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## Development of a new fluorescent Pb<sup>2+</sup> sensor

Lauren Marbella, Barbara Serli-Mitasev, and Partha Basu\*

Department of Chemistry and Biochemistry, Duquesne University, Pittsburgh, Pa 15282

### Keywords

fluorescent probes; lead; chemosensors; sensors; fluorescence spectroscopy

Lead is a persistent environmental contaminant,<sup>[1, 2, 3, Domaille, 2008 #17, 4]</sup> as exposure to very low levels of lead can cause neurological, reproductive, cardiovascular, and developmental disorders.<sup>[3, 5, 6]</sup> Children with variants in iron metabolism genes may be more susceptible to lead absorption and accumulation.<sup>[7], [8]</sup> The US Center for Disease Control (CDC) set standards stating that a 10–19 µg/dL blood lead level poses a potential threat and diagnostic testing is recommended.<sup>[7]</sup> Of particular interest is Pb<sup>2+</sup> as it interferes with enzymatic heme production.<sup>[9]</sup>

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

Fluorescence based sensors offer unparalleled sensitivity and thus, have garnered significant interest.<sup>[4]</sup> Most fluorescent probes for detecting Pb<sup>2+</sup> use peptides,<sup>[14]</sup> proteins,<sup>[15]</sup> or DNazymes.<sup>[3, 6, 16–18]</sup> These probes lack the simplicity that a small molecular probe can offer. In addition, non specific interaction and background fluorescence often act as a deterring factor, which underscores the necessity of a selective lead sensor that can function in aqueous environments.<sup>[1–3, 6]</sup> To this end, a water soluble fluorescence based small molecule Pb<sup>2+</sup> sensor (Leadfluor-1) has showed promise in understanding cellular Pb<sup>2+</sup> trafficking.<sup>[2]</sup> 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-4H-5-oxa-1,3-dithia-6,11-diazacyclopenta[a] anthracen-2-one, Leadglow (LG, **7**).

LG has a thiol-based binding site, which differs from other fluorophores with more hard donors such as oxygen or nitrogen. Lead is a soft metal and therefore favors sulfur-rich binding sites.<sup>[19]</sup> The proposed molecule can serve as a highly sensitive and selective fluorescent lead sensor in aqueous samples. LG fluoresces at 465 nm. In the presence of Pb<sup>2+</sup>, LG fluoresces at 423 nm, with a 5-fold increase in emission intensity, indicating a turn-on response to lead in aqueous solution.

\*Fax: (+) 412 396 5683, basu@duq.edu, Homepage ((optional)): <http://www.science.duq.edu/faculty/basu.html>.

The synthetic procedure to LG is shown in Scheme 1. The reaction of 2-methyl-3-butyn-2-ol and 3,4-dihydro-2H-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. Reaction of the  $\alpha$ -keto ester **2** with 4-phenyl 1,3-dithiolane-2-thione allowed us to introduce the protected dithiolene moiety. Direct reaction of **2** with the 4-phenyl 1,3-dithiolane-2-thione affords the intermediate molecule **3** which was transformed to the pyrandione **4** upon addition of trifluoroacetic acid (TFA). Conversely when the same reaction was performed in xylene, the pyrandione **4** was isolated directly in moderate yields. The thione sulfur in **4** was replaced with oxygen using mercury(II) acetate giving the pyrandione, **5**, in good yield. The reaction of **5** with *o*-phenylenediamine in methanol afforded almost quantitatively the quinoxaline compound, **6**. Addition of benzylchloroformate and triethylamine to **6** leads to the formation of compound **7** (Leadglow, LG) in good yield. LG was characterized by infrared, NMR ( $^1\text{H}$  and  $^{13}\text{C}$ ), and UV-visible spectroscopies, and mass spectrometry.

All spectroscopic measurements were performed in 2.5% MeOH and water.  $\text{NET}_4\text{OH}$  was added to the solution (2:1  $\text{NET}_4\text{OH}$ :LG) to hydrolyze the carbonyl group and expose the thiolato binding site. LG exhibits an absorption band at 415 nm ( $\epsilon = 1.3 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ ) and an emission band of low intensity ( $\phi = 0.12$ ) at 465 nm. Upon incubation of a solution of LG with lead acetate solution, the absorption band shifts to 389 nm ( $\epsilon = 1.1 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ ). The emission band also shifts to 423 nm, with a 5-fold increase in the fluorescence intensity ( $\phi = 0.63$ ), thus acting as a 'turn-on' sensor (Figure 1). In addition, LG exhibits a shift in the emission energy characteristic feature of a wavelength-ratiometric probe (blue shifted by 42 nm). Thus, like Leadfluor-1, LG acts not only as a turn on sensor, but also as a ratiometric one<sup>[2]</sup>. Upon binding to lead, Leadfluor-1 exerts a larger increase in the emission intensity (18 fold) with a quantum yield of 0.013, LG offers a higher quantum yield (0.63) for the Pb-bound species. LG is versatile and functions well at a wide pH range (Figure 2). The emission intensity of  $\text{Pb}^{2+}$  bound LG remains near constant in the pH range from 4 to 10.

Binding assays were performed using Job's method of continuous variation,<sup>[20]</sup> which indicates a 1:2  $\text{Pb}^{2+}$ :LG complex. The apparent dissociation constant for a complex,  $K_d$ , for  $\text{Pb}^{2+}$  coordination to LG was found to be 217 nM (at pH 10), using the Hill-1 function. LG is very sensitive to  $\text{Pb}^{2+}$  in aqueous solution. LG binds to  $\text{Pb}^{2+}$  much stronger than Leadfluor-1 ( $K_d$  23  $\mu\text{M}$ ).<sup>[2]</sup> The 'turn-on' feature of the sensor allowed detection of a low level (10 ppb) of  $\text{Pb}^{2+}$ , even in the presence of other metals, using a 1  $\mu\text{M}$  solution of LG. The LG can be used in detecting and determining  $\text{Pb}^{2+}$  in the tested range from 1 ppb to 50 ppb. Thus LG offers a high dynamic range for  $\text{Pb}^{2+}$  detection. To further examine the sensitivity and accuracy of the sensor, we used a NIST standard of trace elements in water (SRM<sup>®</sup> 1643e) in the concentration range of 1–50 ppb  $\text{Pb}^{2+}$  and probed with 1  $\mu\text{M}$  LG. In this case, accurate fluorescence responses were observed from 50 ppb to as low as 10 ppb. LG was also used in quantitating the concentration of  $\text{Pb}^{2+}$  in solutions prepared from a lead standard (NIST SRM<sup>®</sup> 3128). These results were compared with those obtained from ICPMS measurement. The precisions of the two methods were found to be comparable by F-test and t-test analyses.

LG is extremely selective for  $\text{Pb}^{2+}$  against other common metal ions tested. The fluorescence response of 5  $\mu\text{M}$  LG in the presence of  $\text{Pb}^{2+}$  and other ions in aqueous solution are shown in Figure 3. No change in the fluorescence was observed when a solution of LG containing  $\text{Pb}^{2+}$  was incubated with 2 mM  $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , or  $\text{Mg}^{2+}$ , thus exhibiting a similar properties to Leadfluor-1.<sup>[2]</sup> These metal ions were tested with higher concentration as they are highly abundant in mammalian cells. Similarly, the fluorescence intensity of LG containing  $\text{Pb}^{2+}$  remains unchanged in the presence of 3d transition metals.

Thus, 75  $\mu\text{M}$   $\text{Fe}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{As}^{3+}$ ,  $\text{Sn}^{2+}$  or  $\text{Mn}^{2+}$  ions cause no difference in fluorescence intensity. This clearly demonstrates high selectivity of LG towards  $\text{Pb}^{2+}$  which is important for a viable sensor whether investigating an environmental sample (where common heavy metal contamination includes  $\text{Cd}^{2+}$ ,  $\text{As}^{3+}$  and  $\text{Hg}^{2+}$ ) or biological sample as plausible cellular targets of toxic lead accumulation include calcium- and zinc-dependent proteins.<sup>7,17</sup>

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

## Experimental Section

### Synthetic Materials and Methods

Potassium hydroxide, carbon disulfide, and mercuric acetate were purchased from Fisher Scientific and were used as they were received without further purification. The rest of the chemicals were purchased from Acros and were used as they were received without further purification. Standard Reference Material<sup>®</sup> 1643e Trace Elements in Water and 3128 Lead Standard Solution were purchased from National Institute of Standards and Technology and used as received. Column chromatography was performed on Silica gel P60 (Sorbent Technologies).  $\text{CH}_2\text{Cl}_2$  was distilled over  $\text{CaH}_2$ , diethyl ether over Na wire/benzophenone and MeOH over Mg. All the other solvents were used without further purification.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were obtained in  $\text{CDCl}_3$  or  $\text{CD}_3\text{OD}$  at 25°C on a Bruker 400 MHz spectrometer. Infrared spectra were obtained on a Nicolet 380 FT-IR (Thermo) spectrometer. APCI-MS were recorded in methanol on a Waters ZMD mass spectrometer set in positive mode (solvent: methanol; cone voltage: 20 V; corona 2.7 kV; source temperature: 130°C; flow rate: 100  $\mu\text{L}/\text{min}$ ) or negative mode (solvent: methanol; cone voltage: –20 V; corona 2.5 kV; source temperature: 130°C; flow rate: 100  $\mu\text{L}/\text{min}$ ). ESI-MS were recorded on a Waters ZMD mass spectrometer set in the negative ionization mode (solvent: methanol; cone voltage: 20 V; capillary voltage: 2.9 kV; source temperature: 130°C; flow rate: 150  $\mu\text{L}/\text{min}$ ). Inductively coupled plasma mass spectrometry was conducted in an Agilent 7500ce equipped with a Shield Torch SYSTEM.

### 3-Methyl-3-tetrahydropyranloxy-butyne (1)

3-methyl-3-tetrahydropyranloxy-butyne was prepared according to literature procedure.<sup>[21]</sup> Dihydropyran (14.7 mL, 160.71 mmol) was added to a cooled (–20°C) solution of 2-methyl-3-butyne-2-ol (9.2 g, 112.53 mmol) in 80 mL of dry  $\text{CH}_2\text{Cl}_2$ . A few crystals of *p*-toluenesulphonic acid were added to the solution. The solution was stirred for 3 h and then washed with a saturated solution of  $\text{NaHCO}_3$  (4×30 mL) and the organics were dried over  $\text{MgSO}_4$ . The solvent was removed at reduced pressure and the resulting oil was purified by vacuum distillation. Yield: 15 g (90%).  $^1\text{H}$  NMR spectrum in  $\text{CDCl}_3$  (ppm):  $\delta$  5.06 (m, 1H), 3.95 (m, 2H), 3.50 (m, 2H), 2.43 (s, 1H), 1.85 (m, 2H), 1.70 (m, 2H), 1.51 (s, 6H).  $^{13}\text{C}$  NMR spectrum in  $\text{CDCl}_3$  (ppm):  $\delta$  95.97, 86.25, 86.24, 71.78, 70.72, 63.12, 31.80, 30.47, 29.68, 25.29, 20.29. IR spectrum (neat,  $\text{cm}^{-1}$ ): 3295, 2941, 2108, 1466, 1380.

### Ethyl-5-methyl-2-oxo-5-(tetrahydro-2H-pyran-2-yloxy)hex-3-ynoate (2)

A solution of 3-methyl-3-tetrahydropyran-2-yloxy-butyne (3.3 g, 19 mmol) in 30 mL Et<sub>2</sub>O was cooled to 0°C. *n*-Butyllithium (11.5 mL, of a 2.5 M solution in hexane, 28 mmol) was added to the previous solution. The resulting solution was stirred for 30 min at 0°C, then cooled to -78°C and diethyl oxalate (4.3 mL, 29.4 mmol) was added. The reaction was followed by TLC (hexane/EtOAc 90:10). After ca. 2 h the reaction mixture was poured into a cold aqueous solution of NH<sub>4</sub>Cl. The aqueous layer was extracted with Et<sub>2</sub>O (3×25 mL). The organics were dried over MgSO<sub>4</sub>. The solvent was removed under reduced pressure and the resulting yellow oil was purified via chromatography (silica gel, hexane/EtOAc 90:10) to afford **2** as a pale yellow liquid. Yield: 2.3 g (45%). <sup>1</sup>H NMR spectrum in CDCl<sub>3</sub> (ppm): δ 5.10 (m, 1H), 4.38 (q, 2H), 4.36 (m, 2H), 3.96 (m, 2H), 3.54 (m, 2H), 1.85 (m, 2H), 1.75 (m, 2H), 1.64 (s, 3H), 1.59 (s, 3H), 1.40 (t, 3H). <sup>13</sup>C NMR spectrum in CDCl<sub>3</sub> (ppm): δ 169.3, 158.7, 101.4, 96.2, 81.6, 70.5, 63.1, 31.5, 29.2, 28.9, 25.2, 19.9, 13.8, 13.8. IR spectrum (neat, cm<sup>-1</sup>): 2209, 1741, 1689. MS-APCI calculated for C<sub>14</sub>H<sub>20</sub>O<sub>5</sub> [M]<sup>-</sup>: 268.12, found 267.96.

### 4,4-Dimethyl-2-thioxo-4H-[1,3]dithiolo[4,5-c]pyran-6,7-dione (4)

4-phenyl-1,3-dithiolane-thione was prepared according to literature procedure.<sup>[22]</sup> Ethyl-5-methyl-2-oxo-5-(tetrahydro-2H-pyran-2-yloxy)hex-3-ynoate (1.0 g, 3.7 mmol) and 4-phenyl-1,3-dithiolane-thione (2.0 g, 9.3 mmol) were dissolved in xylene (15 mL). The deep yellow solution was refluxed at 140°C for 8 h under Ar. The open intermediate, **3**, was treated with a few catalytic drops of trifluoroacetic acid (TFA). The reaction was followed by TLC, eluent: CH<sub>2</sub>Cl<sub>2</sub>. The solvent was removed under reduced pressure and purified via chromatography (silica gel, CH<sub>2</sub>Cl<sub>2</sub>) to afford **4** as a yellow solid. Yield: 0.31 g (34%). <sup>1</sup>H NMR spectrum in CDCl<sub>3</sub> (ppm): δ 1.88 (s, 6H). <sup>13</sup>C NMR spectrum in CDCl<sub>3</sub> (ppm): δ 205.3, 167.7, 162.7, 157.8, 153.6, 83.2, 31.9. IR spectrum (neat, cm<sup>-1</sup>) 1747, 1681, 1557, 1460. MS-ESI calculated for C<sub>7</sub>H<sub>8</sub>O<sub>4</sub>S<sub>2</sub>Na [M+Na]<sup>+</sup>: 268.96, found 268.79.

### 4,4-Dimethyl-4H-[1,3]dithiolo[4,5-c]pyran-2,6,7-trione (5)

Mercuric acetate (181 mg, 0.568 mmol) was added to 4,4-dimethyl-2-thioxo-4H-[1,3]dithiolo[4,5-c]pyran-6,7-dione (100 mg, 0.406 mmol) in CH<sub>2</sub>Cl<sub>2</sub>/AcOH (3:1) and stirred for 30 min. The reaction mixture was filtered through a celite pad to remove the mercury salts. The resulting solution was washed with water (3×15 mL) and saturated NaHCO<sub>3</sub> (5×10 mL) and dried over MgSO<sub>4</sub>. The solvent was removed under reduced pressure to afford **5** as a beige solid. Yield: 40 mg (43%). <sup>1</sup>H NMR spectrum in CDCl<sub>3</sub> (ppm): δ 1.88 (s, 6H). <sup>13</sup>C NMR spectrum in CDCl<sub>3</sub> (ppm): δ 184.5, 163.5, 160.4, 153.5, 128.3, 84.2, 31.8. IR spectrum (neat, cm<sup>-1</sup>) 1746, 1693, 1641, 1552, 1453. MS-ESI calculated for C<sub>7</sub>H<sub>8</sub>O<sub>4</sub>S<sub>2</sub>Na [M+Na]<sup>+</sup>: 252.96, found 252.79.

### 3-(5-(2-Hydroxypropan-2-yl)-2-oxo-1,3-dithiol-4-yl)quinoxalin-2(1H)-one (6)

*o*-phenylenediamine (50 mg, 0.46 mmol) was added to a stirred solution of 4,4-dimethyl-4H-[1,3]dithiolo[4,5-c]pyran-2,6,7-trione (100 mg, 0.43 mmol) in methanol (15 mL). The solution was stirred overnight. The solvent was removed under reduced pressure and the residue was purified via crystallization from CH<sub>2</sub>Cl<sub>2</sub>/hexane affording pure **6** as a light orange solid. Yield: 143 mg (98%). <sup>1</sup>H NMR in CDCl<sub>3</sub> (ppm): δ 10.40 (bs, 1H), 7.88 (d, 1H), 7.62 (t, 1H), 7.43 (d, 1H), 7.28 (t, 1H), 4.28 (s, 1H), 1.61 (s, 6H). <sup>13</sup>C NMR spectrum in CD<sub>3</sub>OD (ppm): δ 194.6, 158.7, 156.0, 148.2, 136.0, 132.6, 127.9, 125.5, 123.6, 122.3, 119.2, 77.3, 33.8. IR spectrum (neat, cm<sup>-1</sup>) 1667, 1598. MS-ESI calculated for C<sub>14</sub>H<sub>11</sub>N<sub>2</sub>O<sub>3</sub>S<sub>2</sub> [M-H]<sup>-</sup>: 319.03, found 318.87.

### 4,4-Dimethyl-4H-5-oxa-1,3-dithia-6,11-diaza-cyclopenta[a]anthracen-2-one (Leadglow, LG) (7)

3-(5-(2-hydroxypropan-2-yl)-2-oxo-1,3-dithiol-4-yl)quinoxalin-2(1H)-one (140 mg, 0.439 mmol) was partially dissolved in  $\text{CH}_2\text{Cl}_2$  (10 mL). Benzylchloroformate (125  $\mu\text{L}$ , 0.81 mmol) and triethylamine (120  $\mu\text{L}$ ) were added and the resulting solution was stirred overnight. The volume of the solution was reduced to ca. 3 mL and it was purified via chromatography (silica gel,  $\text{CH}_2\text{Cl}_2$ ) to give pure Leadglow, **7**. Yield: 92 mg (70%).  $^1\text{H}$  NMR spectrum in  $\text{CDCl}_3$  (ppm):  $\delta$  7.96 (d, 1H), 7.83 (d, 1H), 7.66 (t, 1H), 7.60 (t, 1H), 1.84 (s, 6H).  $^{13}\text{C}$  NMR spectrum in  $\text{CDCl}_3$  (ppm):  $\delta$  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. IR spectrum (neat,  $\text{cm}^{-1}$ ): 1705, 1664, 1624, 1461, 1409. MS-APCI calculated for  $\text{C}_{14}\text{H}_{11}\text{N}_2\text{O}_2\text{S}_2$   $[\text{M}+\text{H}]^+$ : 303.02, found 302.93. UV-vis in MeOH ( $\lambda_{\text{max}}$ , nm ( $\epsilon$ ,  $\text{M}^{-1}\text{cm}^{-1}$ ): 256 (10988), 367 (11194), 385 (9568). Fluorescence in MeOH: Excitation = 367, 385 nm. Emission = 415 nm. Fluorescence in 2.5% MeOH and water and  $\text{NET}_4\text{OH}$  (1:2, LG:  $\text{NET}_4\text{OH}$ ): Excitation = 415 nm. Emission = 465 nm.

### Spectroscopic Materials and Methods

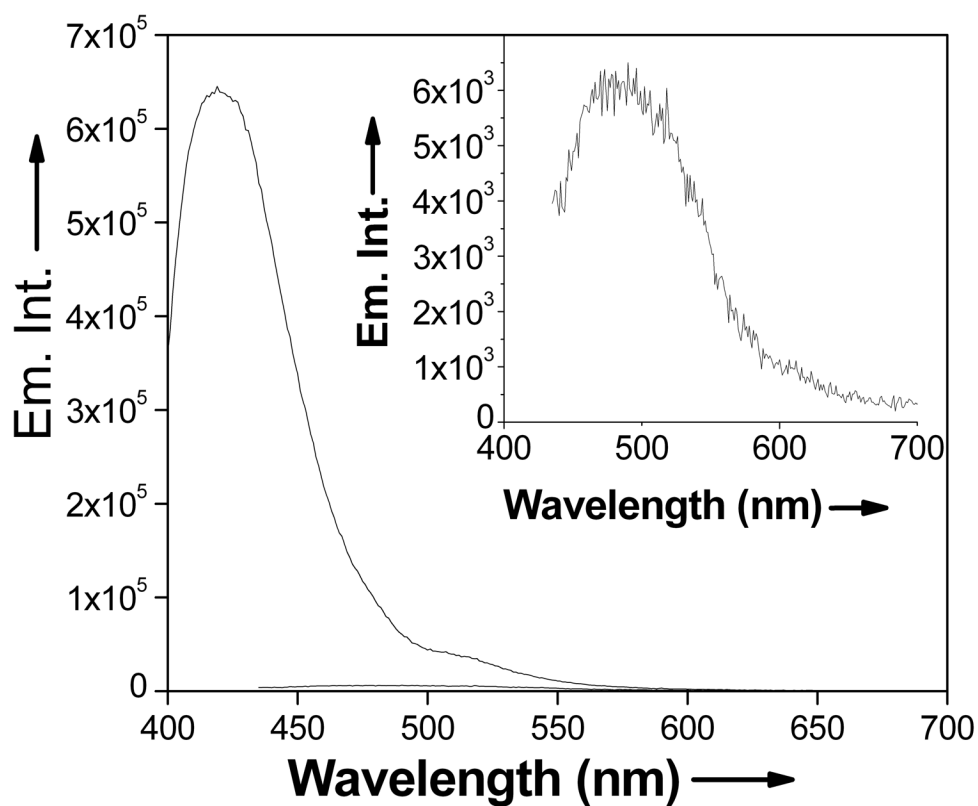
All spectroscopic measurements were recorded in a solution of 2.5% methanol (for solubility reason) and water. HPLC grade MeOH and distilled water were used for each measurement. Absorption spectra were measured on a Varian Cary 300 spectrometer. Samples for absorption measurement were performed in 1-cm  $\times$  1-cm quartz cuvettes (3.5 mL volume, Starna). Fluorescence spectra were measured on a Photon Technology International Quanta Master<sup>TM</sup> 4 spectrofluorometer. Fluorescence measurements were performed in 1-cm  $\times$  1-cm quartz cuvettes (3.5 mL volume, NSG Precision Cells). Fluorescence quantum yields were determined in reference to fluorescein in 0.1 NaOH ( $\phi = 0.95$ ).<sup>[23]</sup> The binding ratio was determined using Job's method of continuous variation.<sup>[20]</sup> The binding affinity of  $\text{Pb}^{2+}$  to LG was also found. Excitation was provided at 389 nm. The dissociation constant,  $K_d$ , was determined by using the Hill function in OriginPro 8, which is as follows:  $y = \text{START} + (\text{END} - \text{START}) \times x^n / (k^n + x^n)$ . START is the first data point where the curve begins, END is the last data point where the curve ends, x is the concentration of  $\text{Pb}^{2+}$ , k is the  $K_d$  for the binding reaction, and n is the Hill coefficient or the cooperativity of the dependence on x. A 10  $\mu\text{M}$  solution (1:2:4  $\text{Pb}^{2+}$ :LG: $\text{NET}_4\text{OH}$ ) was pH adjusted down by adding AcOH and adjusted up by adding  $\text{NET}_4\text{OH}$ . SRM<sup>®</sup>1643e Trace Elements in Water (1–50 ppb  $\text{Pb}^{2+}$ ) was probed with 1  $\mu\text{M}$  LG in  $\text{NET}_4\text{OH}$  (2:1  $\text{NET}_4\text{OH}$ :LG) and 2.5% MeOH and water. LG was able to detect  $\text{Pb}^{2+}$  quantitatively by an increase in fluorescence response from 10–50 ppb  $\text{Pb}^{2+}$ . For ICPMS measurements solutions were scanned for  $^{204}\text{Pb}$ ,  $^{206}\text{Pb}$ ,  $^{207}\text{Pb}$ ,  $^{208}\text{Pb}$  isotopes, the sums of the counts for all isotopes were used for analyses. For quantitative analyses, (ICPMS and fluorescence) calibration curves at pH ~6.6, were created using a multi-element (31 elements) calibration standard (ICP-MSCS-M) obtained from High Purity Standards, Charleston, SC. In both cases, linearity with >99% correlation was maintained with  $\text{Pb}^{2+}$  concentration in the range 1–50 ppm. Using the linear equations, concentrations of  $\text{Pb}^{2+}$  present in samples prepared from lead standard solution (SRM<sup>®</sup> 3128) via serial dilution were determined. The results from two methods (ICPMS and fluorescence) passed the F-test with 95% confidence limit. The t-test with 95% confidence interval showed the results of the methods are statistically equivalent.

### Acknowledgments

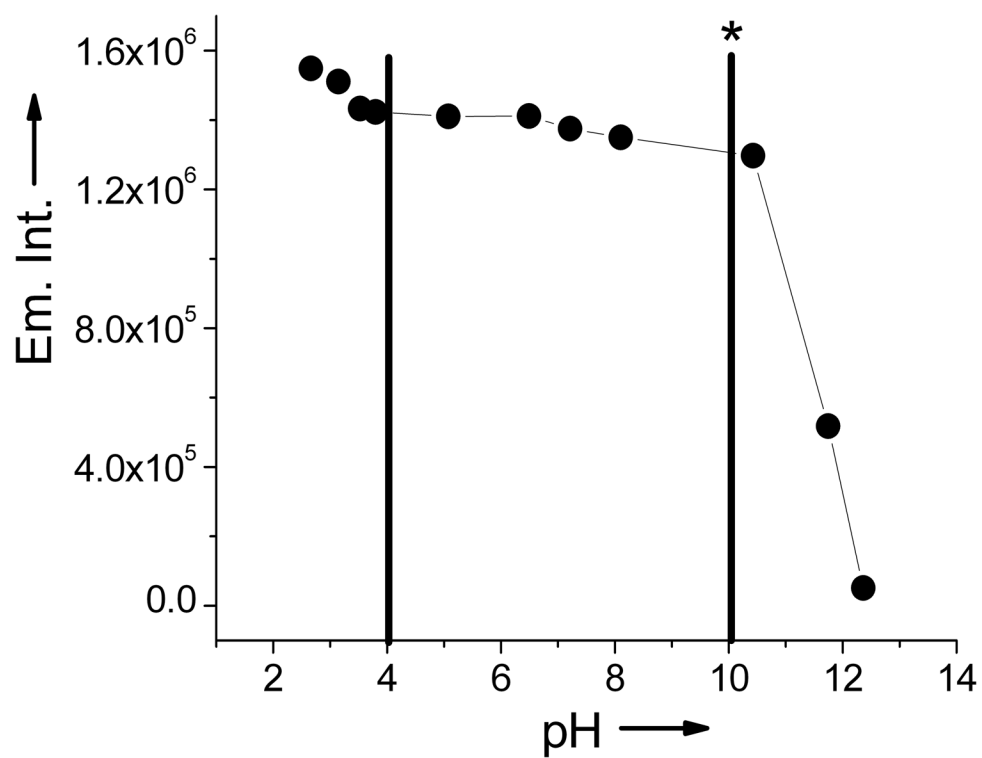
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## References

1. Swearingen CB, Wernette DP, Cropek DM, Lu Y, Sweedler JV, Bohn PW. *Anal Chem.* 2005; 77:442. [PubMed: 15649039]
2. He Q, Miller EW, Wong AP, Chang CJ. *J Am Chem Soc.* 2006; 128:9316. [PubMed: 16848451]
3. Li J, Lu Y. *J Am Chem Soc.* 2000; 122:10466.
4. Domaille DW, Que EL, Chang CJ. *Nat Chem Biol.* 2008; 4:168. [PubMed: 18277978]
5. Jain AK, Gupta VK, Singh LP, Raisonni JR. *Electrochim Acta.* 2006; 51:2547.
6. Xiao Y, Rowe AA, Plaxco KW. *J Am Chem Soc.* 2007; 129:262. [PubMed: 17212391]
7. Hopkins MR, Ettinger AS, Hernandez-Avila M, Schwartz J, Tellez-Rojo MM, Lamadrid-Figueroa H, Bellinger D, Hu H, Wright RO. *Environ Health Persp.* 2008; 116:1261.
8. Bannon DI, Murashchik C, Zapf CR, Farfel MR, Chisolm JJJ. *Clin Chem.* 1994; 40:1730. [PubMed: 8070083]
9. Labbe RF, Vreman HJ, Stevenson DK. *Clin Chem.* 1999; 45:2060. [PubMed: 10585337]
10. Fahrni CJ. *Curr Opin Chem Biol.* 2007; 11:121. [PubMed: 17353139]
11. Aggarwal SK, Kinter M, Herold DA. *Clin Chem.* 1994; 40:1494. [PubMed: 8044988]
12. Bannon DI, Chisolm JJJ. *Clin Chem.* 2001; 47:1703. [PubMed: 11514407]
13. Feldman BJ, Osterloh JD, Hata BH, D'Alessandro A. *Anal Chem.* 1994; 66:1983. [PubMed: 8067521]
14. Deo S, Godwin HA. *J Am Chem Soc.* 2000; 122:174.
15. Chen P, Greenberg B, Taghavi S, Romano C, van der Lelie D, He CA. *Angew Chem Int Ed.* 2005; 44:2715.
16. Liu J, Lu Y. *J Am Chem Soc.* 2003; 125:6642. [PubMed: 12769568]
17. Liu J, Lu Y. *J Am Chem Soc.* 2004; 126:12298. [PubMed: 15453763]
18. Juewen Liu YL. *Angew Chem Int Ed.* 2007; 46:7587.
19. Magyar JS, Weng TC, Stern CM, Dye DF, Rous BW, Payne JC, Bridgewater BM, Mijovilovich A, Parkin G, Zaleski JM, Penner-Hahn JE, Godwin HA. *J Am Chem Soc.* 2005; 127:9495. [PubMed: 15984876]
20. Job P. *Ann Chim.* 1928; 9:113.
21. Baraldi PG, Barco A, Benetti S, Ferretti V, Pollini GP, Polo E, Zanirato V. *Tetrahed.* 1989; 45:1517.
22. Culvenor CCI, Davies W, Pausacker KH. *J Chem Soc.* 1946:1050.
23. Brannon JH, Magde D. *J Phys Chem.* 1978; 82:705.

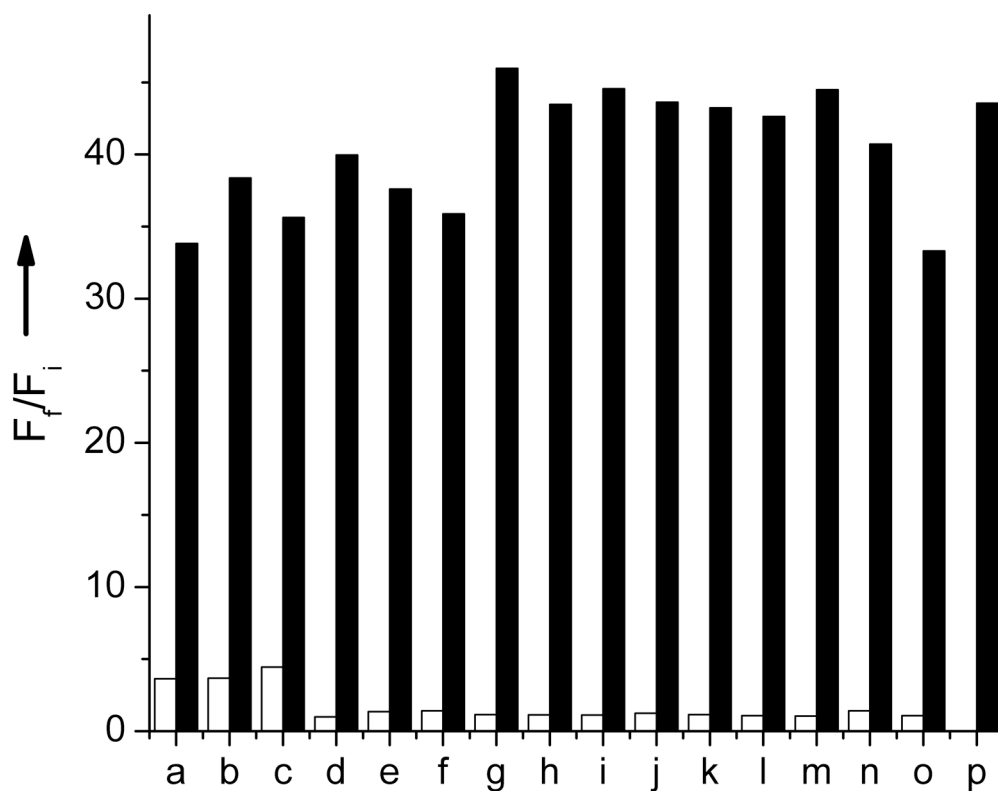


**Figure 1.** Spectra acquired in 2.5% MeOH and water and  $\text{NET}_4\text{OH}$  (2:1  $\text{NET}_4\text{OH}$ :LG). Emission spectra of  $5\ \mu\text{M}$  free LG (red) and  $5\ \mu\text{M}$   $\text{Pb}^{2+}$  bound LG (black). Excitation for free LG provided at 415 nm. Excitation for  $\text{Pb}^{2+}$  bound LG (1:2  $\text{Pb}^{2+}$ :LG) provided at 389 nm. Emission maximum observed at 423 nm with a 5-fold increase in emission intensity. Inset is a magnification of free LG ( $5\ \mu\text{M}$ ) emission spectra.



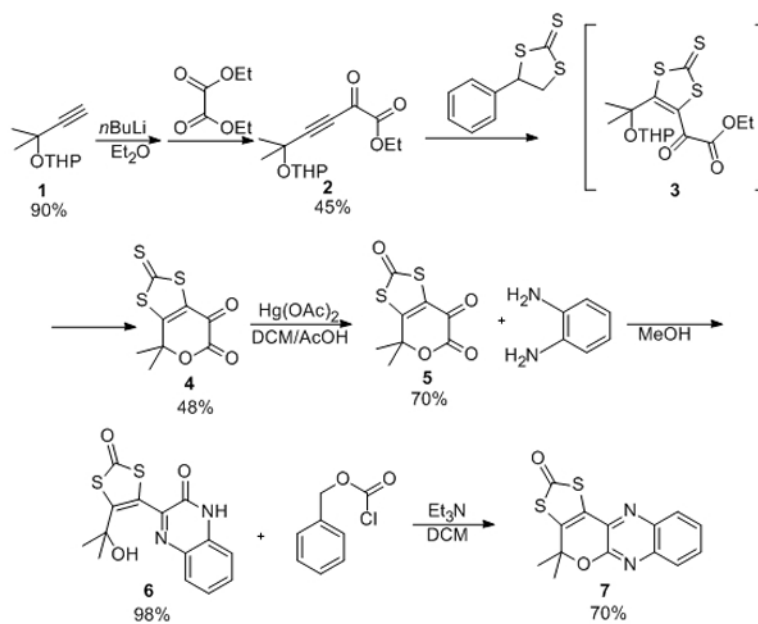
**Figure 2.** pH profile of  $\text{Pb}^{2+}$  bound Leadglow. The complex exhibits a high, constant emission intensity from pH 4 to 10, indicating a wide functional pH range. The asterisk indicates where all selectivity studies were performed.





**Figure 3.**

Spectra acquired in 2.5% MeOH and water and  $\text{NET}_4\text{OH}$  (2:1  $\text{NET}_4\text{OH}$ :LG). Fluorescence response of 5  $\mu\text{M}$  LG to common biologically available cations. The bars represent the final fluorescence response ( $F_f$ ) over the initial fluorescence response ( $F_i$ ). White bars represent the addition of each ion (2 mM for  $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{Mg}^{2+}$  and 75  $\mu\text{M}$  for  $\text{Fe}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{As}^{3+}$ ,  $\text{Sn}^{2+}$ , and  $\text{Pb}^{2+}$ . Black bars represent the addition of 75  $\mu\text{M}$   $\text{Pb}^{2+}$  to the solution. Excitation was provided at 389 nm. a.  $\text{Li}^+$ , b.  $\text{Na}^+$ , c.  $\text{K}^+$ , d.  $\text{Ca}^{2+}$ , e.  $\text{Mg}^{2+}$ , f.  $\text{Fe}^{2+}$ , g.  $\text{Co}^{2+}$ , h.  $\text{Ni}^{2+}$ , i.  $\text{Cu}^{2+}$ , j.  $\text{Zn}^{2+}$ , k.  $\text{Cd}^{2+}$ , l.  $\text{Mn}^{2+}$ , m.  $\text{Hg}^{2+}$ , n.  $\text{As}^{3+}$ , o.  $\text{Sn}^{2+}$ , p.  $\text{Pb}^{2+}$ .



**Scheme 1.**  
Synthetic scheme of Leadglow