Synthesis of triazolyl anthracene as a selective fluorescent chemosensor for the Cu(II) ion

Kris Varazo [a,†], Fang Xie [b], Dana Gulledge [a], Qian Wang [b,*]

[a] Department of Chemistry, Francis Marion University, Florence, SC 29501, USA
[b] Department of Chemistry and Biochemistry, University of South Carolina, Columbia, SC 29208, USA

A R T I C L E   I N F O

A B S T R A C T

The Cu(I)-catalyzed azide-alkyne cycloaddition reaction (CuAAC) has been used to synthesize an anthracene-based fluorescent compound that undergoes strong fluorescence quenching in the presence of Cu(II). Fluorescence studies indicate that the compound forms a 1:1 complex and can be used to quantitatively determine micromolar concentrations of Cu(II) in aqueous solution.

© 2008 Elsevier Ltd. All rights reserved.

Many metal ions have essential implications in human health and regulate many biological processes, and their detection is an important research area in environmental chemistry[1–4] and biology.[5–9] Anthracene, a highly fluorescent fluorophore, has been incorporated into a number of applications for sensing pH,[10] metal ions,[11–16] simple inorganic anions,[17,18] and small organic molecules,[17] as well as for cell-surface labeling[19] and medical diagnosis.[20] Recently, the Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction,[21–23] a prototype reaction of 'click chemistry',[24] has been employed in the synthesis of novel fluorescent compounds.[25–28] The high reaction efficiency of the CuAAC reaction,[21] the bioorthogonality of the alkyne and azide groups,[22] and the unique binding properties of the triazole rings[29] have lead to many practical applications of the resulting fluorescent compounds in sensing,[13,14] biomacromolecule labeling,[26,27,30,31] and nanomaterials synthesis.[32–34] In this work, we have synthesized a novel fluorescent water-soluble sensor incorporating 1,2,3-triazole linkages and an anthracene fluorophore via the CuAAC reaction and evaluated its selectivity and sensitivity for metal-ion detection.

The synthesis of fluorescent sensor 9 is outlined in Scheme 1. Alkyne precursor 3 was prepared via easy transformation from 2,2′-azanediyldiacetic acid 1. Starting from anthracene-9,10-dione 4, aldehyde 6 was synthesized following the reported protocol.[35] Reduction with NaBH₄, followed by mesylation and treatment with a large excess of NaN₃ afforded the diazidomethylanthracene 8. CuAAC reaction of 8 and 3 catalyzed with CuSO₄ and sodium ascorbate followed by saponification with NaOH and subsequent acidification gave the fluorescent sensor 9 in 72% yield. Compound 9 contains a central anthracene core and two triazole ring spacers connecting two iminodiacetate groups. The four carboxylate

groups impart high water solubility and metal-ion binding sites. This compound is structurally similar to a cadmium sensor described by Gunnlaugsson et al.[11] which contains phenyl spacers and exhibits different photophysical properties.

Figure 1 shows a series of fluorescence emission spectra for the spectrophotometric titration of a 2.0 µM aqueous unbuffered solution of sensor with 20 µM CuSO₄, added in 25 µL aliquots. Using 374 nm incident radiation, the spectrum features are characteristic of anthracene, with emission bands at 400, 420, and 450 nm. The fluorescent intensity of 9 maintains a constant level in the pH range 2–7, and decreases dramatically at pH values above 8 (data not shown). Significant fluorescence quenching begins with the addition of the first aliquot, which corresponds to 0.25 µM CuSO₄, and the intensity steadily decreases until the fluorescence is essentially shut off when the concentration of CuSO₄ reaches 2.63 µM. As CuSO₄ was added into the solution, the pH of the solution of 9 only decreased slightly from 5.28 to 5.12. Therefore, the pH change was eliminated as a possible factor contributing to the observed fluorescent intensity changes.

The strong signal modulation observed can serve as the basis for the quantitative detection of Cu(II) ion. Figure 2 shows the results of the spectrophotometric titration of 2.00 mL of a solution containing 10 µM 9 and 10 µM CuSO₄ with 0.10 mM EDTA. Initially, the solution exhibits no fluorescence, but as EDTA is added the fluorescence of 9 is restored. The break in the curve occurs when 1 equiv of EDTA has been added, and corresponds to the equivalence point of the titration, where 2.0×10^{-8} mol of EDTA has been added to completely remove the Cu(II) bound to the sensor and fully restore the fluorescence of the sensor. Importantly, this indicates that the compound binds the Cu(II) ion with 1:1 stoichiometry, since only one equivalent of EDTA was added to completely titrate the complex. The results also suggest that the fluorescence quenching at these low metal ion concentrations is probably a result of static quenching, by the formation of a sensor-metal complex that can participate in photoinduced metal-sensor electron

* Corresponding author. Tel.: +1 803 777 8436; fax: +1 803 777 9521.
E-mail addresses: kvarazo@fmarion.edu (K. Varazo), wang@mail.chem.sc.edu (Q. Wang).
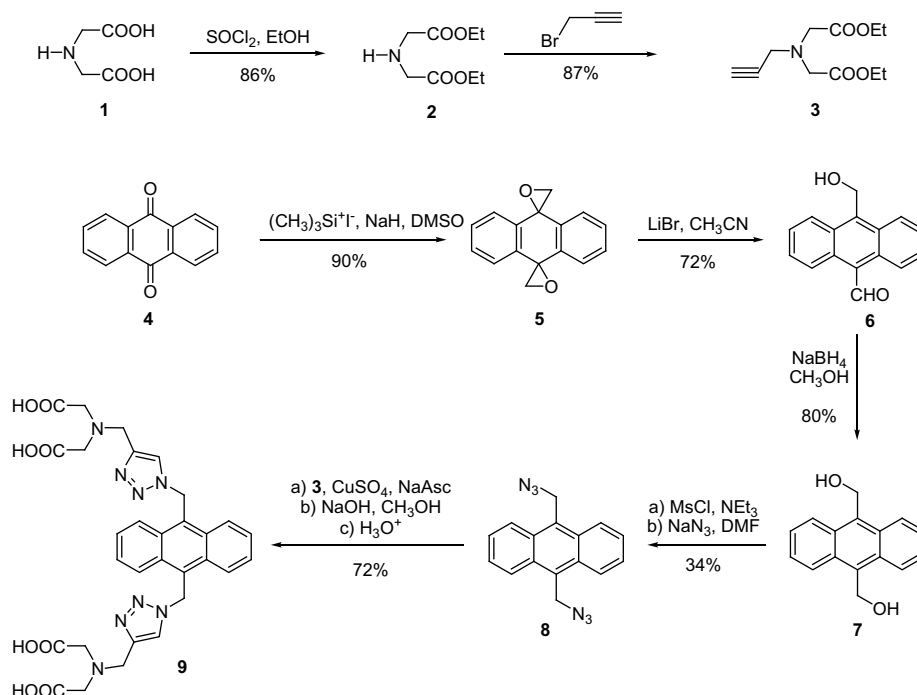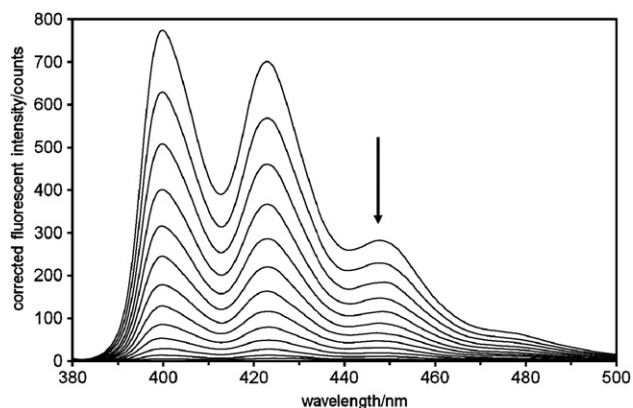† Tel.: +1 843 661 1450; fax: +1 843 661 4616.

Scheme 1. Synthesis of fluorescent sensor **9**.

Figure 1. Fluorescence emission spectra of a 2.0 µM aqueous solution of **9**, as a function of increasing CuSO$_4$ concentration, from 0.25 µM to 2.63 µM (λ_{ex} = 374 nm).

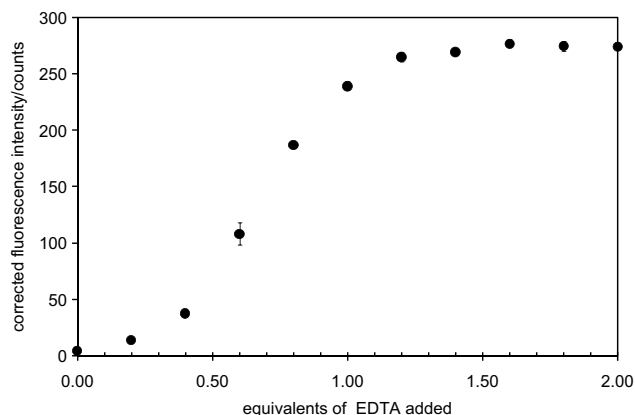transfer.[36,37] The addition of EDTA complexes the ion, which liberates the sensor and restores fluorescence.

Additional support for the 1:1 stoichiometry of the sensor-Cu(II) complex was provided by a mole-ratio plot, shown in Figure 3. The fluorescence of a solution containing 3.1×10^{-9} moles of **9** was monitored as a solution of 20 µM CuSO$_4$ was added in 10 µL increments. As expected, the increase in Cu(II) concentration quenches the fluorescence of **9**, with no significant additional decrease after the mole ratio of sensor to metal reaches a value of approximately one, and is indicated by the intersection of the two linear portions of the data.

The binding selectivity of the sensor was examined using other metal ions that included main group metals and transition metals. Figure 4 shows the relative fluorescence ratio F_o/F (in the absence (F_o) and presence (F) of 10 µM concentrations of **9** and metal ions) for each of the metal ions. The sensor shows the highest selectivity toward Cu^{2+}, with a relative fluorescence five times greater than
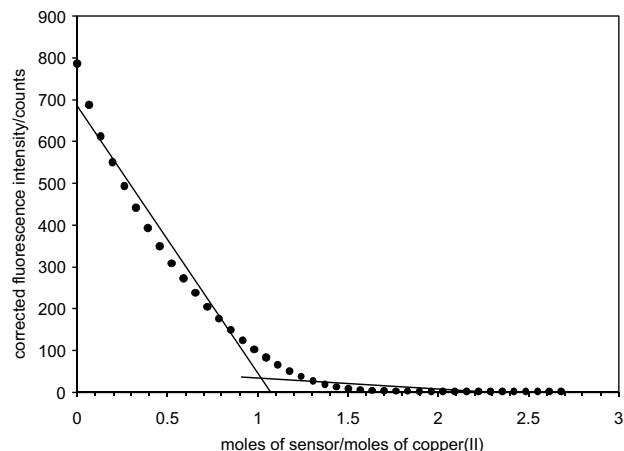

Figure 2. Spectrophotometric titration of an aqueous solution containing 10 µM **9** and 10 µM CuSO$_4$ with 0.10 mM EDTA.

Figure 3. Plot of sensor **9**–Cu(II) complex fluorescence at 400 nm as a function of **9**/Cu(II) molar ratio.
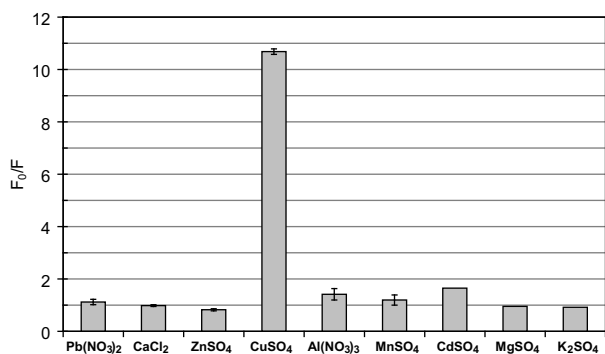
Figure 4. Relative fluorescence ratios (F_0/F) for different metal ions. Each solution contains 10 μM of **9** and an individual metal ion.

that for any other metal ion. Besides the weak binding toward Cd^{2+} and Al^{3+}, **9** does not respond significantly to any of the other ions. The lack of sensitivity toward Mg^{2+}, Zn^{2+}, and Ca^{2+} makes the sensor promising for use in biological solutions which contain large quantities of these ions. Figure 5 shows a series of solutions of **9** in the presence of equimolar concentrations of the metal ions, imaged under UV illumination. Fluorescence quenching is easily seen for the vials containing copper (**E**) and cadmium (**H**).

The binding strength between **9** and copper(II) ion was determined by preparing a binding curve from fluorescence titration data (Fig. 6). A plot of the relative fluorescence (F_0/F) versus pM (negative logarithm of metal ion concentration) shows a sigmoidal shape that is consistent with 1:1 binding.[38] The binding constant can be taken as the break in the curve, and a value of ~$10^{5.5}$ was obtained, indicating fairly strong binding between **9** and Cu(II). The formation constant for copper(II)–EDTA is ~10^{19}, and the larger value is consistent with the ability of EDTA to release copper ions from the dye.[39]

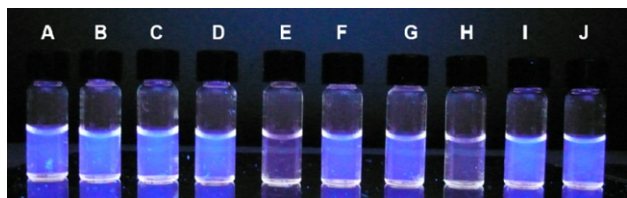
Figure 5. Photograph of solutions under UV illumination containing 10 μM **9** with 10 μM of (A) $Pb(NO_3)_2$, (B) $CaCl_2$, (C) $ZnSO_4$, (D) nothing, (E) $CuSO_4$, (F) $Al(NO_3)_3$, (G) $MnSO_4$, (H) $CdSO_4$, (I) $MgSO_4$, and (J) K_2SO_4.
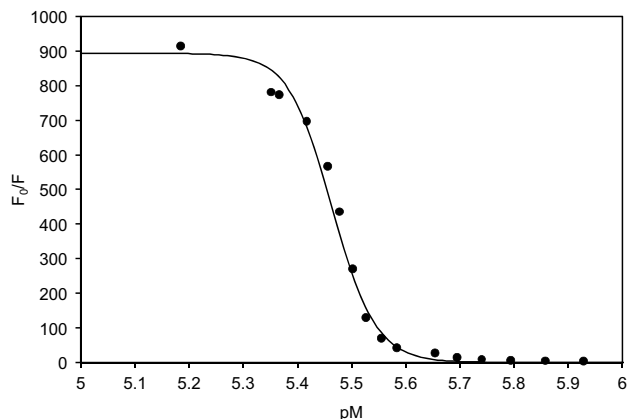

Figure 6. Binding curve plot of relative fluorescence (F_0/F) as a p-function of copper(II) ion concentration.

To conclude, we have synthesized a novel, fluorescent aqueous metal-ion sensing dye containing two 1,2,3-triazole spacers that responds to micromolar concentrations of Cu(II) by undergoing strong fluorescence quenching. Binding curve studies and spectrophotometric titration support the 1:1 stoichiometry of the sensor-metal complex and the strong affinity of the sensor for Cu(II). The EDTA titration also demonstrates the application of the sensor for quantitative analysis. At this point, the role of the triazole ring in metal-ion coordination is not clear. In a recent report of a Zn(II)-sensing fluorescent probe,[13] X-ray studies show that the nitrogen atoms in the triazole ring coordinate the zinc in a distorted trigonal bipyramidal geometry. Our current efforts are focused on obtaining crystals for X-ray analysis to determine the role of the 1,2,3-triazole ring in metal ion binding and evaluate the selectivity of the sensor with other metal ions such as Cu(I), which is the dominant form of copper in biological systems.[40]

Acknowledgments

References and notes

1. Nguyen, B. T.; Anslyn, E. V. *Coord. Chem. Rev.* **2006**, *250*, 3118.
2. Bargossi, C.; Fiorini, M. C.; Montalti, M.; Prodi, L.; Zaccheroni, N. *Coord. Chem. Rev.* **2000**, *208*, 17.
3. Butler, A. *Science* **1998**, *281*, 207–209.
4. de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515–1566.
5. Wolfbeis, O. S. *Angew. Chem., Int. Ed.* **2007**, *46*, 2980–2982.
6. Meloni, G.; Faller, P.; Vasak, M. *J. Biol. Chem.* **2007**, *282*, 16068–16078.
7. Lutsenko, S.; Barnes, N. L.; Bartee, M. Y.; Dmitriev, O. Y. *Physiol. Rev.* **2007**, *87*, 1011–1046.
8. Jiang, P.; Guo, Z. *Coord. Chem. Rev.* **2004**, *248*, 205.
9. Finney, L. A.; O'Halloran, T. V. *Science* **2003**, *300*, 931–936.
10. Ihmels, H.; Meiswinkel, A.; Mohrschladt, C. J.; Otto, D.; Waidelich, M.; Towler, M.; White, R.; Albrecht, M.; Schnurpfeil, A. *J. Org. Chem.* **2005**, *70*, 3929–3938.
11. Gunnlaugsson, T.; Lee, T. C.; Parkesh, R. *Tetrahedron* **2004**, *60*, 11239–11249.
12. Kim, K. S.; Jun, E. J.; Kim, S. K.; Choi, H. J.; Yoo, J.; Lee, C. H.; Hyun, M. H.; Yoon, J. *Tetrahedron Lett.* **2007**, *48*, 2481–2484.
13. Huang, S.; Clark, R. J.; Zhu, L. *Org. Lett.* **2007**, *9*, 4999–5002.
14. Chang, K. C.; Su, I. H.; Senthilvelan, A.; Chung, W. S. *Org. Lett.* **2007**, *9*, 3363–3366.
15. Lee, Y. J.; Seo, D.; Kwon, J. Y.; Son, G.; Park, M. S.; Choi, Y.-H.; Soh, J. H.; Lee, H. N.; Lee, K. D.; Yoon, J. *Tetrahedron* **2006**, *62*, 12340.
16. Martinez, R.; Zapata, F.; Caballero, A.; Espinosa, A.; Tarraga, A.; Molina, P. *Org. Lett.* **2006**, *8*, 3235–3238.
17. Bohne, C.; Ihmels, H.; Waidelich, M.; Yihwa, C. *J. Am. Chem. Soc.* **2005**, *127*, 17158–17159.
18. Ghosh, K.; Sarkar, A. R.; Masanta, G. *Tetrahedron Lett.* **2007**, *48*, 8725.
19. Yang, W.; Fan, H.; Gao, X.; Gao, S.; Karnati, V. V. R.; Ni, W.; Hooks, W. B.; Carson, J.; Weston, B.; Wang, B. *Chem. Biol.* **2004**, *11*, 439.
20. Magri, D. C.; Brown, G. J.; McClean, G. D.; de Silva, A. P. *J. Am. Chem. Soc.* **2006**, *128*, 4950–4951.
21. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596–2599.
22. Wang, Q.; Chan, T. R.; Hilgraf, R.; Fokin, V. V.; Sharpless, K. B.; Finn, M. G. *J. Am. Chem. Soc.* **2003**, *125*, 3192–3193.
23. Tornoe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057–3064.
24. Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004–2021.
25. Sivakumar, K.; Xie, F.; Cash, B. M.; Long, S.; Barnhill, H. N.; Wang, Q. *Org. Lett.* **2004**, *6*, 4603–4606.
26. Sawa, M.; Hsu, T.-L.; Itoh, T.; Sugiyama, M.; Hanson, S. R.; Vogt, P. K.; Wong, C.-H. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 12371–12376.
27. Xie, F.; Sivakumar, K.; Zeng, Q.; Bruckman, M. A.; Hodges, B.; Wang, Q. *Tetrahedron* **2008**, *64*, 2906–2914.
28. Zhou, Z.; Fahrni, C. J. *J. Am. Chem. Soc.* **2004**, *126*, 8862–8863.
29. Mindt, T. L.; Struthers, H.; Brans, L.; Anguelov, T.; Schweinsberg, C.; Maes, V.; Tourwe, D.; Schibli, R. *J. Am. Chem. Soc.* **2006**, *128*, 15096–15097.
30. Beatty, K. E.; Liu, J. C.; Xie, F.; Dieterich, D. C.; Schuman, E. M.; Wang, Q.; Tirrell, D. A. *Angew. Chem., Int. Ed.* **2006**, *45*, 7364–7367.
31. Beatty, K. E.; Xie, F.; Wang, Q.; Tirrell, D. A. *J. Am. Chem. Soc.* **2005**, *127*, 14150–14151.
32. O'Reilly, R. K.; Joralemon, M. J.; Hawker, C. J.; Wooley, K. L. *Chem. Eur. J.* **2006**, *12*, 6776–6786.

33. Bruckman, M. A.; Kaur, G.; Lee, L. A.; Xie, F.; Sepulveda, J.; Breitenkamp, R.; Zhang, X.; Joralemon, M.; Russell, T. P.; Emrick, T.; Wang, Q. *ChemBioChem* **2008**, *9*, 519–523.
34. Malkoch, M.; Schleicher, K.; Drockenmuller, E.; Hawker, C. J.; Russell, T. P.; Wu, P.; Fokin, V. V. *Macromolecules* **2005**, *38*, 3663–3678.
35. Lin, Y.; Lang, S. A.; Seifert, C. M.; Child, R. G.; Morton, G. O.; Fabio, P. F. *J. Org. Chem.* **1979**, *44*, 4701–4703.
36. Valverde-Aguilar, G.; Wang, X.; Nelsen, S. F.; Zink, J. I. *J. Am. Chem. Soc.* **2006**, *128*, 6180–6185.
37. Akkaya, E. U.; Huston, M. E.; Czarnik, A. W. *J. Am. Chem. Soc.* **1990**, *112*, 3590–3593.
38. Conners, K. A. *Binding Constants: The Measurement of Molecular Complex Stability*; Wiley Interscience: New York, 1987.
39. Martell, A. E.; Smith, R. M.; Motekaitis, R. J. *NIST Critically Selected Stability Constants of Metal Complexes*. NIST Standard Reference Database 46, Gaithersburg, MD, 2001.
40. Zeng, L.; Miller, E. W.; Pralle, A.; Isacoff, E. Y.; Chang, C. J. *J. Am. Chem. Soc.* **2006**, *128*, 10–11.