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Dicarboxylated ethynylarenes as buffer-dependent chemosensors for Cd(II), Pb(II), and Zn(II)

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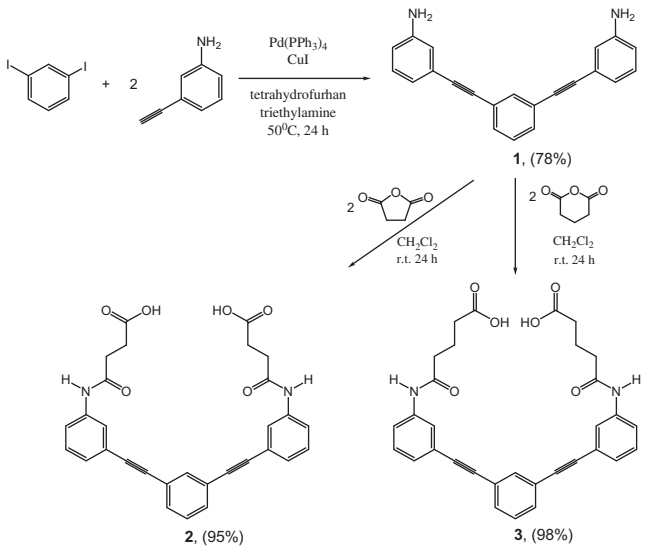
ABSTRACT

Two dicarboxylated ethynylarenes were prepared efficiently from condensation of 1,3-bis(3-aminophenylethynyl)benzene with 2 equiv of either succinic anhydride or glutaric anhydride. These compounds behave as fluorescent chemosensors selective for Cd(II), Pb(II), and Zn(II) cations under buffered aqueous conditions, with analyte binding observed as bathochromically shifted, intensified fluorescence. It was noteworthy that the fluorescence responses varied significantly with buffer identity. A conformational restriction mechanism involving reversible interactions between the fluorophore, metal cation, and buffer itself is proposed.

Because the majority of transition metal cations cannot be observed directly by spectroscopic measurement, the indirect detection of such analytes through the use of fluorescence chemosensors constitutes an ongoing area of research.[1] As the most abundant transition metal in the body, Zn(II) is an important target,[2] as are Cd(II) and Pb(II) due to their strong toxicity and persistence as environmental pollutants.[3] Selective detection of such analytes in aqueous environments is challenging.[4]

Arene-based sensors operating via conformational restriction mechanisms can be used to detect a variety of cationic,[5,6] anionic,[7] and small molecule[7c,8] analytes. Ethynylarenes are also attractive templates for constructing such fluorescent chemosensors[9] due to their efficient and modular construction, well-defined optoelectronic properties, and rotationally mobile π-systems.[10] Conformational restriction sensing relies on analyte binding at a peripheral recognition site leading to rigidification of the covalently connected fluorophore unit.[6a,11] This results in an intensification of fluorescence signal relative to the analyte unbound state. If coplanarity between the fluorophore's arene units is simultaneously perturbed, hypsochromic or bathochromic shifts in emission can also be observed.[6]

The goal of this investigation was to develop a new family of fluorescence chemosensors comprised of ethynylarene fluorophore units and carboxylic acid containing analyte recognition units. These molecules were designed to operate via a conformational restriction mechanism. In the absence of analyte the arene subunits can freely rotate at the alkyne bonds, while cooperative binding of divalent cation analytes between sensor's peripheral carboxylate groups causes rigidification and increased coplanarity in the ethynylarene fluorophore.



Scheme 1. Preparation of ethynylarene sensors.

Scheme 1 summarizes the simple preparation of the two dicarboxylated ethynylarenes used in this investigation. Sonogashira

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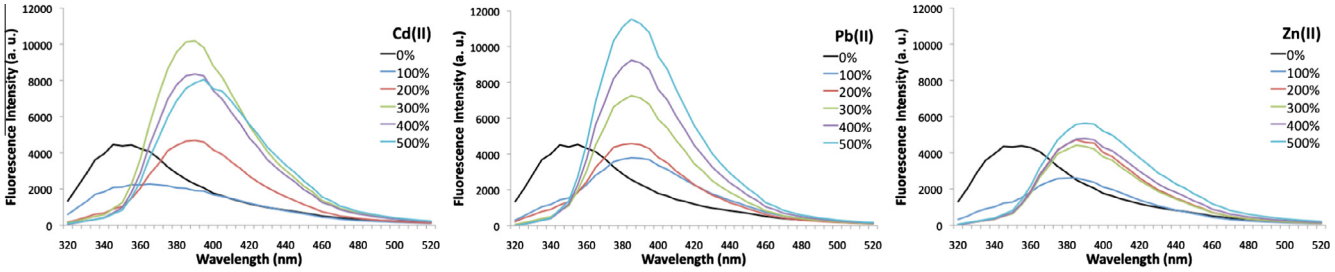

Figure 1. Changes in fluorescence emission of **2** (50 μM) upon titration with Cd(II), Pb(II), and Zn(II) with TRIS (pH 7.6) buffer.

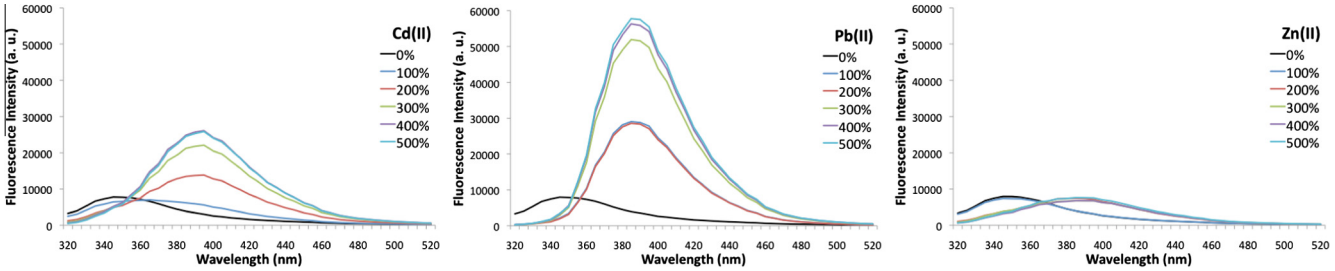

Figure 2. Changes in fluorescence emission of **3** (50 μM) upon titration with Cd(II), Pb(II), and Zn(II) with TRIS (pH 7.6) buffer.

coupling[12] between 1,3-diiodobenzene and two equivalents of *meta*-ethynylanaline produced diamine **1** as previously reported.[13] Condensation of **1** with two equivalents either succinic or glutaric anhydride resulted in dicarboxylated ethynylarenes **2** and **3**, respectively. These reactions were conducted in CH_2Cl_2 solvent, and as each reaction progressed the carboxylated products precipitated from solution. Reaction progress was monitored by HPLC, where condensation products displayed longer retention times than reactant **1** (Supplementary data). Reaction completion was also assessed by ^1H NMR in CD_3OD, where aromatic region signals of **2** and **3** displayed significant downfield shifts relative to reactant **1**. Each of these products was soluble in aqueous buffer at pH 7.6 and upon excitation at λ_{max} = 300 nm displayed fluorescence emission at λ_{max} = 350 nm.

High-throughput fluorescence screening[1c,14] was used to examine whether these compounds would display the predicted spectroscopic responses upon exposure to cationic analytes in aqueous solution. TRIS buffered aqueous 100 μM stock solutions of **2** and **3** were mixed with varying ratios of 100–500 μM aqueous metal chloride salt solutions in 96 well plates. The resulting solutions of 50 μM sensor mixed with 50–250 μM metal cations were analyzed for fluorescence changes induced by increasing metal concentrations. Among the twelve metal cation analytes studied, only Cd(II), Pb(II), and Zn(II) produced bathochromic shifts in fluorescence output when mixed with **2** (Fig. 1) or **3** (Fig. 2). Concurrent signal intensification varied significantly among metal/sensor combinations. These observations are consistent with a conformational restriction mechanism of signal generation. Enabled by the 30–40 nm shift in emission wavelength upon analyte binding, a ratiometric comparison of emission intensities (390/340 nm) was used to define the 'turn-on' sensor response (Fig. 3).

In comparing the results of **2** and **3** it is evident that the single methylene unit difference between succinic and glutaric units significantly impacts signal generation. Compound **2** shows a greatly enhanced signal strength for Zn(II) relative to **3**, while **3** shows a strong selectivity toward Pb(II). The significant bathochromic responses observed for **2** and **3** enabling ratiometric interpretation of data positions the 1,3-bis(arylethynyl)benzene motif as an attractive template to modularly incorporate a diversity of peripheral analyte binding units for future chemosensor development.

At this stage of the study, the hypothesized mechanism for analyte binding was cooperative intramolecular chelation between each carboxylate unit of the sensor and the divalent cation analyte. Unexpectedly, when TRIS buffer was replaced with phosphate during subsequent control studies, no 'turn-on' signals were observed despite the identical pH 7.6 conditions of the assays (Supplementary data). Because TRIS has been shown to perturb the results of metal binding assays by coordinating metal cations,[15] a survey of buffers was conducted to evaluate the influence of buffer identity on chemosensor performance.

Figure 4 summarizes the commercially available buffers and control compounds selected for this survey. These can be organized into three families: the trihydroxy-containing TRIS family, the morpholine-containing MOPS family and the piperazine-containing HEPES family. Buffers were selected so that the impact of any minor structural differences on sensor performance could be identified. The small organic molecules triethanolamine (TEA), *N*-methylmorpholine (NMM), and dimethylpiperazine (DMP) were also employed as buffers in this study to gain additional insight on structure–property relationships.

Aqueous solutions of **2** and **3** were prepared using each of these buffers adjusted to pH 7.6 with HCl or NaOH. High throughput screening was then performed against the same panel of cations as the initial TRIS study. Varying the buffer identity did not lead to any significant 'turn-on' signals for analytes beyond Cd(II), Pb(II), and Zn(II), but there was surprising variation in sensor output observed for **2** (Fig. 5) and **3** (Supplementary data).

The modest size difference in the carboxylate chains of **2** and **3** significantly impacted signal output for the buffers surveyed, with **2** displaying generally weaker signal strengths than **3**. A notable feature of this system is that analyte selectivity can be tuned simply by changing buffer identity. For example, while **2** + TRIS detected each of Cd(II), Pb(II), and Zn(II), **2** + TAPS was selective for Cd(II) and **2** + DMP was selective for Zn(II).

Because **2** and **3** are each inactive when using phosphate or citric acid buffers, amine functionality appears necessary for 'turn-on'

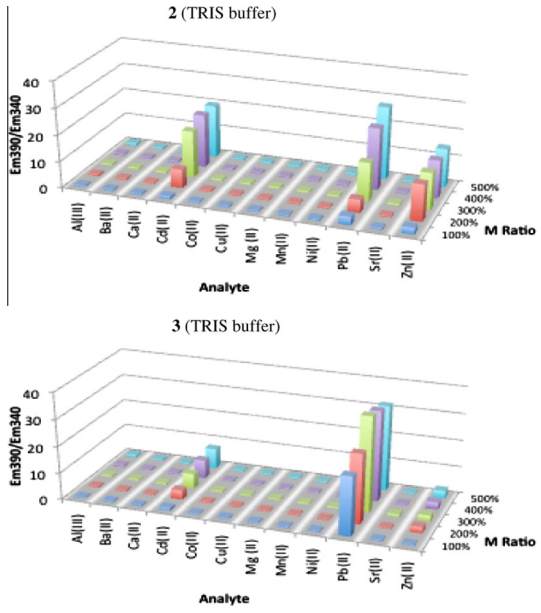

Figure 3. Ratiometric analysis for assays of **2** and **3** with 1–5 equiv of metal cation analytes, showing 'turn-on' responses for Cd(II), Pb(II), and Zn(II).

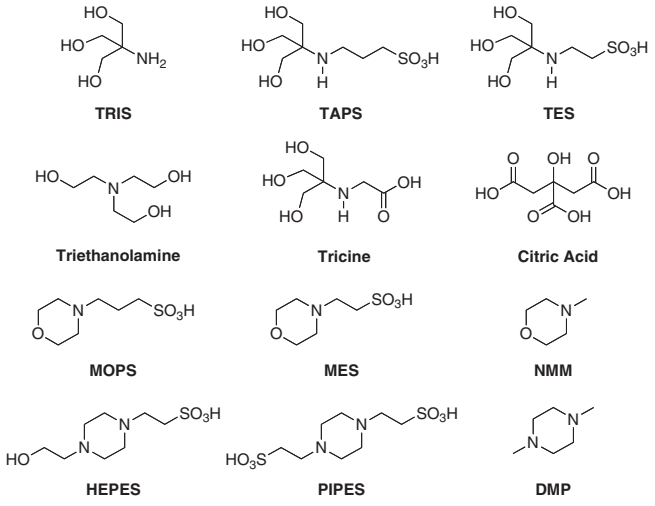

Figure 4. Identities of buffers surveyed.

signal generation. It is proposed that the amine containing buffers interact electrostatically with the sensor carboxylate units via their ammonium groups present at pH 7.6, supported by the observation that all sensors failed to operate at either high (pH 10) or low (pH 3) pH levels. Optimal 'turn-on' signals were observed for buffer:sensor ratios of 1000:1 or 500:1 but were lost at ratios of 100:1 or less. Hence, this weak electrostatic interaction driving buffer/sensor binding in these assays is likely enforced by the large statistical excess of buffer relative to sensor.

The importance of this electrostatic interaction between buffer and sensor is also supported by the inactivity of tricene buffered sensors, where buffer carboxylate groups could compete with sensor carboxylate groups for ammonium affinity. In contrast, buffers possessing sulfonate groups were able to generate 'turn-on' signals. Responses by buffers with either one or zero sulfonate groups (such as MOPS vs NMM and HEPES vs DMP) were nearly identical, while the buffer with two sulfonate groups (PIPES) showed no

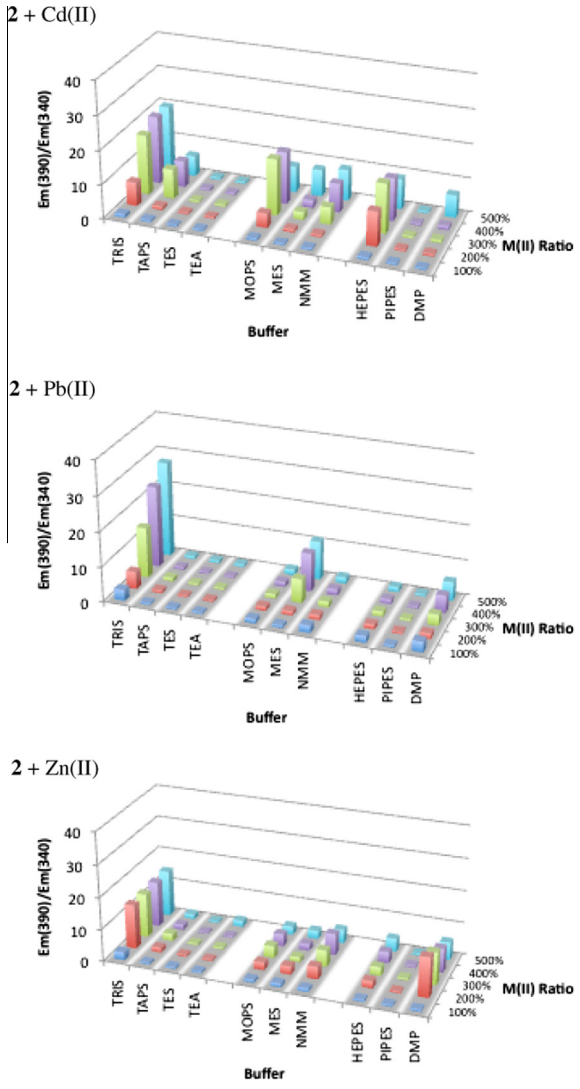

Figure 5. Ratiometric sensing output for **2**, comparing impact of buffer identity.

'turn-on' responses. In comparing TES with TAPS and MES with MOPS, longer spacing between ammonium and sulfonate groups in the buffer correlates to stronger 'turn-on' signals for most analytes. These observations collectively indicate that electrostatic repulsion between buffer sulfonate and sensor carboxylate groups destabilize analyte binding, and results in an interesting diversity of 'turn-on' sensing patterns toward Cd(II), Pb(II), and Zn(II) at the concentrations studied.

In the absence of analyte there were no observed differences in the fluorescence emission of **2** and **3** among the buffers studied. So while the data generated from this buffer survey suggests that a direct interaction between sensor and buffer is necessary for 'turn-on' signal generation, these two constituents alone are unable to generate fluorescence changes. 'Turn-on' signals were only observed when metal cation analytes were present, supporting a three-component binding event that leads to conformational restriction and increased π-system coplanarity in **2** and **3**. It is proposed that the hydroxy, ether, and amine functionalities present in the TRIS, MOPS, and HEPES families of sensors are essential for metal analyte binding, but additional study is needed to precisely define these collective binding interactions.

To identify the detection limits afforded by **2** and **3** in various buffers, serial dilution assays were performed on those

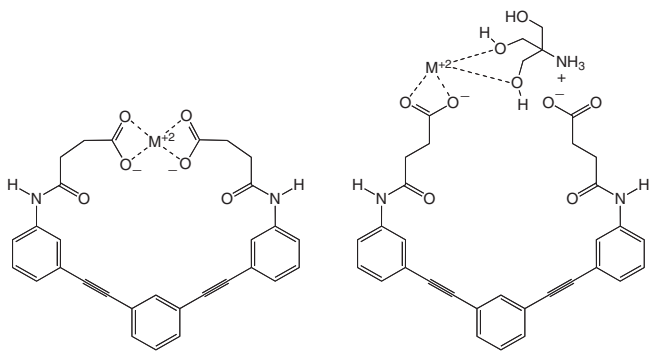

Figure 6. Illustrations of binding mechanisms for **2**. Left: initially hypothesized binary interaction between sensor and metal. Right: possible ternary interaction between sensor, metal, and buffer (TRIS example shown).

sensor/buffer/metal combinations showing strong 'turn-on' signals (Supplementary data). The reversibility of analyte binding is supported by the design of these dilution experiments where pre-saturated 'turn-on' signals were lost upon dilution. The metal concentration at which the bathochromic shift became absent was defined as the lower detection limit for each system. Buffer identity impacted binding affinity up to two orders of magnitude, and **3** generally showed 10-fold stronger binding affinity than **2** for analogous buffers. Several buffer–metal combinations for **2** and **3** continued to generate 'turn-on' signals at the 1 μM lower detection limit of the assay, establishing the ability of this chemosensing motif to detect aqueous Cd(II), Pb(II), and Zn(II) at biologically relevant concentrations.

It is proposed that the 'turn-on' fluorescence chemosensing responses of **2** and **3** for micromolar Cd(II), Pb(II), and Zn(II) analytes in aqueous solution involves the direct participation of buffer molecules as part of a three-component binding event (Fig. 6). This study serves as a cautionary example that buffers themselves can significantly perturb fluorescence output, and that an evaluation of buffer influence is advisable when studying chemosensors targeting metal cations in buffered aqueous environments. High throughput screening used in this study exploited such buffer influence as an additional variable for discovering new sensor motifs with interesting patterns of affinity for metal cation analytes. Future work will aim to more precisely define the noncovalent interactions driving metal cation binding by sensor and buffer in these systems, to examine how modular changes to the ethynylarene unit impact sensor performance, and to explore sensor/metal combinations able to selectively detect amine-containing small molecule analytes.

Acknowledgments

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Supplementary data

Supplementary data (details of synthetic procedures and characterization of **1**, **2**, and **3**, fluorescence titration assay results, and table of detection limits) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.07.111.

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