Lead Sensors

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Development of a Fluorescent Pb^{2+} Sensor**

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Lead is a persistent environmental contaminant.[1–4] Even exposure to very low levels of lead can cause neurological, reproductive, cardiovascular, and developmental disorders.[3,5,6] Children with variants in iron metabolism genes may be more susceptible to lead absorption and accumulation.[7,8] The US Center for Disease Control (CDC) set standards stating that a 10–19 $\mu g\,dL^{-1}$ level of lead in blood poses a potential threat and that diagnostic testing is recommended.[7] Of particular interest is Pb^{2+}, as it interferes with enzymatic heme production.[9]

Poisoning by heavy metals, such as lead, has prompted demand for new techniques to selectively identify and study the actions of these metal ions.[7,4,10] Currently, the most common methods of detection of lead include atomic absorption spectrometry,[8] inductively coupled plasma mass spectrometry,[11] and anodic stripping voltammetry;[12] these instrumentally intensive methods[6,13] measure only total lead content,[1] and often require extensive sample preparation. Thus, a simple and inexpensive method for not only detecting, but also quantifying Pb^{2+} is desirable in real-time monitoring of environmental, biological, and industrial samples.

Fluorescence-based sensors offer unparalleled sensitivity and thus have garnered significant interest.[4] Most fluorescent probes for detecting Pb^{2+} use peptides,[14] proteins,[15] or DNAzymes.[3,6,16–18] These probes lack the simplicity that a small-molecule probe can offer. Moreover, nonspecific interactions and background fluorescence often act as a deterring factor, which underscores the necessity of a selective lead sensor that can function in aqueous environments.[1–3,6] To this end, a water-soluble fluorescence-based small-molecule Pb^{2+} sensor (leadfluor-1) has showed promise in the study of cellular Pb^{2+} trafficking.[2] In addition to solubility and sensitivity, selectivity is an important criterion for the success of a sensor. Ideally, the sensor should have high selectivity with a high dynamic range. Herein we present the design, synthesis, and characterization of a new turn-on ratiometric fluorescent lead sensor, 4,4-dimethyl-4*H*-5-oxa-1,3-dithia-6,11-diazacyclopenta[*a*]anthracen-2-one, leadglow (**7**), which has a thiol-based binding site and therefore differs from other fluorophores with harder donors such as oxygen or nitrogen. Lead is a soft metal and therefore favors sulfur-rich binding sites.[19]

The synthesis of **7** is shown in Scheme 1. The reaction of 2-methyl-3-butyn-2-ol and 3,4-dihydro-2*H*-pyran in the presence of a catalytic amount of *p*-toluenesulfonic acid results in the protected alcohol **1** in excellent yield. Deprotonation of **1** followed by the addition of diethyl oxalate at low temperature affords **2** in moderate yield. The reaction of **2** with 4-phenyl-1,3-dithiolane-2-thione allowed us to introduce the protected dithiolene moiety. The intermediate compound **3** was transformed into pyrandione **4** upon addition of trifluoroacetic acid (TFA). Conversely when the same reaction was performed in xylene, pyrandione **4** was isolated directly in moderate yields. The thione sulfur atom in **4** was replaced with oxygen by using mercury(II) acetate to give pyrandione **5** in good yield. The reaction of **5** with *o*-phenylenediamine in methanol afforded almost quantitatively quinoxaline **6**. Addition of benzyl chloroformate and triethylamine to **6** leads to the formation of compound **7** in good yield; the product was characterized by infrared, NMR (^1H and ^{13}C), and UV/Vis spectroscopy, as well as mass spectrometry.

All spectroscopic measurements were performed in 2.5 % MeOH/water. NEt_4OH was added to the solution (2:1 NEt_4OH/**7**) to hydrolyze the carbonyl group and expose the thiolate binding site. Leadglow exhibits an absorption band at 415 nm ($\varepsilon = 1.3 \times 10^5\,M^{-1}cm^{-1}$) and an emission band of intensity $\Phi = 0.12$ at 465 nm. Upon incubation of a solution of **7** with lead acetate solution, the absorption band shifts to 389 nm ($\varepsilon = 1.1 \times 10^5\,M^{-1}cm^{-1}$). The emission band also shifts to 423 nm with a fivefold increase in the fluorescence intensity ($\Phi = 0.63$); the compound thus acts as a "turn-on" sensor (Figure 1). The shift in emission energy of **7** is characteristic of a wavelength-ratiometric probe (blue shift of 42 nm). Thus, like leadfluor-1, leadglow acts not only as a turn-on sensor, but also as a ratiometric one.[2] Leadflour-1 exerts a larger increase in the emission intensity upon binding to lead (18-fold) and a quantum yield of 0.013; however, **7** has a higher quantum yield (0.63) for the Pb-bound species. Leadglow is versatile and functions well over a wide pH range (Figure 2). The emission intensity of Pb^{2+} bound **7** remains almost constant in the pH range from 4 to 10.

Binding assays were performed by using Job's method of continuous variation,[20] which indicate a 1:2 Pb^{2+}/**7** complex. The apparent dissociation constant, K_d, for a Pb^{2+}–**7** complex was found to be 217 nM (at pH 10), using the Hill-1 function. Leadglow is very sensitive to Pb^{2+} in aqueous solution and binds to Pb^{2+} much stronger than leadfluor-1 ($K_d = 23\,\mu M$).[2] The "turn-on" feature of **7** allowed detection of a low level (10 ppb) of Pb^{2+}, even in the presence of other metals, by using a 1 μM solution. Leadglow can be used in detecting and determining Pb^{2+} in the tested range from 1 ppb to 50 ppb. Thus, this sensor offers a high dynamic range for Pb^{2+}

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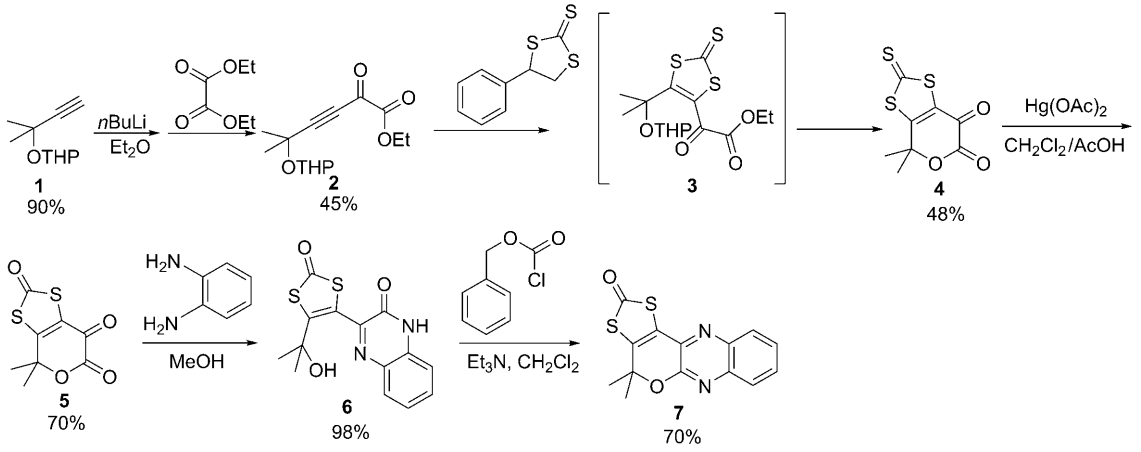

Scheme 1. Synthesis of leadglow (**7**).

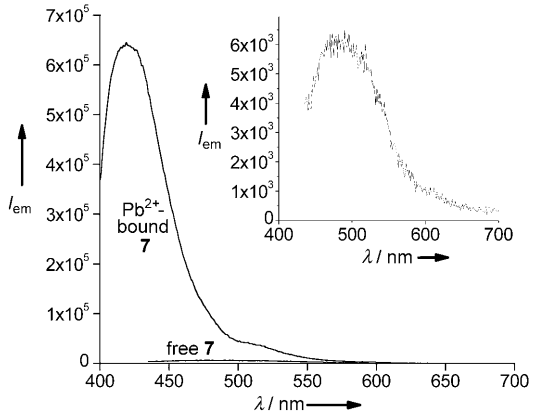

Figure 1. Emission spectra acquired in 2.5% MeOH/water and NEt_4OH (2:1 NEt_4OH/**7**) of 5 μM free **7** and 5 μM Pb^{2+}-bound **7**. Excitation of free **7**: 415 nm; excitation of Pb^{2+}-bound **7** (1:2 Pb^{2+}/**7**): 389 nm; emission maximum observed at 423 nm with a fivefold increase in emission intensity. The inset shows a magnification of the emission spectrum of free **7** (5 μM).

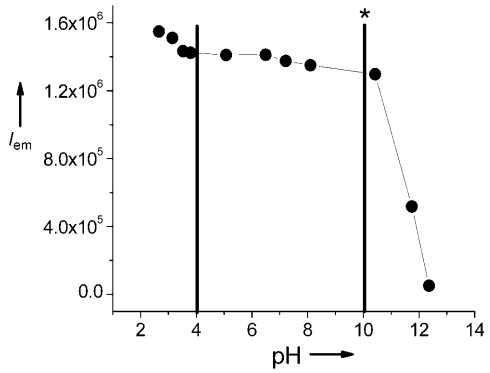

Figure 2. Dependence of emission of Pb^{2+}-bound **7** on pH. The complex exhibits a high, constant emission intensity from pH 4 to 10, indicating a wide functional pH range. The asterisk indicates the pH value at which the selectivity studies were performed.

detection. To further examine the sensitivity and accuracy of the sensor, we used a NIST standard of trace elements in water (SRM 1643e) in the concentration range 1–50 ppb Pb^{2+} and probed with 1 μM **7**. In this case, accurate fluorescence responses were observed from 50 ppb to as low as 10 ppb. Leadglow was also used to determine the concentration of Pb^{2+} in solutions prepared from a lead standard (NIST SRM 3128). These results were compared with those obtained from ICPMS measurements. The precisions of the two methods were found to be comparable by F-test and t-test analyses.

Leadglow is extremely selective for Pb^{2+} against other common metal ions tested. The fluorescence response of 5 μM **7** in the presence of Pb^{2+} and other ions in aqueous solution is shown in Figure 3. No significant change in the fluorescence was observed when a solution of **7** was incubated with 2 mM Li^+, Na^+, K^+, Ca^{2+}, or Mg^{2+}, followed by addition of Pb^{2+}; thus, **7** exhibits similar properties to those of leadfluor-1.[2] These metal ions were tested with higher concentrations as they are highly abundant in mammalian cells. Similarly, the

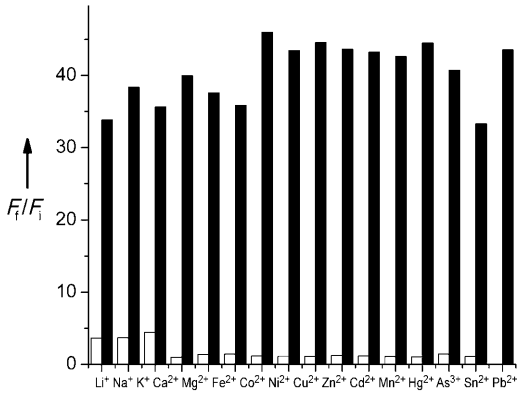

Figure 3. Fluorescence response of 5 μM **7** to common biologically available cations in 2.5% MeOH/water and NEt_4OH (2:1 NEt_4OH/**7**). The bars represent the ratio of the final fluorescence response (F_f) over the initial fluorescence response (F_i). The white bars represent the response to the addition of the given ions (2 mM for Li^+, Na^+, K^+, Ca^{2+}, and Mg^{2+}; and 75 μM for Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Mn^{2+}, Hg^{2+}, As^{3+}, and Sn^{2+}), the black bars that to the addition of 75 μM Pb^{2+} to the respective solution. Excitation wavelength: 389 nm.

fluorescence intensity of **7** in the presence of Pb^{2+} remained unchanged in the presence of metals and metalloid (Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, As^{3+}, Sn^{2+}, Mn^{2+}). This result clearly demonstrates the high selectivity of **7** towards Pb^{2+}, which is important for a viable sensor whether investigating an environmental sample (in which common contaminants include Cd^{2+}, As^{3+}, and Hg^{2+}) or a biological sample as plausible cellular targets of toxic lead accumulation, including calcium- and zinc-dependent proteins.[7,17]

In conclusion, **7** is a new fluorescent sensor that can detect Pb^{2+} in aqueous solution over a wide pH range (4–10) and in a mixture of several other metals at a concentration as low as 10 ppb. This sensor is advantageous because of its sensitivity for Pb^{2+} at concentrations below the limit set by the US Environmental Protection Authority (EPA), its turn-on and ratiometric detection of Pb^{2+} over other biologically as well environmentally abundant cations, its visible excitation and emission profiles, and its high optical brightness. This molecular system offers a wide variety of choices from tuning the excitation to specific tagging through selective substitution.

Experimental Section

For details, see the Supporting Information. Compound **1** and 4-phenyl-1,3-dithiolane-2-thione were prepared according to literature procedures.[21,22] Fluorescence quantum yields were determined in reference to fluorescein in 0.1 M NaOH ($\Phi = 0.95$).[23]

Synthesis of **7**: 3-[5-(2-Hydroxypropan-2-yl)-2-oxo-1,3-dithiol-4-yl]quinoxalin-2-one (140 mg, 0.439 mmol) was partially dissolved in CH_2Cl_2 (10 mL). Benzyl chloroformate (125 μL, 0.81 mmol) and triethylamine (120 μL) were added, and the resulting solution was stirred overnight. The solution was reduced in volume to around 3 mL and purified by chromatography (silica gel, CH_2Cl_2) to give pure **7**. Yield: 92 mg (70%); ^{1}H NMR (25 °C, $CDCl_3$): $\delta = 7.96$ (d, 1H), 7.83 (d, 1H), 7.66 (t, 1H), 7.60 (t, 1H), 1.84 ppm (s, 6H); ^{13}C NMR (25 °C, $CDCl_3$): $\delta = 189.0$, 152.5, 141.0, 140.7, 139.6, 133.7, 130.5, 128.6, 128.1, 127.5, 124.2, 81.2, 30.0 ppm; IR (neat): $\tilde{\nu} = 1705$, 1664, 1624, 1461, 1409 cm^{-1}. MS calcd for $C_{14}H_{11}N_2O_2S_2$ $[M+H]^+$: 303.02, found 302.93; UV/vis (MeOH): λ_{max} (ε in $M^{-1}cm^{-1}$) = 256 (10988), 367 (11194), 385 nm (9568); fluorescence (MeOH): excitation = 367, 385 nm; emission = 415 nm; fluorescence in 2.5% MeOH/water and NEt_4OH (1:2 **7**/NEt_4OH): excitation = 415 nm; emission = 465 nm.

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