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Responsive Two-Photon Induced Europium Emission as Fluorescent Indicator for Paralytic Shellfish Saxitoxin

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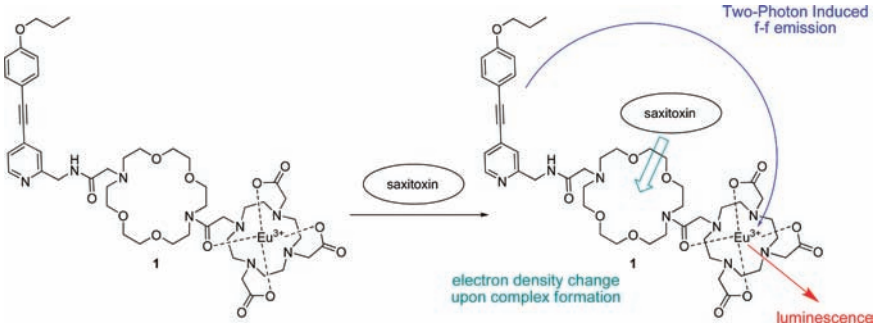
ABSTRACT



A water-soluble europium(III) complex (1) has been synthesized and demonstrated to be a specific fluorescence probe for the paralytic shellfish toxin saxitoxin, a neurotoxin that blocks the voltage-gated sodium channels on cell membranes. Saxitoxin binds to the europium complex ($K_B = 6.1 \times 10^4\ M^{-1}$) and triggers a two-photon induced f−f emission enhancement by over 100% and increases the two-photon absorption cross-section from 9 to 36 GM.

There is an increasing public concern about the contamination of seafood and drinking water resources by algal toxins. Recent studies in Southeast Asian and Pacific waters have identified algal toxins as one of the major threats to the local fisheries, aquaculture industry, marine environmental quality, and public health of the region. Chemical detection of algal toxins in natural water is an analytically challenging task, as these toxins are cationic and highly hydrophilic and do not possess any chromophores or fluorophores.[1]

Among the various aquatic biotoxins, paralytic shellfish poisoning (PSP) toxins are probably the most difficult to detect in natural water. The neurotoxins responsible for PSP are saxitoxin (STX) and its analogues, such as

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neosaxitoxin and decarbamoylsaxitoxin.[2] They belong to a class of highly potent algal neurotoxins that owe their toxicity to their ability to block voltage-gated sodium channels on cell membranes.[3] There are several methods to detect STX, such as high-performance liquid chromatography (HPLC) coupled with chemical derivatization or mass spectrometric (MS) detection. The postcolumn fluorescent derivatization HPLC method by Oshima et al. is the most widely adopted analytical protocol for PSP determination.[4] However, the current analytical process is tedious, and the results obtained are not always reproducible. Immunoassays are another alternative to the chemical qualitative determination of PSP toxins. The major drawback is that it requires toxin-specific antibodies, which are expensive and have short storage lives. This renders the immunoassay incapable of screening more than a few PSP toxin variants.[5] Thus, a molecular sensor that can directly respond to PSP toxins in water would be useful.

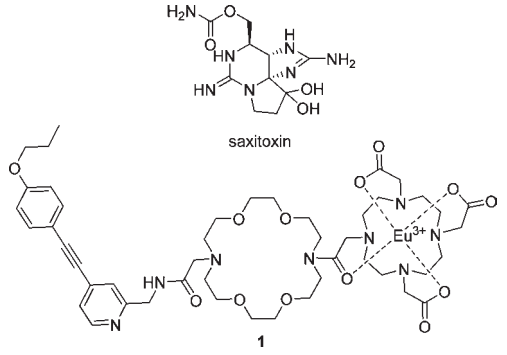
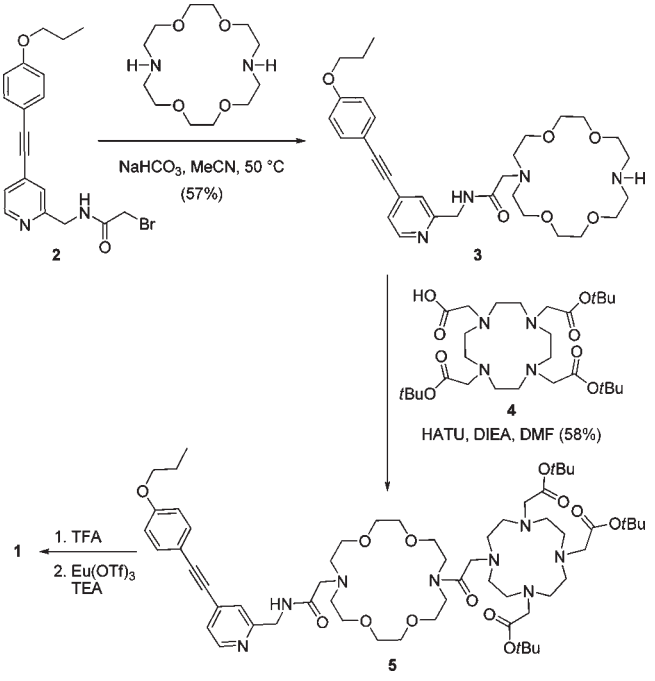


Figure 1. Structure of europium complex **1** and saxitoxin.

One of the promising alternatives to the existing detection methods is chemosensing, which enables real-time and in situ monitoring of an analyte. The molecular sensing elements involved are generally robust and reusable. Recently, several optical chemosensors featuring the diaza-18-crown-6 moiety demonstrated their specificity for STX.[6] However, the emission bands of these materials for STX are usually broad with short lifetimes in the nanosecond range and have low signal-to-noise ratios. Incorporation of lanthanides is an attractive approach for developing STX sensors since lanthanide complexes generally exhibit long-lived luminescence lifetimes, large Stokes shifts, and sharp emission peaks.[7] In addition, there are various examples of $4f^N-4f^N$ luminescence sensitized by the antenna effect following multiphoton absorption of tripodal amide ligands.[8] These phenomena are particularly useful for addressing the low signal-to-noise ratios in existing STX in situ tracers. Herein, we report the development of a novel water-soluble emissive europium complex (**1**), which can be excited in the biologically relevant near-infrared region through multiphoton absorption, for STX sensing.

Scheme 1. Synthesis of Europium Complex **1**



As shown in Figure 1, europium complex **1** contains an amide-substituted 1,4,7,10-tetraazacyclododecane ligand and its pendant arms with carboxylic acid groups, a highly conjugated organic chromophore and a diaza-18-crown-6 derivative. The carboxylic groups should help to improve the solubility of the complex for the detection of STX in water/food samples, and the diaza-18-crown-6 moiety should provide the binding site for "trapping" the STX.

Complex **1** was readily prepared from the 2-bromoacetamide (**2**).[9] As shown in Scheme 1, monoalkylation of

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diaza-18-crown-6 with **2** using sodium bicarbonate in acetonitrile at 50 °C afforded **3** in 57% yield. Amide coupling of **3** with acid **4**[10] finished the synthesis of ligand **5**, which is fully characterized by ^{1}H and ^{13}C NMR and HRMS. After acid hydrolysis of the *tert*-butyl esters with TFA, the crude product was treated with Eu(III) triflate. Recrystallization of the crude complex with CH_3CN afforded **1** in 50% yield. The structure of europium complex **1** was characterized unambiguously by HRMS.

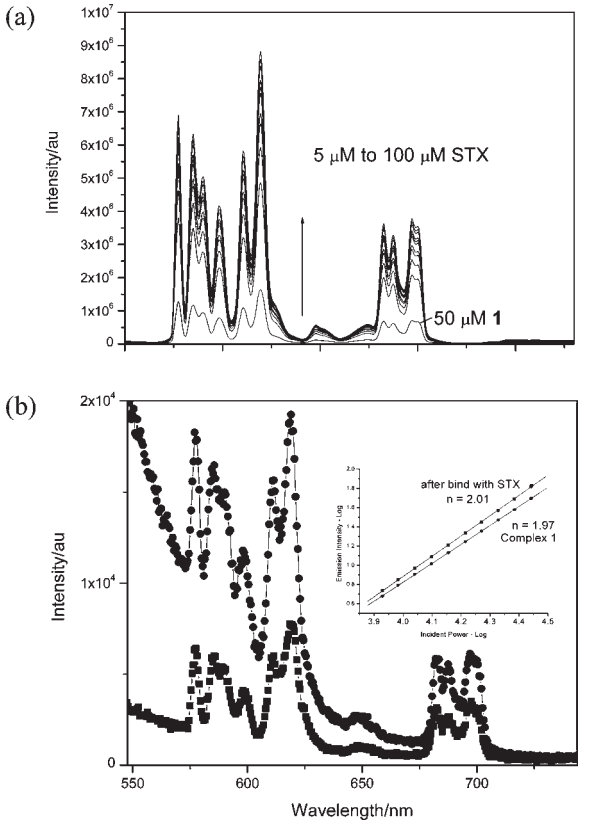

Figure 2. Enhancement of europium emission intensities of complex **1** (50 μM in water) upon binding with STX: (a) linear excitation, λ_{ex} = 350 nm, [STX] = 5−100 μM; (b) two-photon excitation, λ_{ex} = 750 nm. Squares: [STX] = 0 (9 GM), circles: [STX] = 15 μM (36 GM). The inset of (b) shows the power dependence of two-photon induced f−f emission of complex **1** upon boning with STX.

With complex **1** prepared, its photophysical properties in water were studied. The conjugated chromophore of **1** demonstrated a strong two-photon absorption and appropriate triplet states (21739 cm^{-1}) which provide an efficient pathway for the energy transfer from the ligand to the excited state ($^{5}D_0$) of the europium. Four structural red f−f ($^{5}D_0 \rightarrow {}^{7}F_J$, J = 1−6 Hz) emission bands can be obtained from complex **1** upon excitation at 350 and 750 nm. The absolute quantum yield (Φ) and the emission lifetime (τ) of complex **1** are 0.03 and 0.56 ms, respectively. The two-photon absorption cross section of complex **1** is around 9 GM (GM = 10^{-50} cm^4 s $photon^{-1}$ $molecule^{-1}$).

When complex **1** was titrated against STX in water, a significant enhancement of the luminescence signals was observed with both linear and two-photon excitation (Figure 2), indicating complex formation between complex **1** and STX. After complex **1** binds with STX, the emission intensity and quantum yield increased by more than 50% (Φ = 0.08). The binding ratio, constant and rate between complex **1** and STX have been determined by the hypersensitive $^{5}D_0 \rightarrow {}^{7}F_2$ emission intensity at various concentrations of STX. Although the binding constant of complex **1** (K_B = 6.1 × 10^4 M^{-1}) is smaller than that reported by Gawley et al., complex **1** demonstrated long lifetime emission and its potential for developing as a time-resolved detection system and aim for the improvement of signal-to-noise ration during the sensing process.[6] Moreover, an observable color change from pale yellow to red was observed upon binding of STX in aqueous solutions (insert of Figure 3).

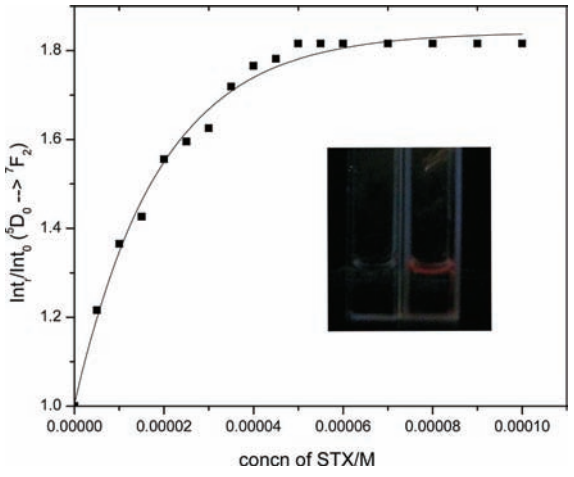

Figure 3. Binding isotherms for titration of STX against europium emission ($^{5}D_0 \rightarrow {}^{7}F_2$) response for complex **1** (50 μM in water). The curve fit assumes a 1:1 binding stoichiometry. (K_b = 6.1 × 10^4 M^{-1}). The inset shows the observable color change of complex **1** upon binding with STX in aqueous solutions.

Since UV excitation has the disadvantages of low signal-to-noise ratio and toxicity in situ detection, two-photon induced emission is one of the promising solutions to these problems. Europium complex **1** demonstrated real-time two-photon induced f−f emission response to STX (Figure 2b). STX serving as the trigger for the two-photon induced f−f emission of complex **1**. After "trapping" the STX by the crown moiety, the electron localization in the conjugated system is changed probably due to hydrogen bondings and electrostatic interactions, which led to a dramatic increase of the two-photon induced emission intensity (three times) with two-photon absorption cross section increaed from 9 to 36 GM (Figure 2b).

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The luminescence lifetime, quantum yield, and the number of water molecules in the first coordination sphere of complex **1** are summarized in Table 1. There are more water molecules directly bound to the chelated europium metal in the absence of STX than in the presence of STX. The number of inner-sphere water molecules decreases from 0.84 to 0.1 upon addition of STX, and the result is in good agreement with the change in hypersensitive europium emission in an aqueous environment.[11]

Table 1. Luminescence Lifetimes (τ), Number of Inner-Sphere Water Molecules (q), Quantum Yield (φ), and Two-Photon Absorption Cross-Section (δ) of Complex **1** (50 μM in Water) in the Presence and Absence of STX

complex	$\tau(H_2O)$	$\tau(D_2O)$	q^a	$\varphi(H_2O)^b$	δ/GM $(H_2O)^c$
1	0.56	1.23	0.84	0.03	9
1 + STX	0.87	1.22	0.1	0.08	36

[a] Derived hydration numbers, q (±20%) $q^{Eu} = 1.2[(k_{(H_2O)} - k_{(D_2O)}) - (0.25 + 0.07x)]$ (x = number of carbonyl-bound amide NH oscillators),[12] decay curve monitored at 616 nm ($^5D_0 \rightarrow {}^7F_2$, λ_{ex} = 350 nm).[10] [b] λ_{em} = 560−700 nm, λ_{ex} = 350 nm. [c] Two-photon absorption cross-section (GM = 10^{-50} cm ^{4} s photon^{-1} molecule^{-1}, λ_{em} = 580−700 nm, λ_{ex} = 750 nm).

The selectivity of europium complex **1** for STX over other bioactive cations (K^+, Na^+, Ca^{2+}) and motif PSPs (neosaxitoxin, decarbamonylsaxitoxin, and decarbamonylneosaxitoxin) have been determined. As shown in Figure 4, complex **1** shows high specificity toward STX. These results suggested that the carbamate and the guanidine moieties of STX are both important for binding with complex **1**. This selectivity is particularly important because these cations in food samples can bind competitively with selectivity crown ethers and interfere with the response of cations. The f−f emission enhancement of complex **1** for STX is greater than that for other common bioactive cations. The significant f−f emission enhancement can be visualized under UV-excitation after complex **1** binds with STX (Figure 2).

In conclusion, we have designed and synthesized europium complex **1** with a modified STX diaza-18-crown binding site which was demonstrated to be highly specific and responsive europium emission for STX through linear and nonlinear (near-infrared) excitation. The binding of STX triggered and enhanced the two-photon-induced visible f−f emission of the europium complex. Based on these findings, we can further establish a new STX tracer that is less phototoxic and can visualize a specific toxin within the "biological window" of excitation (700−1000 nm). The utility of the tracer in biological systems will be explored both in vitro and in vivo. Our results demonstrate the great potential of the europium complex (**1**) as a new generation of biotracer for STX, as they have strong binding affinity ($K_B = 6.1 \times 10^4$ M^{-1}) and long lifetime emission. This tracer could find applications both for STX detection for research purposes as well as in the marketplace.

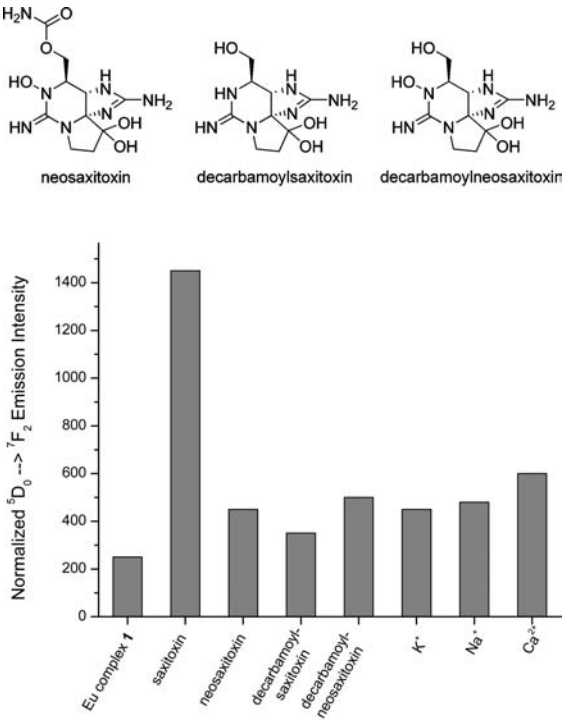

Figure 4. Response of europium emission variation ($^5D_0 \rightarrow {}^7F_2$) of complex **1** ($10^{-5}$ M) to different PSP toxins (15 μM) and metal ions 60 μM, λ_{ex} = 350 nm).

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Supporting Information Available. Experimental details of synthesis, NMR spectra and HRMS of compound **3** and ligand **5**, and HRMS spectra of complex **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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