Bioorganic & Medicinal Chemistry Letters 26 (2016) 1651–1654

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

A metal-free turn-on fluorescent probe for the fast and sensitive detection of inorganic azides

Ke Wang [a], Frédéric Friscourt [b,†], Chaofeng Dai [a], Lifang Wang [a], Yueqin Zheng [a], Geert-Jan Boons [b,*], Siming Wang [a], Binghe Wang [a,*]

[a] Department of Chemistry and Center for Diagnostics and Therapeutics, Georgia State University, Atlanta, GA 30302-3965, USA
[b] Complex Carbohydrate Research Center, University of Georgia, Athens, GA 30602, USA

ARTICLE INFO

Article history:
Received 18 December 2015
Revised 19 February 2016
Accepted 22 February 2016
Available online 23 February 2016

Keywords:
Sodium azide
Click chemistry
Fluorescent detection
Quantitative analysis

ABSTRACT

Sodium azide is toxic and widely used in agricultural, commercial products, and research laboratories. Thus it is of a significant environmental concern and there is a need for the development of a rapid detection method. A fluorogenic dibenzylcyclooctyne derivative (Fl-DIBO) is herein described as a fluorescent probe for the rapid detection of inorganic azide via Strain-Promoted Azide-Alkyne Cycloaddition (SPAAC). Fl-DIBO was found to be highly selective toward NaN_3 in comparison to other common anions with good sensitivity and detection limit of 10 μM.

Published by Elsevier Ltd.

Sodium azide is a colorless, tasteless, odorless and salt-like solid that is widely employed in automobile airbags, airplane escape chutes, pest control, agriculture, and research laboratories.[1] It is also often used as a preservative and biocide.[2] Contamination by sodium azide is therefore a significant environmental concern. The US Environmental Protection Agency (EPA) and the Office of Occupational Health and Safety Administration (OSHA) have specified exposure limits at 0.3 mg/m^3.[3,4] Exposure over 0.7 g (10 mg/kg) is considered lethal.[5] Even at lower doses (0.004–2 mg/kg), sodium azide can cause substantial harm to human health.[6] Azide is known to block cytochrome *c* oxidase leading to inhibition of mitochondrial respiration, and consequently impaired memory.[7–9] Because of its acute toxicity, several azide-poisoning cases have been reported. In 1998, sodium azide was deliberately added to a teapot in Niigata, Japan, poisoning nine persons.[10] Later, a Harvard University poisoning case in 2009[11] and a Dallas Richardson restaurant's tea poisoning case in 2010[12] were also reported as azide poisoning incidents. According to a report of the Center for Disease Control and Prevention (CDC), the FBI spent more than 5 months to figure out the chemical nature of the poison[12] in the Harvard University case because of the limited ability for sodium azide detection. Symptoms of azide-induced mitochondrial poisoning are known to be similar to that of Alzheimer's disease. Thus azide has been used in creating animal models of Alzheimer's disease.[13] For all these reasons, there is a need for a fast and accurate azide detection method.

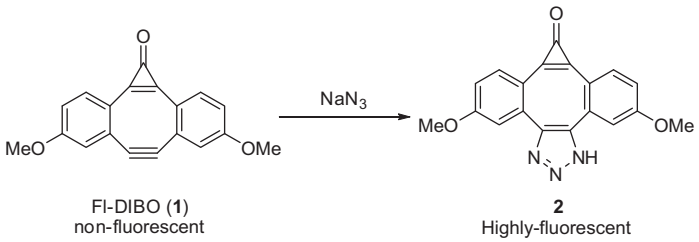
Current laboratory methods for sodium azide determination mainly include chromatography[14–17] and electrochemical detection.[18–20] Various fluorescent probes for the detection of NaN_3 have been recently reported.[21–23] However, selectivity for NaN_3 in the presence of other inorganic anions is an issue.[21,23] Metal complex-based methods have been reported.[21] Herein we describe a metal-free fluorescent method for the detection of inorganic azide.

Although organic azido compounds have been widely known to react with terminal alkynes via copper(I)-catalyzed cycloaddition (CuAAC)[24–26] and strained alkyne without the use of Cu(I),[27] inorganic azide does not readily undergo the same reaction except in rare cases.[15] 4-Dibenzocyclooctynol (DIBO)[28,29] and its sulfated analog S-DIBO[30] have been used as probes for studying the subcellular location of glycoconjugates in living cells and labeling of azido-tagged proteins via SPAAC. Because of the fluorescent nature of the cycloaddition products, we were interested in examining whether they could be used to react with inorganic azide, and thus for their detection. Such work is along the line of our long interest in developing chemosensors for biologically important molecule such as hydrogen sulfide,[31,32] fluoride,[33] and etc.

Since azide anion is known to react differently from their organic azido counterparts, we first examined the reactivity of the strained cyclooctyne Fl-DIBO (**1**) with sodium azide (Scheme 1). Upon mixing Fl-DIBO with NaN_3 at room temperature in a mixture

* Corresponding authors. Tel.: +1 404 413 5544 (B.W.).
E-mail address: wang@gsu.edu (B. Wang).
† Current address: Institut Européen de Chimie et Biologie, Université de Bordeaux, INCIA, CNRS UMR 5287, 2 rue Robert Escarpit, 33607 Pessac, France.

http://dx.doi.org/10.1016/j.bmcl.2016.02.069
0960-894X/Published by Elsevier Ltd.



Scheme 1. The reaction between inorganic azide and Fl-DIBO.

of dioxane and HEPES buffer (1:1), a new fluorescent product was formed within minutes. The reaction was complete within 1 h (Scheme 1 and Fig. 1). ^{1}H and ^{13}C NMR and mass spectrometric analyses of the isolated fluorescent product confirmed the formation of the desired triazole **2** (see SI for details).

Triazole **2** exhibits a hypsochromic fluorescence emission as compared to 1-substituted triazole derivative with the emission maximum at 469 nm (λ_{ex} 363 nm). Next, reaction kinetics was studied by following fluorescence intensity changes at 469 nm. In a typical pseudo first-order experiment, 10 μM of probe **1** was stirred with various amounts of NaN_3 ranging from 1 mM to 10 mM at room temperature in a mixture of dioxane and HEPES buffer (1:1) and the fluorescence emission was recorded. The pseudo first-order rate constants were linearly dependent on the concentration

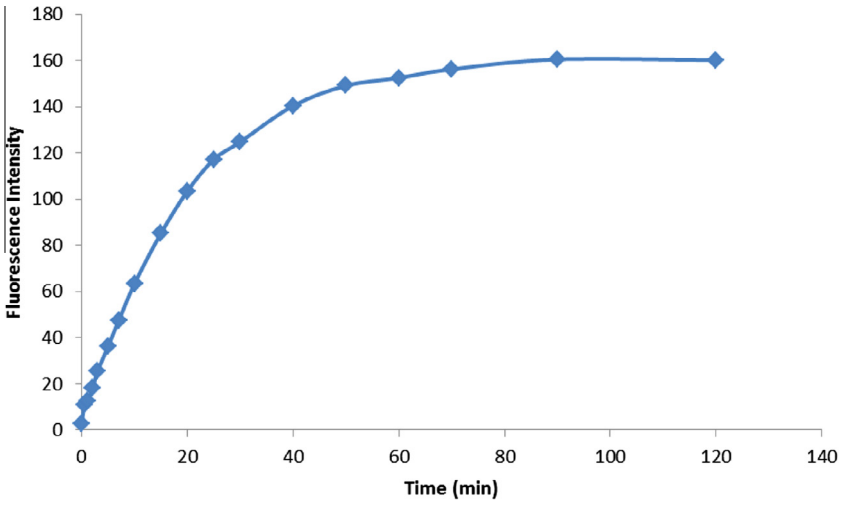


Figure 1. Time-dependent fluorescence intensity changes (λ_{ex} = 363 nm, λ_{em} = 469 nm) of a mixture of Fl-DIBO (10 μM) and sodium azide (10 mM) at room temperature (25 °C) in a mixture of 50 mM HEPES buffer and dioxane (1:1) at pH 7.4.

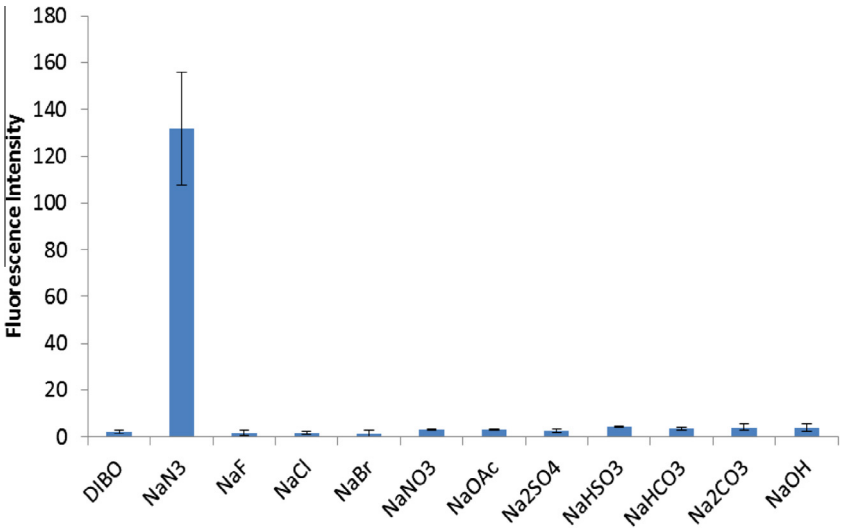


Figure 2. Fluorescence responses of Fl-DIBO to various salts. Fl-DIBO 10 μM, salts 1 mM in a mixture of 50 mM HEPES buffer and dioxane (1:1) at pH 7.4. Fluorescence intensities were recorded after 10 h of incubation with the addition of salts at 25 °C. Data represents the average of three independent experiments.