Tetrahedron 65 (2009) 4293–4297

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Aminoisoquinolines as colorimetric Hg^{2+} sensors: the importance of molecular structure and sacrificial base

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ARTICLE INFO

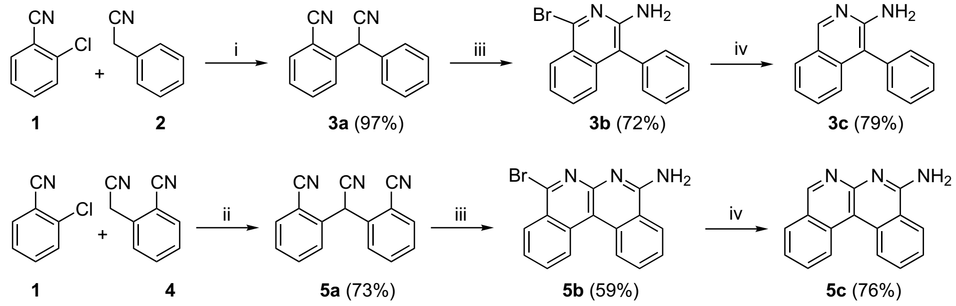
Article history:
Received 6 January 2009
Received in revised form 9 March 2009
Accepted 13 March 2009
Available online 27 March 2009

ABSTRACT

Here it is shown that 3-phenyl-2-amino isoquinoline acts as a simple mercury sensor. It is simple to synthesize. The molecule requires base/buffer to bind in a 1:1 stoichiometry with mercury ion, however. Otherwise, it acts as a sacrificial base, presumably to pick up a proton liberated during binding. This binding is characterized quantitatively.

1. Introduction

There is an increasing public concern over the severe risk of heavy metal pollution and poisoning in environment, food, and products.[1] Mercury ion is of particular interest because of its high toxicity and wide variety of damage to kidneys and digestive and neurological systems.[2] Thus, a simple yet highly selective routine of detecting mercury ions in both organic and aqueous media has become an urgent call and has drawn a lot of research attention.[3] Most mercury sensors that have been recently developed fall into one of the four categories: nano-particle based sensors, DNA or other bio-molecule based sensors, polymer based sensors, or organic dye based sensors. While the first three categories usually have the advantage of lower detection limit, their applications are highly constrained by the susceptibility to a series of factors, including but not limited to, temperature, pH, presence of other salts, etc. On the other hand, organic dye based sensors are usually synthesized organic molecules with high fluorescence and/or remarkable optical changes upon binding to target metal ions. These dyes ideally have the advantages of low cost, easy synthesis and storage, and more tolerance toward different experimental conditions. However, organic dye based mercury sensors often suffer from one or several disadvantages—particularly the requirement for a multi-step synthesis with a low overall yield, a low binding constant and/or poor sensitivity and/or selectivity.



Scheme 1. Synthesis of the compounds. (i) KO^{t}Bu, DMF, rt, 1 h; (ii) KO^{t}Bu, DMF, 70 °C, 18 h; (iii) HBr/HOAc; (iv) $NaBH_4$, Pd/C.

We wondered what the simplest chromophore and binding group for mercuric ion could be. Furthermore, we asked, which synthetic approaches could most efficiently incorporate simple but effective chemical functionalities into these molecules. To these

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doi:10.1016/j.tet.2009.03.063

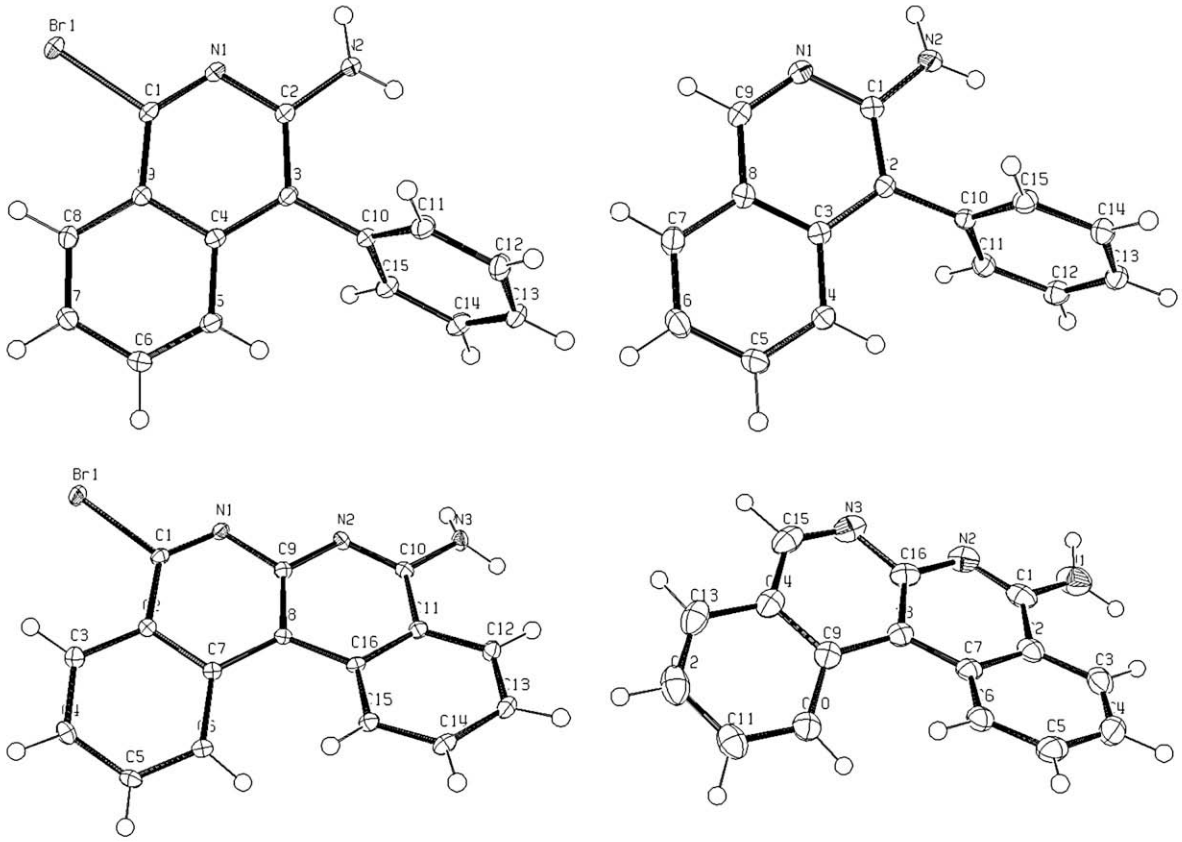


Figure 1. ORTEP diagrams of **3b**, **3c**, **5b**, and **5c**.

ends, we show here that a simple reaction sequence can produce a viable, small molecule mercury sensor. The way the molecule binds to mercury via deprotonation is novel and potentially useful in further sensor design paradigms.

2. Results and discussion

The synthesis of the molecules studied here involves three, straightforward steps from commercial starting materials (Scheme 1). It has been shown that isoquinoline derivatives can be synthesized via acid-promoted cyclization,[4–9] and this methodology could be adapted to the synthesis of **3b**.

Interestingly, this methodology could be extended to a double cyclization to form **5b** in reasonable yield. Note that the benzylic CH group spontaneously tautomerized to NH to result in an entirely aromatic system. To our knowledge, this is the first example of cyclization/aromatization of this kind. This type of cascade cyclization has potential for the preparation of larger, fused aromatic molecules, and this chemistry is under further exploration for that purpose. To determine the effect of the bromine atom on the photophysical properties of these molecules, the dehalogenated molecules **3c** and **5c** were prepared via simple reduction.

Crystals of **3b**, **3c**, **5b**, and **5c**, suitable for X-ray diffraction were obtained (Fig. 1). These data confirmed the closure of multiple aromatic rings. The isoquinoline region of **3b** and **3c** was planar. Molecules **5b** and **5c** were notably nonplanar.

The absorbance, fluorescence, and quantum yields of the four molecules were measured. Each has a long wavelength absorbance with a reasonable extinction coefficient ($>1000\ M^{-1}\,cm^{-1}$) that is potentially suitable as a colorimetric sensor. Also, all compounds fluoresce with a quantum yield that is much higher in the absence of the heavy bromine atom (Table 1).

Upon addition of mercuric ions, both the fluorescence and absorbance of the compounds changed. This change was most notable in molecule **3c**, so all further studies are reported on this molecule. Qualitatively (Fig. 2), **3c** exhibited very high selectivity toward mercury ion, with an obvious color change from colorless to dark yellow, as well as significant quenching of fluorescence. No other cation examined exhibited the same color change or fluorescence quenching. Also, the more conjugated **5c** provided no advantage (e.g., larger change in absorbance or fluorescence upon addition of mercury ion) suggesting that extended conjugation is not required for these type of molecules in this application. Finally, commercially available 2-amino isoquinoline mixed with mercuric ion as above displayed no color changes. The pendant phenyl group is thus necessary for the colorimetric response.

Quantitatively, a ca. 40 nm red shift in the UV absorption peak was observed when **3c** was added to mercuric ion. A poor fit was found, however, when absorbance changes were graphed as a function of mercuric ion concentration using a Benesi–Hildebrand plot.[10] A Job plot indicated an unexpected 2:1 binding stoichiometry between **3c** and mercuric ion in acetonitrile.[11] This initially puzzling observation had a simple explanation, however. Ammonia is known to bind to mercuric ion in a fashion that consumes 2 equiv of ammonia per mercuric ion to form a 1:1 complex. The second

Table 1
Photophysical data (solutions in CH_3CN)

Compound	Absorbance λ_{max} (nm) (ε_{max} ($M^{-1}\,cm^{-1}$))	Fluorescence λ_{max} (nm) (Θ)
3b	380 (3269)	447 (0.02)
3c	370 (3497)	436 (0.87)
5b	350 (3827)	450 (0.03)
5c	346 (3208)	432 (0.15)

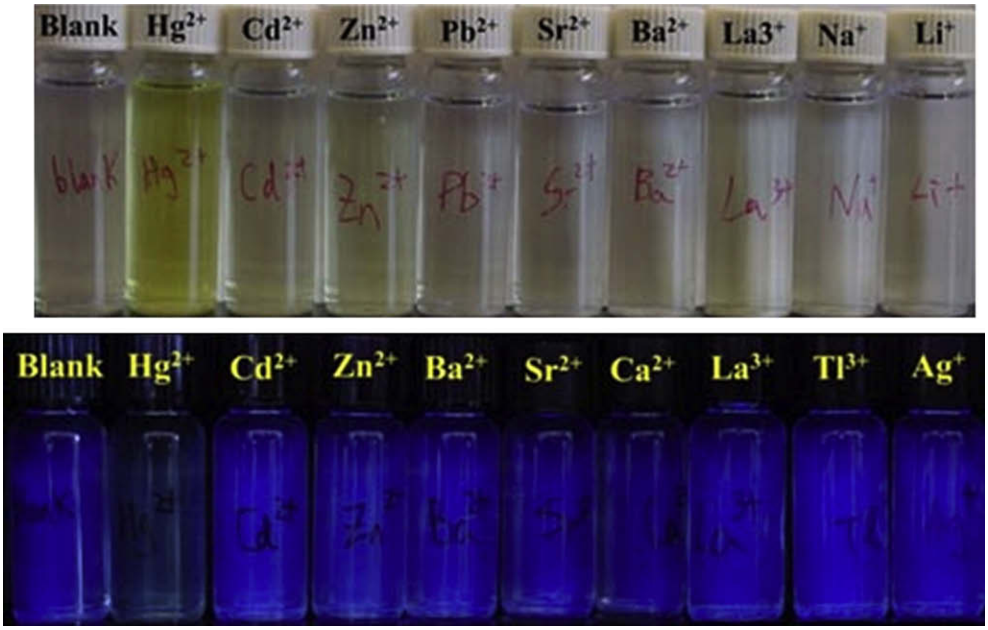


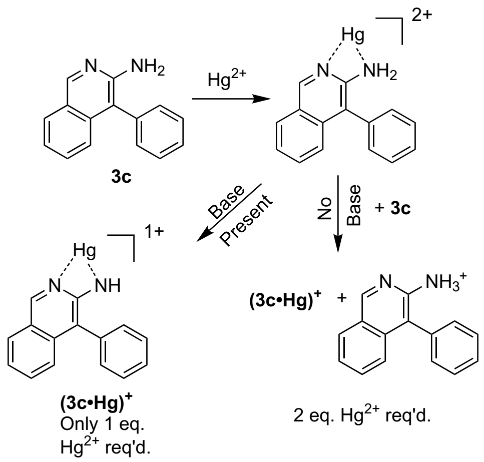
Figure 2. Color change and fluorescence quenching (under UV light) when **3c** binds to Hg^{2+} ion, compared to binding to other cations. (Top) Photograph of acetonitrile solutions of 2 mM **3c** plus 2 mM of different metal cations under day light. (Bottom) Photograph of 0.1 mM **3c** plus 0.1 mM of different metal cations in acetonitrile under a UV lamp.

equivalent of ammonia merely serves as a base, and the resulting complex is a mercuric–amido complex (Scheme 2).

When the same Job plot experiment was rerun in a 99:1 mixture of acetonitrile and phosphate buffer at pH 7.0, a 1:1 binding stoichiometry was determined. Presumably in this case, the phosphate acted as the proton acceptor and an amido complex was formed. Ancker et al. reported an X-ray structure of a neutral, 1:1 complex between HgCl and 2-(trimethylsilyl amino)-6-methyl pyridine, supporting this idea.[12]

A similar red shift in the absorbance and fluorescence quenching upon addition of Hg^{2+} to **3c** in 99:1 acetonitrile/buffer was observed (Fig. 3). From a Benesi–Hildebrand plot, a K_a of $5.0\times10^4\ M^{-1}$ was measured for the absorbance change upon binding and from a Stern–Volmer plot, a K_a of $1.8\times10^4\ M^{-1}$ was measured. Thus, a reasonable agreement was found between these two values. These values are also reasonably large, making **3c** a good candidate as a small molecule mercuric ion sensor.

Molecule **3c** showed a good, naked eye detection limit of ca. 0.1 ppm (0.5 μM) in both organic (CH_3CN) and semi-aqueous ($DMSO/H_2O$ 4:1) media (S/N=3:1). At this concentration, only a 3:1 ratio of signal to noise was measured. Moreover, it showed a high



Scheme 2. Rationalization of the 2:1 and 1:1 binding stoichiometries.

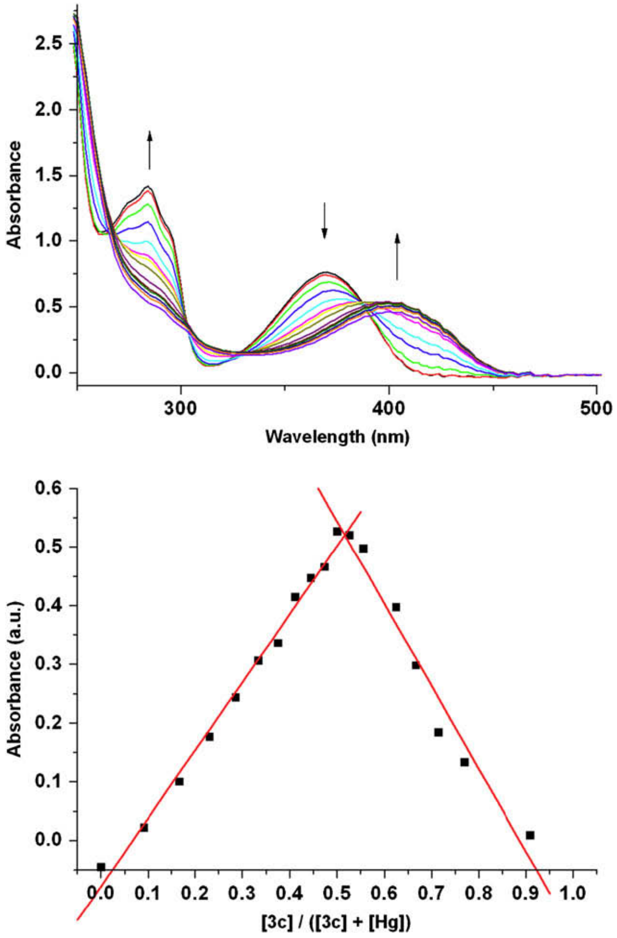


Figure 3. (Top) Overlay plot of UV–vis absorption spectra in 99:1 acetonitrile/buffer as Hg^{2+} was added to **3c**. Increases and decreases in relative absorbance are shown with vertical arrows. (Bottom) Invariant concentration Job plot of molecule **3c** and Hg^{2+}.

selectivity for binding with Hg^{2+}. As can be observed qualitatively in Figure 2, little change in color or fluorescence was observed when **3c** was mixed with other cations. The second strongest complex measured was between **3c** and Zn^{2+} with a K_a value of $6.6\times10^2\ M^{-1}$, which is >20 times smaller than that with Hg^{2+}. Addition of all other cations gave an undetectable absorbance change in the UV–vis spectrum of **3c**. Furthermore, in a mixed ion sensing experiment, where **3c** and Hg^{2+} were mixed with the same concentration of Li^+, Na^+, K^+, Ag^+, Cd^{2+}, Pb^{2+}, Co^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, La^{3+}, and Tl^{3+}, the changes in absorbance were the same as in the absence of these added cations.

3. Conclusions

In conclusion, molecule **3c** is a simple, conveniently prepared, colorimetric mercury ion sensor. Comparison to **3b**, **5b**, and **5c** indicates that, from a structural perspective, it possesses the minimum number of features required. Indeed, when commercially available 2-amino isoquinoline was mixed with mercuric ion as above, no color changes were observed. This observation suggests that changes in relative twist of the pendant, rotatable arene ring of **3c** are likely important to the color and fluorescence changes upon binding. Thus, this molecule represents a reasonable optimization of synthetic steps and structural features for a simple mercuric ion sensor.

4. Experimental

4.1. General methods

All chemicals were purchased from Aldrich or Acros. Acetonitrile was dried by distillation over CaH_2.

^1H NMR spectra were referenced to residual ^1H shift in $CDCl_3$ (7.24 ppm). $CDCl_3$ (77.0 ppm) was used as the internal reference for ^{13}C NMR. The following abbreviations were used to explain the multiplicities: s=singlet, d=doublet, t=triplet, q=quartet, br s=broad singlet.

Reactions were monitored by thin-layer chromatography (TLC) on commercial silica precoated plates with a particle size of 60 Å. Developed plates were viewed by UV lamp (254 nm). Flash chromatography was performed using 230–400 mesh silica gel. Microwave heating was performed by a microwave reactor of model The Discover instrument by CEM Corp., Matthews, N.C., operating at a power of 300 W. Temperature was measured via an internal IR-sensor. Specified reaction times refer to total heating time. Ramp times are specified for each individual solvent and temperature. All reactions were performed in sealed vessels.

Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC 708184–708187. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

4.2. Synthesis of 2-(cyano(phenyl)methyl)benzonitrile (3a)

To a solution of potassium *tert*-butoxide (823 mg, 7.35 mmol) in 10 mL dimethyl formamide (DMF) immersed in an ice bath, a mixture of 2-chloro-benzonitrile (**1**) (656 mg, 4.77 mmol) and 2-phenylacetonitrile (**2**) (430 mg, 3.67 mmol) in 6 ml DMF was added dropwise. The mixture was stirred at room temperature for 1 h, after which 20 mL saturated aqueous ammonium chloride was added to quench excess potassium *tert*-butoxide. The solution was extracted with diethyl ether, washed with deionized water, and then dried over anhydrous sodium sulfate. After removal of solvent and column purification with dichloromethane/methanol (DCM/MeOH), light yellow crystals of 2-(cyano(phenyl)methyl)benzonitrile (**3a**), were obtained in 97% yield (780 mg). Alternatively, compound **3a** could be obtained from recrystallization in hot ethanol (6.6 g, 71% yield). ^1H NMR ($CDCl_3$): 5.50 (s, 1H), 7.26–7.67 (m, 9H). ^{13}C NMR ($CDCl_3$): 40.1, 112.1, 117.1, 118.5, 127.8, 129.0, 129.2, 129.6, 133.7, 134.0, 134.3, 139.7. Mp: 84–87 °C. Elemental Analysis calcd: C, 82.55; H, 4.62; N, 12.84. Found: C, 82.41; H, 4.64; N, 12.85.

4.3. Synthesis of 1-bromo-4-phenylisoquinolin-3-amine (3b)

A solution of 7 mL of 45% HBr in acetic acid was added to 626 mg (2.87 mmol) of **3a** in a flask. The solution turned yellow upon the addition of HBr, and was stirred at room temperature for another 2 h. A yellow solid was precipitated out by the addition of 10 mL diethyl ether, and this solid was collected via filtration and washed with more ether. This solid was dissolved in 40 mL ethyl acetate, neutralized by shaking with aqueous saturated sodium hydrogen carbonate and dried over anhydrous sodium sulfate. After removal of solvent and column purification with DCM/MeOH, yellow crystals of 1-bromo-4-phenylisoquinolin-3-amine (**3b**) were obtained in 72% yield (620 mg). Alternatively, compound **3b** could be obtained from recrystallization in hot ethanol (4.0 g, 60% yield). ^1H NMR ($CDCl_3$): 4.40 (s, 2H), 7.24–7.59 (m, 9H), 8.13 (d, J=8.7 Hz, 1H). ^{13}C NMR ($CDCl_3$): 123.8, 124.1, 128.4, 128.9, 129.7, 130.7, 131.1, 135.0, 139.2, 151.0, 154.5. Mp 164–168 °C. Elemental Analysis Calcd: C, 60.22; H, 3.71; N, 9.36. Found: C, 60.24; H, 3.65; N, 9.39.

4.4. Synthesis of 4-phenylisoquinolin-3-amine (3c)

To a mixture of 2.0 mL of 4 N potassium hydroxide and 2.0 mL methanol in Schlenk flask, 107 mg (0.36 mmol) of **3b** was added slowly under nitrogen protection. To this solution, a mixture of 60 mg (0.028 mmol) 5% Pd on activated carbon and 60 mg (1.59 mmol) sodium borohydride was added, and the suspension was stirred at room temperature overnight. The solution was filtered and the solids were washed with tetrahydrofuran (THF). The resulting solution was dried over anhydrous sodium sulfate. After removal of solvent and column purification with DCM/MeOH, a light yellow solid, 4-phenylisoquinolin-3-amine (**3c**), was obtained in 79% yield (741 mg). ^1H NMR ($CDCl_3$): 4.36 (s, 2H), 7.23–7.59 (m, 8H), 7.83 (d, J=8.4 Hz, 1H), 8.8418.91 (s, 1H). ^{13}C NMR ($CDCl_3$): 111.7, 112.9, 123.2, 124.1, 128.1, 128.2, 129.6, 130.6, 130.8, 136.0, 137.5, 151.5, 152.0. Mp 144–147 °C. Elemental Analysis Calcd: C, 81.79; H, 5.49; N, 12.72. Found: C, 81.21; H, 5.57; N, 12.48. HRMS: Calcd: 220.100. Found: 220.100.

4.5. Synthesis of molecule 5a

To a mixture of 2-chloro-benzonitrile (768 mg, 5.58 mmol), 2-cyanophenyl acetonitrile (1.21 g, 8.38 mmol), and potassium *tert*-butoxide (1.94 g, 17.3 mmol) in a Schlenk flask, 12 mL anhydrous THF was added through a syringe. The solution was then refluxed under dinitrogen at 70 °C with stirring for 18 h. A solution of aqueous saturated ammonium chloride (30 mL) was added to quench excess potassium *tert*-butoxide. The solution was extracted with DCM and dried over anhydrous sodium sulfate. After removal of solvent and column purification with DCM/MeOH, a red solid (**5a**), was obtained in 73% yield (0.99 g). ^1H NMR ($CDCl_3$): 5.89 (s, 1H), 7.52–7.78 (m, 8H). ^{13}C NMR ($CDCl_3$): 39.8, 112.9, 116.4, 129.8, 129.9, 133.9, 134.4, 136.7. Mp 171–176 °C. Elemental Analysis Calcd: C, 79.00; H, 3.73; N, 17.27. Found: C, 78.73; H, 3.67; N, 17.25.

4.6. Synthesis of molecule 5b

A mixture of 10 mL 45% HBr in acetic acid and 10 mL DCM was added to 435 mg (1.79 mmol) of **5a** in a flask, and stirred at room temperature overnight. Then 100 mL of diethyl ether was added, and a yellow solid precipitated out and was filtered. This solid was redissolved in 50 mL THF, neutralized by addition of aqueous, saturated sodium hydrogen carbonate, and dried over anhydrous sodium sulfate. After removal of solvent and column purification with DCM/MeOH, a light yellow solid (**5b**), was obtained in 59% yield (341 mg). ^{1}H NMR (DMSO): 5.76 (s, 2H), 7.63–7.99 (m, 4H), 8.36 (d, J=8.1 Hz, 1H), 8.48 (d, J=9.3 Hz, 1H), 8.86 (q, J=8.7 Hz, 2H). ^{13}C NMR (DMSO): 106.8, 119.1, 124.8, 125.0, 125.3, 126.3, 126.9, 127.3, 129.2, 131.4, 132.2, 133.1, 134.3, 144.7, 150.6, 157.7. Mp decomp. Elemental Analysis Calcd: C, 59.28; H, 3.11; N, 12.96. Found: C, 59.44; H, 3.06; N, 12.81.

4.7. Synthesis of molecule 5c

To a mixture of 2.0 mL of 4 N potassium hydroxide and 2.0 mL methanol in Schlenk flask, 76 mg (0.23 mmol) of **5b** in 1 mL of THF was added slowly under nitrogen protection. To this solution, a mixture of 48 mg (0.044 mmol) 5% Pd on activated carbon and 90 mg (2.38 mmol) sodium borohydride was added, and the suspension was stirred at room temperature for 3 days. The solution was filtered and the solids were washed with tetrahydrofuran (THF). The resulting solution was dried over anhydrous sodium sulfate. After removal of solvent and column purification with DCM/MeOH, a light yellow solid (**5c**), was obtained in 76% yield (439 mg). ^{1}H NMR ($CDCl_3$): 4.11 (s, 2H), 7.46 (t, J=6.9 Hz, 1H), 7.56 (t, J=7.5 Hz, 1H), 7.72 (q, J=7.5 Hz, 2H), 7.96 (d, J=7.8 Hz, 1H), 8.06 (d, J=7.8 Hz, 1H), 8.70 (d, J=8.7 Hz, 1H), 8.81 (d, J=8.4 Hz, 1H), 9.10 (s, 1H). ^{13}C NMR ($CDCl_3$): 108.5, 119.3, 124.1, 124.8, 125.2, 126.3, 127.0, 127.1, 129.1, 130.9, 131.3, 133.4, 134.3, 151.0, 153.8, 157.1. Mp decomp. HRMS: Calcd: 245.095. Found: 245.095.

4.8. Photophysical measurements

Quantum yields of **3b**, **3c**, **5b**, and **5c** were measured by using 2-aminopyridine as standard.[13]

Binding constants were measured from absorbance changes using the Benesi–Hildebrand method. Stock solutions of 2 mM Hg^{2+} and 0.1 mM **3c** were prepared in 99:1 acetonitrile/buffer. A series of samples were prepared containing 1 mL mercury stock solution, different volumes of **3c** stock solution (added in 0.1 mL increments) and enough additional solvent to make a total volume of 2 mL. The corresponding absorbance was measured and the values 1/A were plotted against 1/[**3c**], in which A is the absorbance at corresponding concentration of molecule **3c**. K_a was calculated as the ratio of the Y-intercept/slope for a linear fit of these data.[10]

Binding constants were also measured from fluorescence changes via a Stern–Volmer plot. Stock solutions of 50 μM Hg^{2+} and 50 μM **3c** were prepared in 99:1 acetonitrile/buffer. A series of samples were prepared containing 1 mL **3c** stock solution, different volumes of **3c** stock solution (added in 0.1 mL increments) and enough additional solvent to make a total volume of 2 mL. The corresponding fluorescence emission was measured from 375 to 600 nm (excited at 370 nm), and different values of $(F_0/F-1)/[Hg^{2+}]$ were plotted against their corresponding mercury concentrations, in which F_0 is the initial fluorescence without mercury ion, F are fluorescence intensities at corresponding mercury concentrations. The value of K_a was calculated as absolute value of the Y-intercept.[14]

The binding ratios were calculated using Job plots. Stock solutions of 0.25 mM Hg^{2+} and 0.25 mM **3c** were prepared in 99:1 acetonitrile/buffer. The first series of samples was prepared containing 2 mL **3c** stock solution, and different volumes of mercury stock solution (0.1 mL increments). The second series of samples was prepared containing 2 mL mercury stock solution, and different volumes of **3c** stock solution (added in 0.1 mL increments).[10] The corresponding absorbance was measured and the values A were plotted against [**3c**]/([**3c**]+[Hg^{2+}]), where A is the corresponding absorbance of different fraction of molecule **3c** in the mixed solutions. The peak of this plot shows the binding ratio, at which neither molecule **3c** nor mercury ion is in excess.

Acknowledgements

We would like to thank Dr. Lin He for suggestions on fluorescence measurements. This work was supported by the Department of Energy (Grant DE-FG02-05ER46238). Mass spectra were obtained at the NCSU Department of Chemistry Mass Spectrometry Facility. Funding was obtained from the North Carolina Biotechnology Center, and the NCSU Department of Chemistry.

Supplementary data

Additional photophysical data. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2009.03.063.

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