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2-(1,2,3-Triazol-4-yl)pyridine-containing ethynylarenes as selective 'turn-on' fluorescent chemosensors for Ni(II)

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Abstract

A series of ethynylarene compounds containing 2-(1,2,3-triazol-4-yl)pyridine chelating units were studied as fluorescent chemosensors for metal cations in aqueous solution. Analogs possessing two chelating units bridged by either 1,4-diethynylphenyl or 2,7-diethynylnaphthyl subunits displayed large hypsochromic shifts coupled with signal intensification when exposed to increasing concentrations of Ni(II), a unique response among 22 metal cation analytes. This response was shown to be reversible, and is proposed to derive from disruption of aggregate formation upon Ni(II) binding at the peripheral chelating units.

Keywords

Sensor; Fluorescent; Ratiometric; Ni(II) detection; 1,2,3-Triazole

Fluorescence chemosensing has long stood as an attractive approach for the detection of analytes lacking their own spectroscopic signatures. Signal generation can derive from a variety of mechanisms, with systems capable of sensitive and selective detection being highly desirable.¹ Sensors tailored for metal ion detection typically contain both metal binding and fluorescence emission subunits. An obvious advantage to developing sensors from small molecules is the ability to systematically modulate molecular features using traditional organic synthesis to engineer desired performance.

An essential nutrient at trace levels, Ni(II) is commonly found in elevated levels in the environment due to a variety of industries such as electroplating, electronics cleaning and rechargeable batteries.² Small molecule based fluorescence chemosensors typically suffer from poor selectivity and the fluorescence quenching properties of Ni(II).³ While recent progress has been made in this area,⁴ there remains a need to develop new tools for the detection of aqueous Ni(II) analytes.

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Supplementary Material

Details of synthetic procedures and characterization of compounds **1-7** as well as fluorescence titration assay results are available.

Ethynylarenes appended with metal-binding peripheral subunits have been shown to be effective fluorescence chemosensors for a variety of metal cation analytes.⁵ Variation of metal-binding subunit identity enables the selectivity of metal ion binding to be varied modularly. Following the advent of click chemistry in 2002, the past decade has seen a surge of interest in studying the metal-binding properties of 1,2,3-triazole-containing chelators.⁶ We report herein the preparation of a family of 1,2,3-triazole containing ethynylarene compounds and the ability of two such derivatives to serve as selective chemosensors for Ni(II) analytes in an aqueous environment.

Scheme 1 summarizes the synthesis of the two active sensors used in this investigation. A tandem Sharpless-Meldal click chemistry approach⁷ was employed to join 4-iodobenzyl bromide, sodium azide, and 2-(trimethylsilylethynyl)pyridine to produce **1**. This tandem reaction involves azide substitution and trimethylsilylalkyne deprotection, followed by Cu-catalyzed Huisgen 1,3-dipolar cycloaddition between the resulting organic azide and terminal alkyne intermediate products.⁸ Sonagashira coupling⁹ of two equivalents of **1** and either *p*-diethynylbenzene or 2,7-diethynylnaphthalene¹⁰ resulted in the dimeric 2-(1,2,3-triazol-4-yl)pyridine-containing ethynylarenes **2** and **3**, respectively.¹¹ Each product showed limited solubility in common organic solvents and was purified by simple washing with methanol. Product identity was assessed by ¹H NMR, ¹³C NMR, and MALDI-TOF MS.

The 2-(1,2,3-triazol-4-yl)pyridine motif is known to bind a variety of metal cations, including Ni(II).¹² Both **2** and **3** were designed to operate as conformational restriction chemosensors, possessing two metal-binding subunits covalently attached to, but not electronically conjugated with, a central ethynylarene unit capable of fluorescence emission. Similar arene-bridged triazole chelators have been shown to form stable intermolecular complexes with Ni(II), although such species lack spectroscopic signatures enabling their application to fluorescence chemosensing.^{12b}

These sensors were originally designed as next generation analogs of ethynylarene chemosensors operating via a conformational restriction mechanism,^{5d,5g,14} such that in the absence of analyte the arene subunit would be capable of free rotation about its alkyne bonds resulting in a baseline fluorescence emission signal. Upon binding of analyte a rigidification of the central ethynylarene unit would be enforced that results in an increase in fluorescence intensity and a possible change in emission wavelength depending on the conformation of the ethynylarene bonds in the analyte bound state.¹³ Due to the distance between the chelating units in the sensor, such rigidification would likely not be driven by cooperative interaction between chelating units of the same molecule, but rather by cooperative intermolecular binding of multiple sensor molecules via analyte coordination.^{12b}

Because the goal of this study was to identify sensors capable of sensing aqueous solutions of metal cation analytes, it was important to evaluate **2** and **3** in an aqueous environment. While each compound is insoluble in water itself, both **2** and **3** can be pre-dissolved in DMSO and diluted 1:1 or 1:2 into water without precipitating. Significant differences in the UV-visible absorbance and fluorescence emission spectra for **2** and **3** are observed in comparing DMSO and DMSO:water mixtures (Supplementary Material). Under aqueous conditions the fluorescence emission for each was significantly red shifted and quite broad

relative to the DMSO samples, while the absorbance spectra for each also varied significantly. It is proposed that the differences in emission for **2** and **3** under varying solvent conditions are due to aggregate formation promoted by the decreased solubility of these compounds in an aqueous environment. Due to the variation in fluorescence emission promoted by environmental changes, these compounds were considered attractive candidates for sensor applications.

High-throughput fluorescence screening^{1c,5g,14} was used to examine the spectroscopic responses of these ethynylarenes in the presence of various cations in DMSO/water solvent systems. DMSO stock solutions of **2** and **3** were mixed with varying concentrations of aqueous metal chloride salt solutions in 96 well plates. The resulting solutions of 50 μ M sensor mixed with 50-250 μ M metal cations in 1:1 DMSO:water and 1:2 DMSO:water for **2** and **3** respectively were analyzed for fluorescence changes induced by the increasing metal concentrations. In these assays, **2** was excited at $\lambda_{\text{max}} = 290$ nm and monitored from 320 nm to 550 nm and **3** was excited at $\lambda_{\text{max}} = 320$ nm and monitored from 350 to 600 nm.

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Among the 22 metal cations evaluated in this study, both **2** and **3** displayed unique responses to the Ni(II) analyte, as illustrated in Figures 1 and 2. As Ni(II) concentrations increased, a large hypsochromic shift and an emission intensity increase was observed for each compound. Ratiometric comparisons of emission intensities for **2** (365/450 nm) and **3** (375/435 nm) were used to define 'turn-on' sensor responses. Ratiometric analysis summaries for all analyte:sensor combinations assayed using **2** and **3** are shown in Figures 3 and 4.

This method of data analysis shows clearly that the only analytes varying significantly from control responses are Ni(II), Cu(II) and V(III). It is noteworthy that the positive ratiometric responses generated for both Cu(II) and V(III) result from simple emission quenching, which is a significantly different spectroscopic response than the blue-shifted signal intensification observed for Ni(II).¹⁵ Based on these observations, it can be claimed that both **2** and **3** serve as selective fluorescence chemosensors for Ni(II) under aqueous conditions.

Two different assays were performed to determine the reversibility of Ni(II) binding with **2** and **3**. Titration of the standard assays for both **2** and **3** with Ni(II) using the competitive chelator EDTA degraded the observed ratiometric signals back to baseline levels.¹⁵ In addition, simple dilution of samples showing turn-on responses from 10-fold to 1000-fold resulted in a return to baseline levels.¹⁵ Each of these results indicate that the interactions of

2 and **3** with Ni(II) generating turn-on signals is reversible. It is noteworthy that the turn-on signal for **2** persisted at significantly lower concentrations than did **3**, suggesting that the identity of the central arene subunit can significantly impact fluorescence output despite these sensors possessing identical chelating subunits.

In an attempt to elucidate the binding mechanism of **2** and **3**, four control compounds (**4-7**) were prepared (Figure 5).¹⁵ These controls were designed to gain insight on three major factors regarding the parameters of analyte binding leading to turn-on signaling: (1) the site of metal binding, (2) the need for two chelating subunits to promote signal generation, and (3) the importance of arene subunit identity on signal generation.

The importance of a bidentate chelating unit for efficient analyte binding is supported by the observation that regioisomer **4** displayed no perturbations in fluorescence signal in the presence of Ni(II).¹⁵ This indicates that the peripheral chelating units are the site of metal binding in **2** and **3** that lead to turn-on signaling. The role that chelating unit cooperativity plays was probed using analog **5**, which contained a single chelating unit. Interestingly, both **2** and **5** show similar hypsochromic shifts in the presence of Ni(II). But unlike the active sensor **2**, control compound **5** does not generate dramatic intensification of emission signal.¹⁵ This could be due to either a less rigid arene environment due to the inability of **5** to cooperatively bind Ni(II) analytes at opposite ends of the molecule, or it could be due to an overall decrease in solubility of **5** relative to **2** when bound to Ni(II).

Control compounds **6** and **7** were prepared to examine how changing the size of the ethynylarene subunit and corresponding intramolecular spacing of chelating subunits might impact the fluorescence output for this chemosensor motif. Excitation of **6** in 1:1 water:DMSO at $\lambda_{\text{max}} = 270$ nm resulted in an emission signal with $\lambda_{\text{max}} = 315$ nm. Assays showed that Co(II), Cu(II), Fe(II) and Ni(II) each caused signal quenching with no observed shift in wavelength.¹⁵ Excitation of **7** at $\lambda_{\text{max}} = 340$ nm gave an emission signal with $\lambda_{\text{max}} = 450$ nm. Assays showed that both Cu(II) and V(III) quenched this signal with no shift in wavelength.¹⁵

Varying the size of the ethynylarene [pi]-system was effective in engineering varying energies of fluorescence output for the compounds themselves, but such changes could not be implemented into creating additional effective sensors. It is proposed that the observed quenching in each of these systems is due to arene precipitation driven by metal binding under the conditions surveyed. Because each of these derivatives share identical metal chelating subunits, it highlights the sensitive influence that the intramolecular spacing of chelating units has on signal generation, despite the fact that the intervening ethynylarene units do not directly participate in the binding of analytes. Hence, it seems in this system that arene identity can not be varied independently of chelator identity, despite the fact that these units are not conjugated with each other.

In conclusion, two new 'turn-on' fluorescence chemosensors for the detection of aqueous Ni(II) analytes have been developed. While a variety of 2-(1,2,3-triazol-4-yl)pyridine-containing ethynylarenes were prepared modularly from readily available precursors and a variety of metal cations were shown to interact with such compounds, only **2** and **3** were

able to generate desirable turn-on signals under the conditions examined. Based on the results presented in this investigation, it is proposed that the turn-on fluorescence signaling of **2** and **3** results from a mechanism of aggregate disruption promoted by metal binding at the 2-(1,2,3-triazol-4-yl)pyridine subunits. The ability of these derivatives to selectively detect aqueous Ni(II) at micromolar concentrations among the 22 metal cations studied is attributed to an optimal balance of solubility and geometry afforded by the ethynylarene subunits of these derivatives. Future studies will examine whether modular variation of chelator unit identity in these ethynylarene systems enables the tuning of analyte selectivity and binding affinity while preserving analogous turn-on fluorescence signaling responses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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11. Preparation of **2**: To a 50 mL test tube was added **1** (0.174 g, 0.48 mmol), *p*-diethynylbenzene (0.030 g, 0.24 mmol), Pd(PPh₃)₄ (0.057 g, 0.05 mmol), and CuI (0.019 g, 0.10 mmol). To a separate test tube was added tetrahydrofuran (20 mL), and triethylamine (5 mL). Each tube was sealed with a rubber septum and purged with a continuous stream of argon (5 min). The contents of the liquids tube were transferred to the solids tube via cannula under argon. The resulting mixture was stirred at 50°C under argon for 24 h. The resulting suspension was extracted between dichloromethane and 5% NH₄OH (aq). The organic layer was separated and dried over MgSO₄. After filtration to remove the drying agent, the solvent was removed via rotary evaporation. The crude product was washed with methanol, isolated by centrifugation and air-dried, yielding an orange-tan powder. Isolated 0.132 g, 0.22 mmol, 93% yield. ¹H NMR (DMSO-*d*₆): δ = 5.75 (m, 4H), 7.36 (m, 2H), 7.43 (d, *J*=8.3 Hz, 4H), 7.61 (s, 4H), 7.62 (d, *J*=8.3 Hz, 4H), 7.91 (m, 2H), 8.05 (m, 2H), 8.61 (m, 2H), 8.74 (s, 2H). ¹³C NMR (DMSO): δ = 53.4, 90.0, 91.7, 120.2, 122.5, 123.1, 123.8, 124.4, 129.0, 132.4, 132.6, 137.6, 138.0, 148.2, 150.4, 150.6. MS (MALDI-TOF): calculated for C₃₈H₂₆N₈ 595, found 596 [M+H]. Mp = 240°C (decomp). Preparation of **3**. Analogous procedure as described for **2** but using 2,7-diethynylnaphthalene resulted in an orange-tan solid. Isolated 0.120 g, 0.186 mmol, 50% yield. ¹H NMR (CDCl₃): δ = 5.65 (s, 2H), 7.30 (m, 2H), 7.56 (d, *J*=8.4 Hz, 4H), 7.615 (m, 2H), 7.62, (d, *J*=8.4 Hz, 4H), 7.83 (d, *J*=8.6 m, 2H), 7.89 (m, 2H), 8.04 (s, 2H), 8.261 (s, 2H), 8.262 (m, 2H), 8.59 (m, 2H). ¹³C NMR (DMSO-*d*₆): δ = 53.4, 90.51, 90.54, 120.2, 121.1, 122.6, 123.8, 124.4, 129.1, 129.9, 131.8, 132.60, 132.64, 133.0, 137.5, 138.0, 148.3, 150.4, 150.6. MS (MALDI-TOF): calculated for C₄₂H₂₈N₈ 645, found 646 [M+H], 668 [M+Na]. Mp = 218-221°C.
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15. See Supplementary Material.

Scheme 1.
Preparation of sensors **2** and **3**

Figure 1. Emission of **2** in response to increasing amounts of Ni(II) analyte in 1:1 DMSO:H₂O solution.

Figure 2.
Emission of **3** in response to increasing amounts of Ni(II) analyte in 1:2 DMSO:H₂O solution.

Figure 3.
Ratiometric analysis for assays of 2 with 0-5 equivalents of metal cation analytes.

Figure 4.
Ratiometric analysis for assays of **3** with 0-5 equivalents of metal cation analytes.

Figure 5.
Identities of control compounds prepared.