expect quick and accurate determination of the existence and concentration of inorganic azide in aqueous and organic solutions using this simple method."

About the authors



Dr. K. Wang

Ke Wang received her B.S. degree in chemistry from Lanzhou University (P. R. of China) in 2010, and then moved to Georgia State University (USA) to pursue her Ph.D. in medicinal chemistry with Dr. Binghe Wang. In 2015, she obtained her Ph.D. degree with research on boronic acid modified nucleotides for diagnostic applications and development of fluorescent chemoprobes for molecules of biological importance.

Dr. F. Friscourt

Frédéric Friscourt received his M.Sc. and chemical engineering diploma from the University of Clermont-Ferrand (France). After completing a Ph.D. in chemistry on asymmetric organometallic and organic catalysis with Professor Pavel Kočovský at the University of Glasgow (UK), he transitioned to the field of chemical biology during his postdoctoral fellowship (2009–2014) in the laboratory of Professor Geert-Jan Boons at the Complex Carbohydrate Research Center (GA, USA), where he developed novel chemical probes for imaging the glycome in living cells. In 2014, he obtained a Junior Chair position from the Excellence Initiative program (IdEx) at the University of Bordeaux (INCIA lab, CNRS UMR 5287, France) and was recently recruited as a group leader at the European Institute of Chemistry and Biology in Bordeaux. His research focuses on using organic chemistry to develop novel tools that can probe the influence of biomolecules in the brain, notably in healthy vs diseased states.

>>

Dr. C. Dai

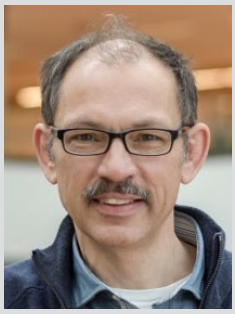
Chaofeng Dai received his B.S. degree in organic chemistry from Lanzhou University (P. R. of China) in 1999, and his Ph.D. degree in organic chemistry from Xiamen University (P. R. of China) in 2007. Then he moved to the USA and joined Professor Binghe Wang's research group first as a postdoctoral research associate and then as a research scientist. His research interests include organic synthesis, medicinal chemistry, bioconjugation chemistry, nucleic acids chemistry, and click chemistry.

Dr. L. Wang

Lifang Wang received her B.S. degree in organic chemistry from Lanzhou University (P.R. of China) in 2000, and her Ph.D. degree in 2007 from Institute of Chemistry, Chinese Academy of Sciences in Shanghai (P. R. of China). She moved to the USA and joined Professor Binghe Wang's research group in 2009 as a postdoctoral research associate where her research interest focused on mass spectrometry. In 2014, she joined Veritas Laboratories LLC, where she is now a senior scientist at the company.

Y. Zheng

Yueqin Zheng was born in 1988 in Fujian (P. R. of China). He received his B.S. degree in materials chemistry from the University of Science and Technology of China (USTC, P. R. of China) in 2011, and joined Professor Binghe Wang's lab as a graduate student at Georgia State University (USA) in 2012. His research interests include prodrugs of gasotransmitters (organic CO prodrugs and organic H_2S prodrugs) and developing novel chemical-reaction-based drug delivery systems.



Prof. G.-J. Boons

Geert-Jan Boons received his M.Sc. and Ph.D. degrees in chemistry from the State University of Leiden (The Netherlands) under the direction of Professor Jacques van Boom. He spent seven years in the UK, first as a postdoctoral fellow at Imperial College London and the University of Cambridge in the research group of Professor Steven Ley, and then as a lecturer and professor at the University of Birmingham. In 1998, he joined the faculty of the Complex Carbohydrate Research Center of the University of Georgia (USA), where he is a Distinguished Professor in Biochemical Science. A hallmark of his research is a seamless integration of method development for complex glycoconjugate synthesis, application of the new methods for the preparation of biologically important targets, and innovative use of the resulting compounds in biological studies.

Dr. S. Wang

Siming Wang is the Director of mass spectrometry facilities at Georgia State University (USA). She obtained her B.S. degree in medicinal chemistry from Beijing Medical College (Now Beijing University Health Sciences Center, P. R. of China) in 1982, and her Ph.D. degree in medicinal chemistry from the University of Kansas, School of Pharmacy (USA), in 1991 (Ph.D. mentor: Professor Robert P. Hanzlik). Subsequently, she did postdoctoral work with Professor Ronald T. Borchardt of the University of Kansas and Professor Francis J. Schmitz of the University of Oklahoma (USA). She then moved to North Carolina (USA) and worked at North Carolina State University, ManTech Corp/US EPA, and the National Institute of Environmental Health Sciences before assuming her current position. Dr. Wang has published over 40 papers in the area of medicinal chemistry, mass spectrometry, and biosensing.

>>

Prof. B. Wang

Binghe Wang is Regents' Professor of Chemistry, Associate Dean for Natural and Computational Sciences in the College of Arts and Sciences, and founding Director of the Center for Diagnostics and Therapeutics at Georgia State University (USA). He also holds an endowed chair as Georgia Research Alliance Eminent Scholar in Drug Discovery and Georgia Cancer Coalition Distinguished Cancer Scholar. Professor Wang obtained his B.S. degree in medicinal chemistry from Beijing Medical College (Now Beijing University Health Sciences Center, P. R. of China) in 1982, and his Ph.D. degree in medicinal chemistry from the University of Kansas (USA), School of Pharmacy, in 1991 (Ph.D. mentors: Professors Matt Mertes and Kristin Bowman-James). Subsequently, he did postdoctoral work with Professor Victor Hruby of the University of Arizona (USA) and Professor Ronald T. Borchardt of the University of Kansas. He started his independent career in 1994 as an Assistant Professor of Medicinal Chemistry at the University of Oklahoma, College of Pharmacy (USA). In 1996, he moved to the Department of Chemistry, North Carolina State University (USA), and was promoted to Associate Professor with tenure in 2000. In 2003, he moved to his current institution at Georgia State University (USA), as Professor of Chemistry, Georgia Research Alliance Eminent Scholar in Drug Discovery, and Georgia Cancer Coalition Distinguished Cancer Scholar. He served as the Chemistry Department chair from 2011–2013 before his current appointment as Associate Dean. His research interests include drug design and delivery, molecular recognition, chemosensing, and new diagnostics. His work has been continuously funded by the NIH for the past 20 years. He was the recipient of the Distinguished Alumni Professor award (2007), which is the highest award that GSU bestows upon a professor for lifetime achievement in scholarly activity, teaching, and service. Professor Wang has published over 230 papers and given over 170 invited lectures worldwide, is the Editor-in-Chief of the high-impact journal Medicinal Research Reviews, and founding serial editor of '*Wiley Series in Drug Discovery and Development*,' which has published over 20 volumes. He has edited books in the areas of drug design, drug delivery, pharmaceutical profiling, chemosensing, and carbohydrate recognition. Internationally, Professor Wang serves on many panels and editorial boards including his current membership on the Synthetic and Biological Chemistry-A Study Section (SBC-A) at the NIH. He has also organized and presided over many international symposia and conferences.

REFERENCES

(1) K. A. Frederick, J. G. Babish *Regul. Toxicol. Pharmacol.* **1982**, *2*, 308.
(2) H. C. Lichstein, M. H. Soule *J. Bacteriol.* **1944**, *47*, 221.
(3) T. Okumura, N. Ninomiya, M. Ohta *Prehosp. Disaster Med.* **2003**, *18*, 189.
(4) L. Gussow *Emergency Med. News* **2010**, *32*, 8.
(5) CDC, *Morbidity and Mortality Weekly Report* **2012**, *61*, 457.
(6) V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless *Angew. Chem. Int. Ed.* **2002**, *41*, 2596.
(7) C. W. Tornøe, C. Christensen, M. Meldal *J. Org. Chem.* **2002**, *67*, 3057.
(8) Q. Wang, T. R. Chan, R. Hilgraf, V. V. Fokin, K. B. Sharpless, M. G. Finn *J. Am. Chem. Soc.* **2003**, *125*, 3192.
(9) H. Oshima, E. Ueno, I. Saito, H. Matsumoto *J. AOAC Int.* **2000**, *83*, 1410.
(10) P. L. Annable, L. A. Sly *J. Chromatogr. A* **1991**, *546*, 325.
(11) S. Kage, K. Kudo, N. Ikeda *J. Anal. Toxicol.* **2000**, *24*, 429.
(12) M. K. Sezgintürk, T. Göktuğ, E. Dinçkaya *Biosens. Bioelectron.* **2005**, *21*, 684.
(13) A. Suzuki, T. A. Ivandini, A. Kamiya, S. Nomura, M. Yamanuki, K. Matsumoto, A. Fujishima, Y. Einaga *Sens. Actuators, B* **2007**, *120*, 500.
(14) D. Leech, F. Daigle *Analyst* **1998**, *123*, 1971.
(15) A. Sahana, A. Banerjee, S. Guha, S. Lohar, A. Chattopadhyay, S. K. Mukhopadhyay, D. Das *Analyst* **2012**, *137*, 1544.
(16) K. Dhara, U. C. Saha, A. Dan, S. Sarkar, M. Manassero, P. Chattopadhyay *Chem. Commun.* **2010**, *46*, 1754.
(17) H. W. Kim, M. G. Choi, H. Park, J. W. Lee, S.-K. Chang *RSC Adv.* **2015**, *5*, 4623.
(18) N. J. Agard, J. A. Prescher, C. R. Bertozzi *J. Am. Chem. Soc.* **2004**, *126*, 15046.
(19) L. Wang, C. Dai, W. Chen, S. L. Wang, B. Wang *Chem. Commun.* **2011**, *47*, 10377.
(20) F. Friscourt, P. A. Ledin, N. E. Mbua, H. R. Flanagan-Steet, M. A. Wolfert, R. Steet, G.-J. Boons *J. Am. Chem. Soc.* **2012**, *134*, 5381.
(21) X. Ning, J. Guo, M. A. Wolfert, G.-J. Boons *Angew. Chem. Int. Ed.* **2008**, *47*, 2253.
(22) F. Friscourt, C. J. Fahrni, G.-J. Boons *J. Am. Chem. Soc.* **2012**, *134*, 18809.
(23) F. Friscourt, C. J. Fahrni, G.-J. Boons *Chem. Eur. J.* **2015**, *21*, 13996.