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ARTICLE

A luminogenic lanthanide-based probe for the highly selective detection of nanomolar sulfide levels in aqueous samples

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Margaret L. Aulsebrook,[a] Suvendu Biswas,[b] Franklin M. Leaver,[c] Michael R. Grace,[a] Bim Graham,[d] Amy M. Barrios[b]* and Kellie L. Tuck[a]*

Dedicated to the memory of our friend and colleague, Prof. Leone Spiccia.

A bimetallic terbium(III)/copper(II) complex (**Tb-1.Cu^{2+}**) for the time-gated luminescent detection of hydrogen sulfide in aqueous samples is reported. The probe shows excellent selectivity towards HS^- over various anions and cations, including the ions common to natural waterways and waste water samples, displaying a 73-fold increase in luminescence in the presence of sulfide. The probe exhibits extremely fast reaction times and a low limit of detection (130 nM). The probe was used to quantify sulfide in an industrial "sour water" sample, with the result in excellent agreement with those from two independent assay methods (methylene blue and AzMC).

Introduction

Hydrogen sulfide (H_2S) is a poisonous, corrosive and flammable gas and its detection has gained much interest recently due to its roles in biology, the environment and the oil industry. H_2S has been implicated in various physiological processes such as the mediation of neurotransmission,[1] relaxation of vascular smooth muscles[2] and inhibition of insulin signalling.[3] Exposure to H_2S can also pose a threat to the environment and to humans with studies suggesting H_2S is a pro-proliferative factor in human colon cancer[4] along with other negative health implications. The naturally occurring H_2S is one of the key contaminants in ground water and crude oil, sparking major concern for the industry.[5,6] Hydrogen sulfide harms product value, produces odour, causes corrosion to infrastructure, compromises environmental safety and poses an exposure hazard for humans. The favoured methods currently used for H_2S detection are the methylene blue test and electrochemical detection.[7] These methods have major drawbacks that limit their efficacy, such as poisoning of reference electrodes by sulfide in electrochemical detection and limited sensitivity in the case of methylene blue (limit of detection, LOD = 3 μM).[8] Consequently, the development of a more robust means of accurately detecting H_2S would benefit a wide range of fields.

To date, there have been a number of fluorescent chemosensors developed for the detection of H_2S. The mechanisms of detection involve copper sulfide precipitation,[9,10] reduction of nitro and azide groups facilitated by sulfide,[11-16] or nucleophilic addition of sulfide.[17] These sensors represent a significant advance, however their broad scale application to the analysis of environmental and biological samples is complicated by the potential for significant interference from fluorescent organic and biological material.

Over the past two decades there has been increasing interest in the development of luminescent lanthanide complex-based sensors, due to the fact that these offer a number of distinct advantages over fluorescence-based designs. Most notably, luminescent lanthanide complexes exhibit extremely long excited state lifetimes (of the order of milliseconds), allowing time-gated measurements to be conducted in which a delay is introduced between sample excitation and emission detection.[18] This eliminates interference from short-lived "background" fluorescence, leading to a dramatic increase in signal-to-noise ratio and, hence, sensitivity. In addition, the lanthanides have characteristically sharp emission spectra with maxima significantly red-shifted compared to organic fluorophores, further minimising background fluorescence. One complication in the design of such complexes is that the lanthanide ions themselves are only very weakly luminescent because the associated *f-f* transitions are parity-forbidden.[19] It has therefore become common practice to encapsulate lanthanide ions within multi-dentate chelating ligands incorporating strongly UV-absorbing "antenna" groups (typically heteroaromatic ring

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[a] *School of Chemistry, Monash University, Clayton, Victoria 3800, Australia. E-mail: Kellie.Tuck@monash.edu, Web: kellietuckgroup.com*
[b] *Department of Medicinal Chemistry, University of Utah College of Pharmacy, Salt Lake City, UT 84108, USA. E-mail: Amy.Barrios@utah.edu*
[c] *Water & Energy Systems Technology, Inc. Kaysville, UT 84037, USA.*
[d] *Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Victoria 3052, Australia.*

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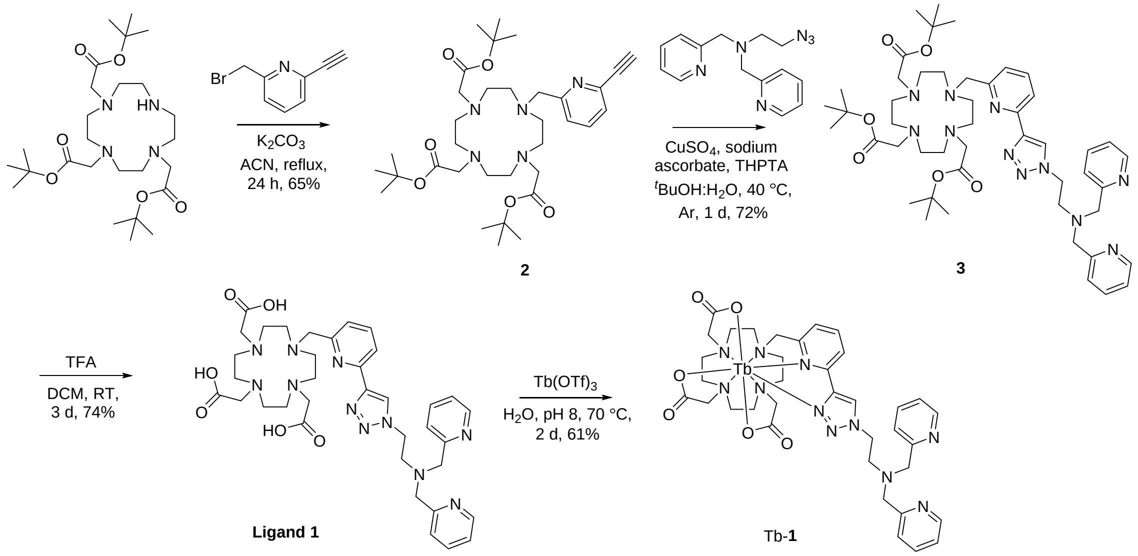

Scheme 1. Synthesis of **Tb-1** complex.

systems). These promote indirect access of the lanthanide to its excited state, leading to enhanced emission, via a process involving excitation of the antenna, intersystem crossing and, finally, energy transfer to the metal centre.[18]

Kinetically inert lanthanide complexes have been developed for luminescent detection of a wide range of chemical species under various conditions.[20,21] There have been reports of luminescent lanthanide-based H_2S sensors, whose mechanism of detection involves either copper sulfide precipitation[22,23] or reduction of azide groups[24-27]. However, with the exception of previous reports from our group, which has demonstrated the utility of azide-bearing lanthanide-based sensors for monitoring sulfide levels in partially refined crude oil,[26,27] the practical utility of such sensors for the analysis of industrial samples has not been demonstrated in the literature. As part of an ongoing program to develop hydrogen sulfide sensors for the petroleum industry, we report herein the synthesis and application of a new "turn-on"-type luminescent lanthanide-based sensor, **Tb-1.Cu^{2+}**, for the detection of H_2S in waste water ("sour water") from an oil refinery. Our design combines use of the copper sulfide precipitation "switch" strategy, initially devised by Chang *et al.*[9] and Nagano *et al.*,[10] with time-gated detection of lanthanide luminescence. The water solubility (at least 15 mg/mL) and sensitivity of this new probe are significantly better than those of our previous lanthanide-based design.[26] The **Tb-1.Cu^{2+}** probe is also superior to the previously reported copper sulfide precipitation-based probes in that it has a significantly improved LOD (130 nM as compared to 2.7 μM)[23] undergoes a much larger luminescence increase upon exposure to sulfide (73-fold *vs* 5-6 fold[23] or 40-fold[22]), and the parent probe **Tb-1** shows no reaction with sulfide in the absence of copper.[22]

Results and discussion

Design and synthesis of Tb-1.Cu^{2+}

The DPA moiety (di(2-picolyl)amine) is known to strongly bind Cu^{2+} ions, and the fluorescence quenching properties of Cu^{2+} ions are well established.[28] Based on these observations, as well as the ability of sulfide to "strip" Cu^{2+} ions from metal complexes, we designed the **Tb-1.Cu^{2+}** probe, which features a DPA group appended to a DO3A-based macrocyclic terbium(III) chelate (DO3A = 1,4,7,10-tetraazacyclododecane-1,4,7-triacetate). The "clicked" pyridinyl pendant arm serves two purposes. Firstly, it acts as an efficient antenna group. Secondly, it provides two terbium(III)-coordinating nitrogen atoms, which eliminates luminescence quenching water molecules from the primary coordination sphere.[29] We anticipated that this would result in a high basal luminescence for **Tb-1**, which would be quenched in the presence of Cu^{2+} ions. Scheme 1 shows the synthetic strategy used to access the terbium(III) complex, **Tb-1**. The **Tb-1.Cu^{2+}** probe was generated *in situ* via the addition of 1 equiv. of Cu^{2+} ions.

Luminescence response of Tb-1.Cu^{2+} to sulfide

Initial analysis of the **Tb-1** complex revealed that it was indeed highly luminescent (ϕ = 0.22). Four distinctive bands were observed in the luminescence spectrum, with emission maxima at 488, 545, 587 and 621 nm. These bands correspond to transitions from the $^5D_4$ excited state to the $^7F_6$, $^7F_5$, $^7F_4$ and $^7F_3$ ground states, respectively.[30] Addition of 1 equiv. of Cu^{2+} led to complete suppression of this luminescence (ϕ = 0, Fig. S2†). These two properties indicated that the probe would be a

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perfect candidate for the “turn-on” luminescence-based detection of hydrogen sulfide via CuS precipitation.

The luminescent intensity of **Tb-1.Cu^{2+}** (5 µM) was found to increase *ca.* 73-fold upon the addition of 2 equivalents of Na_2S (Na_2S exists in the form HS^- at pH 7.4), returning to the level of the initial **Tb-1** complex (Fig. 1).[31] In comparison, previously reported lanthanide-based copper sulfide precipitation probes exhibit significantly smaller changes in luminescence (5–40-fold).[22,23] A linear response was observed over the concentration range 0–10 µM Na_2S. The luminescent signal was found to stabilise immediately,[32] which is a highly desirable feature from the viewpoint of developing a rapid assay for sulfide measurement. The LOD was determined to be 130 nM. Of note, the “on-off” luminescent response of the **Tb-1.Cu^{2+}** probe exhibited a high degree of reversibility over several cycles of sequential addition of Na_2S and Cu^{2+} ions (Fig. S3†), which is a definite advantage over most reaction-based probes.

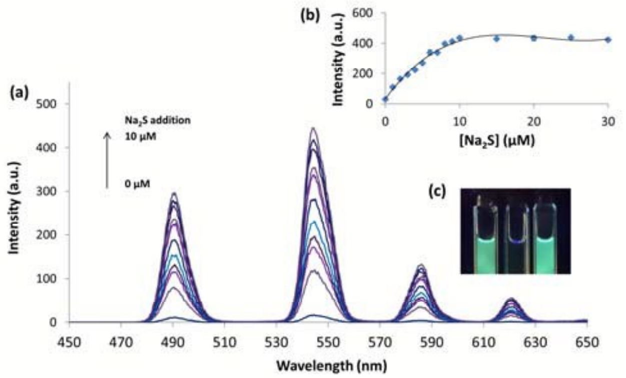

Fig. 1. (a) Changes in the luminescence emission spectrum of **Tb-1.Cu^{2+}** (5 µM) upon the addition of Na_2S (0–10 µM); spectra measured in 10 mM HEPES buffer (pH 7.4) with λ_{ex} = 260 nm. (b) Change in luminescence intensity detected at 545 nm upon the addition of Na_2S (0–30 µM) (average of triplicate experiments). (c) Photograph of solutions of **Tb-1** (left) and **Tb-1.Cu^{2+}** before (middle) and after addition of excess Na_2S (right), irradiated with a common laboratory TLC lamp (254 nm).

Environmental/industrial water samples can contain large amounts of cations and anions. Consequently, the effect of these species on the luminescence response was investigated. The addition of various cations (Mg^{2+}, Na^+, Mg^{2+}, Hg^{2+}, Co^{2+}, K^+, Ni^{2+}, Pb^{2+}, Cd^{2+}, Fe^{3+}, Ca^{2+}, Zn^{2+}) to the **Tb-1.Cu^{2+}** probe resulted in no increase in luminescence (Fig. 2). Importantly, treatment of the probe with Na_2S in the presence of 1 equiv. of the cations gave the same complete recovery of the luminescence as in the absence of cations, except in the case of Ni^{2+}, likely due to the competing formation of NiS. Similar results were obtained in the presence of 10 equiv. of the cations, except in this case both Ni^{2+} and Co^{2+} prevented restoration of luminescence.

Selectivity studies performed with various anions and sulfurous compounds (Cl^-, I^-, HCO_3^-, CO_3^{2-}, ClO^-, NO_2^-, OAc^-, SO_3^{2-}, SO_4^{2-}, $S_2O_3^{2-}$, $S_2O_4^{2-}$, $S_2O_5^{2-}$, lipoic acid and cysteamine) showed that only the addition of cystamine and lipoic acid to **Tb-1.Cu^{2+}** produced an increase in luminescence (Fig. 3). These compounds, however, are not common to natural or industrial waste waters and are therefore not of major concern. All other anions screened, including major anions found in natural waters (HCO_3^-, CO_3^{2-}, SO_4^{2-}, Cl^-), showed no interference with the sulfide-induced luminescence response of the probe.

Detection of sulfide in sour water sample

After establishment of the sensing method, the **Tb-1.Cu^{2+}** probe was applied to sulfide level detection in an industrial waste water sample. As the waste water produced in the oil refining industry can contain very high amounts of sulfide (up to 150 mg/L),[33] ammonia, and other petroleum by-products, the Environmental Protection Agency (EPA) has stipulated that

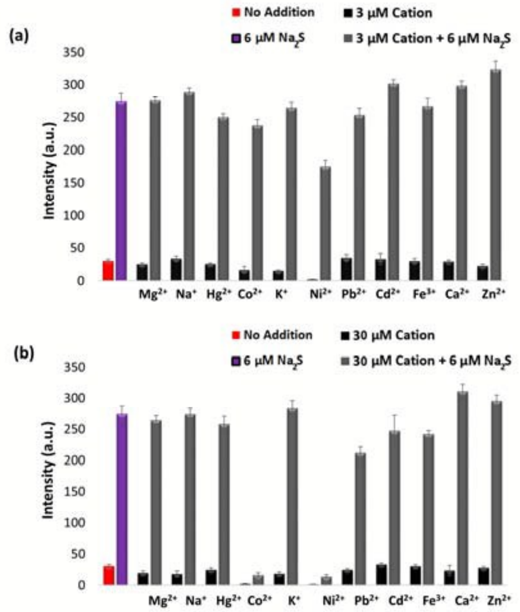

Fig. 2. Changes in luminescent intensity of **Tb-1.Cu^{2+}** (3 µM) detected at 545 nm in the presence of various cations; spectra measured in 10 mM HEPES buffer (pH 7.4) with λ_{ex} = 260 nm. Red bars: **Tb-1.Cu^{2+}** alone (3 µM). Purple bars: **Tb-1.Cu^{2+}** (3 µM) and Na_2S (6 µM). (a) Black bars: **Tb-1.Cu^{2+}** (3 µM) and cation (3 µM). Dark grey bars: **Tb-1.Cu^{2+}** (3 µM), cation (3 µM) and Na_2S (6 µM). (b) Black bars: **Tb-1.Cu^{2+}** (3 µM) and cation (30 µM). Dark grey bars: **Tb-1.Cu^{2+}** (3 µM), cation (30 µM) and Na_2S (6 µM).

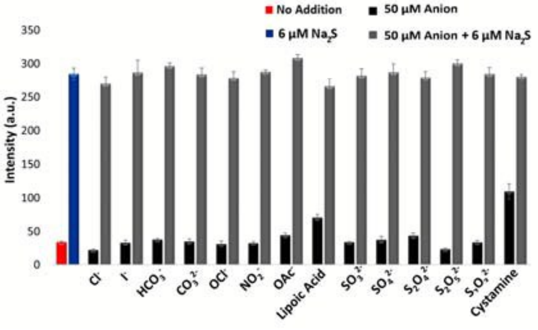

Fig. 3. Changes in luminescent intensity of **Tb-1.Cu^{2+}** (3 µM) detected at 545 nm in the presence of various anions/sulfur compounds; spectra measured in 10 mM HEPES buffer (pH 7.4) with λ_{ex} = 260 nm. Red bar: **Tb-1.Cu^{2+}** alone (3 µM). Blue bar: **Tb-1.Cu^{2+}** (3 µM) and Na_2S (6 µM). Black bars: **Tb-1.Cu^{2+}** (3 µM) and anion/sulfur compound (50 µM). Dark grey bars: **Tb-1.Cu^{2+}** (3 µM), anion/sulfur compound (50 µM) and Na_2S (6 µM).

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the "sour" water must be purified and the hydrogen sulfide in water released into the environment must be below the olfactory detection limit (~ 15 µM). We obtained samples of sour water from a local oil refinery. The sour water was diluted serially in order to obtain a sample with sulfide levels within the measurable linear range of the probe. Analysis of this diluted sample yielded a sulfide concentration of 41 ± 3 mM as measured by **Tb-1.Cu^{2+}**. Two independent quantitative assays – the colourimetric methylene blue assay and an assay employing a fluorogenic probe AzMC previously developed by our group[26] – were also performed to verify our results. These yielded sulfide concentrations of 40.5 ± 2.3 mM and 40 ± 2.5 mM, respectively, in excellent agreement with the value derived using the **Tb-1.Cu^{2+}** probe. Importantly, due to the highly water-soluble nature of our lanthanide-based probe, the addition of organic solvent to the assay mixture is not required, and unlike previous azide-based probes, pre-incubation is not needed. These features, coupled with the excellent sensitivity for sulfide, render the probe superior to many existing colourimetric and fluorogenic methods for the detection of sulfide.

Conclusions

A luminescent lanthanide-based complex, **Tb-1**, has been synthesised and its bimetallic copper(II) counterpart applied in the detection of sulfide. Addition of Cu^{2+} to the complex results in complete quenching of the luminescent signal, which is then restored upon exposure to sulfide. The probe responds selectively to sulfide, with extremely short reaction times and a detection limit in the nanomolar range. The probe has been successfully used to measure the amount of sulfide in a real industrial sour water sample, with the result confirmed by two independent methods, highlighting the potential for the probe to be used in environmental/industrial monitoring of sulfide.

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