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# A water soluble and fast response fluorescent turn-on copper complex probe for $H_2S$ detection in zebra fish

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ABSTRACT

According to the displacement method, herein we reported a water soluble copper complex [Cu(MaT-cyclen)$_2$] as a fluorescent probe for the detection of $H_2S$. For this, 1-((1-((10-methylanthracen-9-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl)-1,4,7,10-tetraazacyclododecane (MaT-cyclen) was synthesized first. To improve its solubility in aqueous media, sodium acetate group was introduced into 8-hydroxy-2-quinoline successfully. MaT-cyclen was chelated with Cu(II) to form [Cu(MaT-cyclen)$_2$] complex, which displayed high sensitivity and selectivity for $H_2S$ over the other possible competitive substances on the basis of forming CuS. Meanwhile, [Cu(MaT-cyclen)$_2$] displayed rapid response ($<1$ min), well reversibility, lowest detection limit (205 nM), and high sensitivity for recognizing $H_2S$ in aqueous solution. Furthermore, its potential utility for biological applications was confirmed by fluorescence imaging of $H_2S$ in live cells as well as in zebra fish.



## 1. Introduction

The development of molecular sensors for the selective detection of various cations, anions, amino acids, or small neutral molecules is an important task for scientific researchers [1,2]. In recent years, anions has attracted significant interest due to its important roles in environmental, clinical as well as in biological applications [3–6]. Sulfide anion is a toxic pollutant and can be widely found in water due to both the industrial processes and microbial reduction of sulfate by anaerobic bacteria or produced from the sulfur-containing amino acids in proteins [7]. Sulfide irritates mucous membranes and causes unconsciousness and respiratory paralysis [8,9]. Once protonated to $HS^-$ or $H_2S$ (hydrogen sulfide), sulfide becomes even more toxic. At a low concentration, $H_2S$ produces personal distress. At higher concentrations, it can result in loss of consciousness, permanent brain damage, or even death through asphyxiation [10]. For centuries, $H_2S$ has been viewed primarily as a noxious chemical species which is naturally produced by geological and microbial activities [11]. $H_2S$ is a weak acid in aqueous solutions ($pKa_1=7.04$, $pKa_2=11.96$) [12], equilibrating mainly with $HS^-$ at physiological pH. It was found that many types of diseases such as chronic kidney disease, liver cirrhosis, and Down's syndrome are associated with abnormal levels of $H_2S$ [13–16]. Therefore, it is important to develop a rapid and sensitive chemosensor for the immediate hydrogen sulfide monitoring in aqueous media and in biological systems.

Transition-metal ions play crucial roles in life processes. Among them, Cu(II) is a important catalytic cofactor for a variety of metalloenzymes such as superoxide dismutase, cytochrome c oxidase, lysyl oxidase, and tyrosinase, etc [17]. Copper-containing enzymes have significant roles in different catalytic processes starting from providing energy for biochemical reactions to assist the formation of cross-links in collagen and elastin, and thereby maintaining and repairing connective tissues related to heart and arteries [18]. Nevertheless, it's overloading exhibits toxicity and a variety of neurodegenerative diseases, for example, Alzheimer's, Menkes, and Wilson's due to its participation in the formation of reactive oxygen species [19].

The classic gravimetric precipitation of CuS from $Cu^{2+}$

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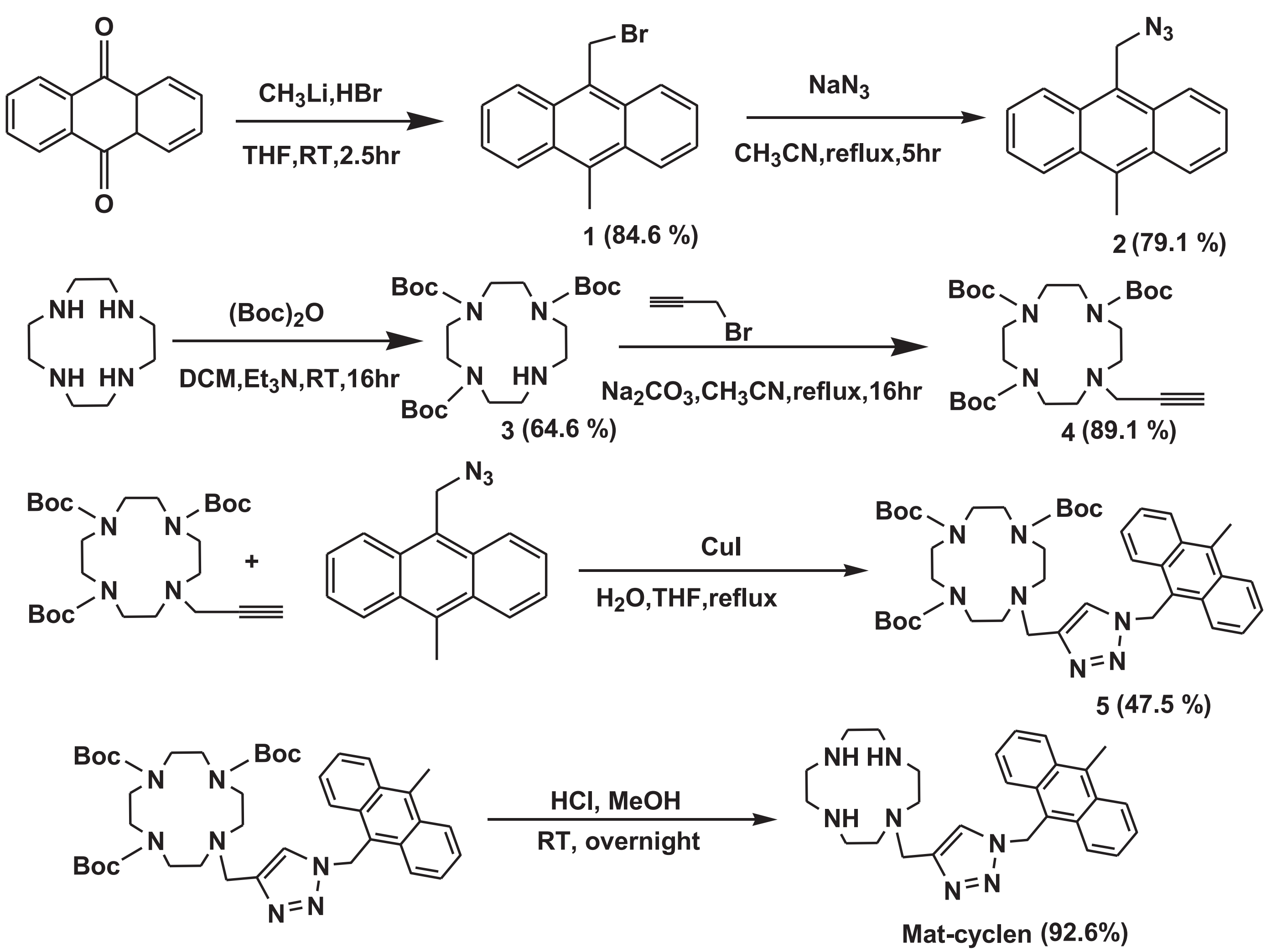
complexes has been successfully employed as a strategy to detect $H_2S$ by a fluorescence response. It is known that heavy and transition metal ions have a strong fluorescence quenching characteristic. Sulfide is known to react with copper ions to form a very stable CuS species, which has a low-solubility product constant $K_{sp}=1.27\times10^{-36}$ [20]. Therefore if the fluorescence of dyes can be effectively quenched by heavy and transition metal ions through complexation, then it may provide a basis for the development of turn-on sensors for $H_2S$ by the displacement method. Sasakura et al. reported a $H_2S$ imaging probe HSip-1, which consists of a cyclen macrocycle attached to fluorescein [21]. Binding of Cu(II) to cyclen receptor quenched the fluorescence. Upon reaction with sulfide donors such as $Na_2S$ or NaHS, CuS gets precipitated and releases unbound cyclen-AF, which displayed enhanced fluorescence. Zeng and Bai have utilized a similar approach in the design of their 8-hydroxyquinoline sulfide probe, which combines a fluorescein with a pendant 8-hydroxyquinoline ligand for copper binding [22]. Even though many reports are available for metal complex based $H_2S$ sensors, most of them have drawbacks including poor detection limit [23,24], longer response time [25], tedious synthetic procedure [26], use of organic solvent [27] or higher amount of co-solvent with water [28]. In addition, only few Cu(II) complex sensors can function in complete aqueous solution [29,30]. Therefore, it is still a challenging task to develop effective fluorescent sensors for $H_2S$ detection in 100% aqueous solution.

Considering that 1,4,7,10-tetraazacyclododecane (cyclen) has been successfully applied in biology, recognition, and imaging applications [31,32], we envisioned that the inorganic-reaction based method could be employed as a platform for the construction of fluorescent sulfide probes potentially useful for hydrogen sulfide detection at physiological pH. In this work, we reported a mono anthracene functionalized cyclen 1-((1-((10-methylanthracen-9-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl)-1,4,7,10-tetraazacyclodode cane (MaT-cyclen), (Scheme 1) as a fluorescent sensor. MaT-cyclen was chelated with Cu(II) to form [Cu(MaT-cyclen)$_2$] complex, which displayed high sensitivity and selectivity for $H_2S$ over the other possible competitive substances on the basis of forming CuS. The metal-ligand stoichiometry was characterized by Job's method and mass spectrometry (ESI-MS). The optical properties of the chemosensor were investigated in detail using UV–visible spectra, fluorescence spectra, mass spectra, and fluorescence imaging in cells and zebra fish.

## 2. Materials and methods

### 2.1. General information and materials

Unless otherwise noted, all the materials used in this study were obtained from commercial sources and used without further purification. All IR spectra were recorded as KBr pellets on a Nicolet Avatar instrument in the frequency range 400–4000 cm$^{-1}$.




**Scheme 1.** Synthesis of MaT-cyclen.