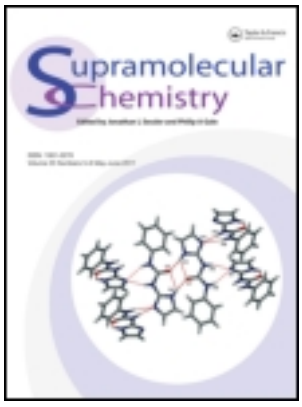


Supramolecular Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gsch20

Reversible fluorescence sensing of Zn^{2+} based on pyridine-constrained *bis*(triazole-linked hydroxyquinoline) sensor

Nagarjun Narayanaswamy [a] , Debabrata Maity [a] & T. Govindaraju [a]
[a] Bioorganic Chemistry Laboratory, New Chemistry Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, Bangalore, 560064, India

Available online: 14 Oct 2011

To cite this article: Nagarjun Narayanaswamy, Debabrata Maity & T. Govindaraju (2011): Reversible fluorescence sensing of Zn^{2+} based on pyridine-constrained *bis*(triazole-linked hydroxyquinoline) sensor, Supramolecular Chemistry, 23:10, 703-709

To link to this article: http://dx.doi.org/10.1080/10610278.2011.611245

Supramolecular Chemistry
Vol. 23, No. 10, October 2011, 703–709

Taylor & Francis
Taylor & Francis Group

Reversible fluorescence sensing of Zn^{2+} based on pyridine-constrained *bis*(triazole-linked hydroxyquinoline) sensor

Nagarjun Narayanaswamy, Debabrata Maity and T. Govindaraju*

Bioorganic Chemistry Laboratory, New Chemistry Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, Bangalore 560064, India

(*Received 2 June 2011; final version received 20 July 2011*)

Zinc ion (Zn^{2+}) is an important and a most useful biological trace nutrient responsible for the activity of several enzymes. Zn^{2+} concentrations in the environment as well as in the human body increase beyond permissible limits as a consequence of its mining and widespread industrial applications. Such excess Zn^{2+} concentrations are toxic to humans and many aquatic organisms. The magnetic inertness and spin paired electronic configuration of Zn^{2+} makes it hard to detect by common analytical techniques. Therefore, fluorometric detection using chemosensor is the most effective tool for the environmental and biological detection of Zn^{2+}. We have developed a novel pyridine-constrained *bis*(triazole-linked hydroxyquinoline) ligand as a reversible fluorescent chemosensor for Zn^{2+}. The symmetrical ligand is highly selective for Zn^{2+} and fluoresces brightly upon complexation compared with other metal ions based on chelation-enhanced fluorescence mechanism. Interestingly, free ligand can be regenerated by treating the ligand–Zn^{2+} complex with aqueous ammonia.

Keywords: zinc; chemosensor; chelation-enhanced fluorescence; click chemistry

Introduction

Zinc is the second most abundant transition metal (*1*). Biologically, zinc ion (Zn^{2+}) is an essential trace nutrient with 2–4 g distributed throughout the human body (*1*, *2*). There exist hundreds of metalloproteins dependent on zinc for their structure and function such as immune response, cell division, transcription and translation (*3–5*). Almost all the zinc in human body present as protein bound form with definitive biological role. However, there also exist unbound zinc at very low concentration in some tissues probably functioning as signalling agents for processes such as apoptosis and neurotransmission (*6*, *7*). Biological functions and the effective storage of such free Zn^{2+} are poorly understood. The permissible dietary intake of zinc for humans is about 8–11 mg/day (*8*). At concentrations higher than the permissible limit, zinc can suppress the uptake of other nutritional trace metals such as copper and iron (*9*). Zn^{2+} toxicity has also been implicated in a variety of human disorders such as Alzheimer's disease (*10*) and diabetes (*11*). Zinc and its compounds are used in alloys, machine parts, batteries, paints, pharmaceuticals, cosmetics and many other industrial applications. The mining and widespread industrial applications are responsible for environmental accumulation and accompanied toxicity of zinc to humans as well as aquatic organisms. Therefore, developing a selective and sensitive technique for the environmental and biological detection of Zn^{2+} is prime importance in monitoring and controlling its safe concentration levels.

Zinc is spectroscopically silent due to its inert electronic configuration of $Zn^{2+}(3d^{10}4s^{0})$. This makes it hard to detect by common analytical techniques such as electron paramagnetic resonance, UV–vis spectroscopy, nuclear magnetic resonance (NMR) and Mössbauer spectroscopy (*12*). Designing chemosensors for transition metal ions that play an important role in biological processes as well as human disorders is the exciting field research. Colorimetric and fluorometric methods are particularly useful for the detection of Zn^{2+}. In addition, designing chemosensors that can effectively differentiate Zn^{2+} from Cd^{2+} has been a great challenge (*13d–f*). These two elements belong to the same group in the periodic table and have similar chemical properties. Hence they exhibit very similar spectral properties with various ligands reported. Few chemosensors have been reported for selective sensing of Zn^{2+}(*13–17*). To our knowledge no chemosensors have been reported to work reversibly, facilitating the recycling of ligands. Therefore, analytical tools which employ reversible fluorescence chemosensors and enable the recovery of free ligand from the Zn^{2+}-bound complex are particularly useful and cost effective. Zn^{2+} complexing fluorescent ligands can be used as probes for studying amyloid-β aggregation and designing potential therapeutic agents (*18*). In this study we report on the synthesis of a novel reversible fluorescence sensor

*Corresponding author. Email: tgraju@jncasr.ac.in

ISSN 1061-0278 print/ISSN 1029-0478 online

http://dx.doi.org/10.1080/10610278.2011.611245
http://www.tandfonline.com

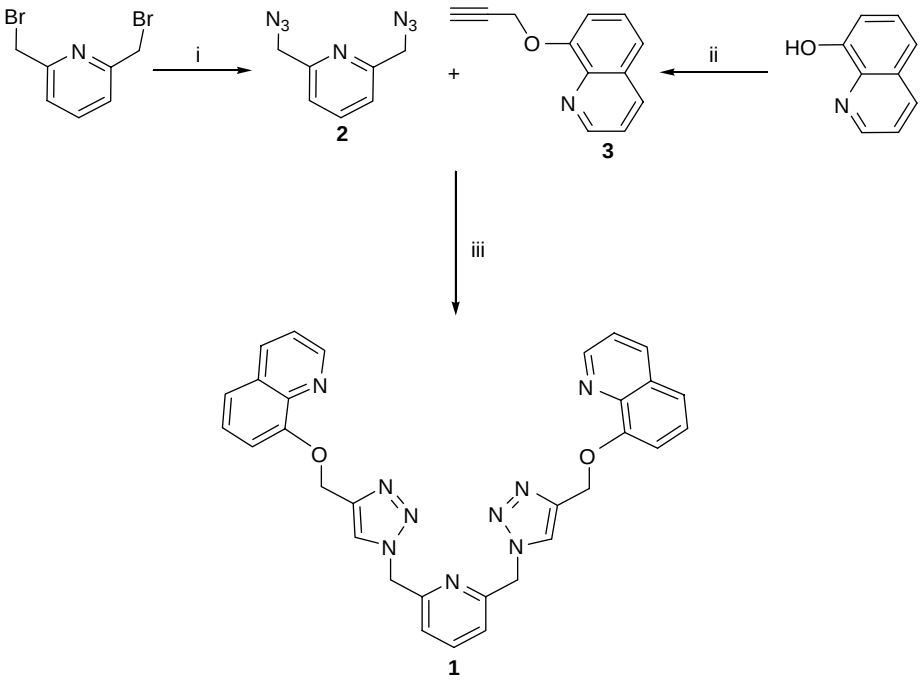
for Zn^{2+} based on chelation-enhanced fluorescence (CHEF) mechanism.

Results and discussions

Hydroxyquinolines have been used as fluorophores in chemosensors for detecting metal ions. The 8-hydroxyquinoline-derived ligands generally show weak fluorescence and upon binding with metal ions exhibit bright fluorescence and good photostability (*19*). In recent years, triazole-forming click reaction has been frequently used to construct ligands with cation and anion chelating ability (*17*, *20–24*). Such synthetic strategies to produce ligands with metal ion coordinating ability offer a new approach for the design of novel chemosensors (*24*). We have designed pyridine-constrained traizole-linked hydroxyquinoline ligand **1** and synthesised using click chemistry as one of the key intermediate reactions (Scheme 1). In the first step, 2,6-*bis*(azidomethyl)pyridine (**2**) was prepared by treating 2,6-*bis*(bromomethyl)pyridine with sodium azide. 8-(Prop-2-ynyloxy)quinoline (**3**) was prepared via propargylation of 8-hydroxyquinoline. Azide **2** and alkyne **3** were subjected to copper(I)-catalysed [3 + 2] cycloaddition to obtain symmetrical ligand **1** in excellent yield.

The photophysical properties of ligand **1** were studied in mixed aqueous medium (10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES):CH_3CN, 1:9, pH 7.2) upon addition of various metal ions (Li^+, Na^+, Mg^{2+}, Sr^{2+}, Al^{3+}, In^{3+}, Hg^{2+}, Pb^{2+}, Cd^{2+}, Mn^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, Ag^+, Fe^{2+} and Fe^{3+}; Figure 1). Ligand **1** exhibits a weak fluorescence emission band at 401 nm. Ligand **1** in presence of metal ions such as Li^+, Na^+, Mg^{2+}, Sr^{2+}, Al^{3+}, In^{3+}, Hg^{2+}, Pb^{2+}, Mn^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Ag^+, Fe^{2+} and Fe^{3+} showed either no change or slight decrease in the emission intensity. Zn^{2+} and Cd^{2+} exhibited enhancement in the fluorescence emission relative to ligand, respectively.

Interestingly between Zn^{2+} and Cd^{2+}, ligand **1** showed complete selectivity for Zn^{2+} over Cd^{2+} (Figure 1, inset). Addition of Zn^{2+} to **1**–Cd^{2+} complex displaces Cd^{2+} with an enhancement in emission intensity due to the formation of **1**–Zn^{2+} complex. We measured the photophysical properties of ligand **1**–Zn^{2+} in presence of tetrabutylammonium salts of anions (F^-, Cl^-, Br^-, I^-, CN^-, SCN^-, NO_3^-, AcO^-, HSO_4^-, $H_2PO_4^-$) in CH_3CN–HEPES solution. Ligand **1**–Zn^{2+} has not shown specificity towards the anions (see Supplementary Information, available online). The fluorescence emission spectra of ligand **1** as a function of Zn^{2+} concentration are shown in Figure 2. The emission maxima of ligand **1** red shifted to 429 nm ($\Delta = 28$ nm) with an isoemissive point (I_e) at 377 nm and overall 6.3-fold increase in emission intensity. Job's plot for the complexation between ligand **1** and Zn^{2+} revealed the formation of ligand **1**–Zn^{2+} complex with 1:2 stoichiometry (Figure 3) (*25*). The response parameter α which is the ratio between free ligand concentrations and the initial concentration of ligand **1** was plotted as a



Scheme 1. Synthesis of pyridine-constrained traizole-linked hydroxyquinoline ligand **1**. (i) NaN_3, DMSO, RT; (ii) Propargyl bromide, K_2CO_3, acetone, reflux and (iii) CuI, sodium ascorbate, toluene/*t*-BuOH, RT.

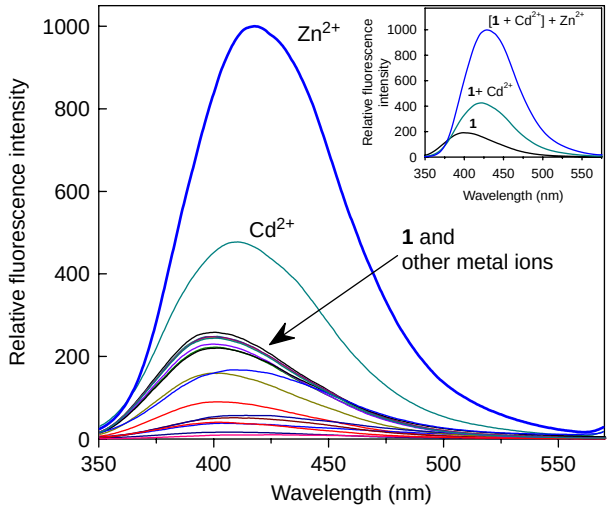


Figure 1. Fluorescence spectra of free ligand **1** (20 μM) and with added metal ions (25 equiv) Li^{+}, Na^{+}, Mg^{2+}, Sr^{2+}, Al^{3+}, In^{3+}, Hg^{2+}, Pb^{2+}, Mn^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Ag^{+}, Fe^{2+}, Fe^{3+}, Cd^{2+} and Zn^{2+}. Inset: Fluorescence spectra of ligand **1**, ligand **1**–Cd^{2+} complex and upon addition of Zn^{2+} to ligand **1**–Cd^{2+} in 10 mM HEPES–CH_3CN (1:9), pH 7.2.

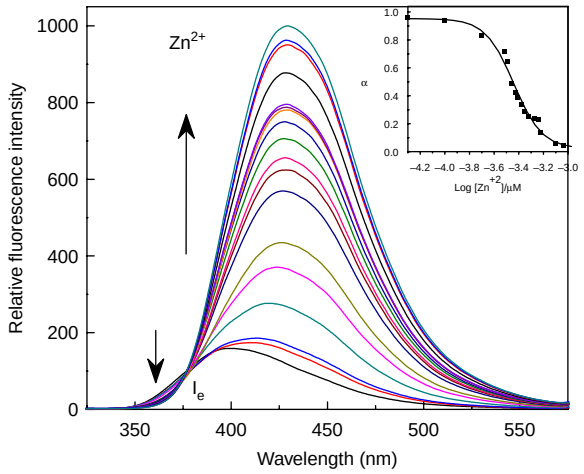


Figure 2. Fluorescence spectra of ligand **1** (20 μM) upon addition of Zn^{2+}(0, 2.5. 5.0, 10.0, 15.0, 16.0, 17.5, 18.5, 19.5, 21.0, 22.5, 24.0, 27.0, 28.5, 30.0, 40.0, 45.5 and 50 equiv) with excitation of 300 nm. Inset: Response parameter (α) as a function of Zn^{2+}.

function of Zn^{2+} (Figure 2). This plot serves as the calibration curve for detection of Zn^{2+}. The association constant (log K) of ligand **1** for Zn^{2+} was calculated as 11.18 from Li's equations (*26*).

Preferential selectivity of ligand **1** towards Zn^{2+} was demonstrated by competitive binding studies in presence of various metal ions. Ligand **1** was treated with 10 equiv of Zn^{2+} in presence of the same concentrations of other metal ions (Figure 4).

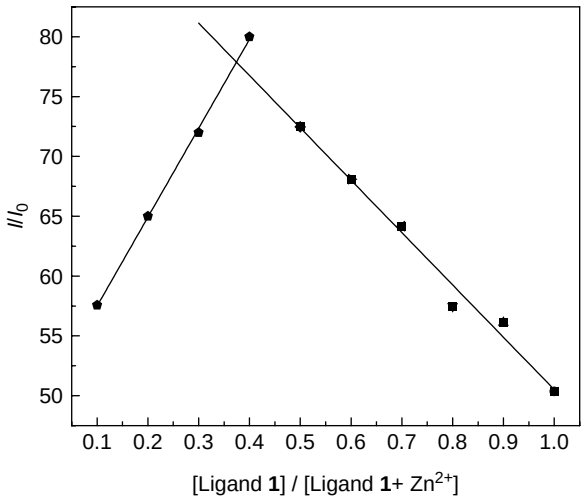


Figure 3. Job's plot showing the 1:2 binding stoichiometry for the formation of ligand **1** and Zn^{2+} complex in 10 mM HEPES–CH_3CN (1:9), pH 7.2.

The competitive study clearly suggests that there is no interference of other metal ions for sensing of Zn^{2+}. Though there is a slight decrease in the signal intensities in case of Ni^{2+}, $Cu^{2+}$and Fe^{2+}, response is very much in the detectable range. Thus Zn^{2+} can be detected in presence of other metal ions using ligand **1**. To determine the binding mode of ligand **1** and Zn^{2+}, ^1H NMR spectra of ligand were recorded in CD_3CN with sequential addition of Zn^{2+}. ^1H NMR spectra change upon addition of total of 3.0 equiv of Zn^{2+} (Figure 5). The main proton signals considered for assigning the binding mode of symmetrical ligand **1**–Zn^{2+} are methylene protons (H_a and H_c), triazole proton (H_b) and protons of quinoline moiety (H_d, H_e, H_f and H_g). These protons undergo downfield shift upon addition of Zn^{2+}. Overall downfield shift of 0.09, 0.08, 0.49, 0.46, 0.40, 0.66 and 0.10 ppm corresponding to H_a, H_b, H_c, H_d, H_e, H_f and H_g, respectively, was observed with addition of 2.0 equiv of Zn^{2+}. Further addition of Zn^{2+} did not lead to any appreciable changes in the chemical shifts of protons under consideration. Notably, no appreciable change in the chemical shifts of pyridyl moiety protons was observed. These data suggest the coordination of Zn^{2+} to ligand through a nitrogen atom and an oxygen atom of hydroxyquinoline moiety and a nitrogen atom from triazole ring. NMR study also shows the 1:2 binding stochiometry of symmetrical ligand **1** and Zn^{2+}, confirming the Job's plot data (Figure 5).

Next we studied the recovery of free ligand **1** by displacing the bound Zn^{2+} ions using a suitable coordinating ligand. Thus the recovered ligand **1** can be reused for the detection of Zn^{2+} and engender this fluorescence-based analytical tool as viable and cost effective. We selected ammonia as the ligand of choice because of its high association constant (log k = 31.16) for zinc(II) (*27*) which

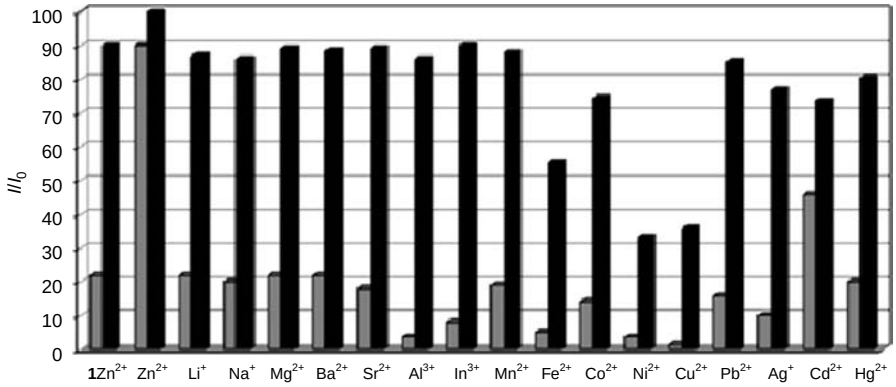


Figure 4. Relative fluorescence of ligand **1** and Zn^{2+} complex in presence of various metal ions. Grey bar: ligand **1** (20 μM) and 10 equiv of metal ions stated. Black bar: ligand **1** (20 μM) and Zn^{2+}(10 equiv) along with other metal ions (10 equiv) stated (for Zn^{2+} effective concentration is 20 equiv).

is ~20 orders more than that of ligand **1**–$2Zn^{2+}$ ($\log k = 11.18$) and as ammonia is available in abundance and relatively inexpensive. Fluorescence emission of ligand **1**–Zn^{2+} was monitored upon sequential addition of ammonia solution. The fluorescence intensity of ligand **1**–Zn^{2+} complex decreased gradually and became constant which corresponds to emission of free ligand **1** (Figure 6) and formation of $[Zn(NH_3)_4]^{2+}$ as supported by mass analysis (see Supplementary Information, available online). The emission maxima of ligand **1** blue shifted to ~400 nm ($\Delta = 30$ nm) with an isoemissive point (I_e) at 378 nm and overall 6.2-fold decrease in emission intensity (Figure 6). Thus using ammonia, free ligand **1** was regenerated from ligand **1**–Zn^{2+} complex which can be recycled for further zinc(II) sensing. Formation of ligand **1**–$2Zn^{2+}$ complex was also supported by mass spectro-

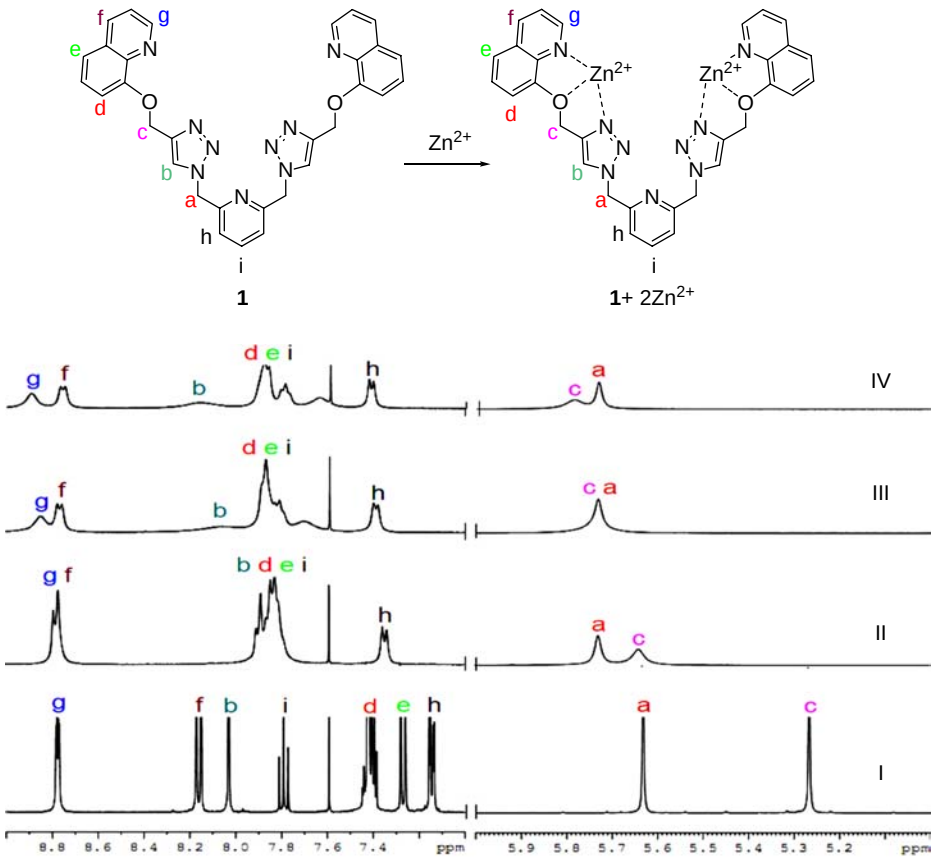


Figure 5. Binding mode of ligand **1**–$2Zn^{2+}$ complex and ^{1}H NMR spectra of ligand **1** upon addition of Zn^{2+} in CD_3CN: (I) ligand **1**, (II) ligand **1** + 1.0 equiv of Zn^{2+}, (III) ligand **1** + 2.0 equiv of Zn^{2+} and (IV) ligand **1** + 3.0 equiv of Zn^{2+}.

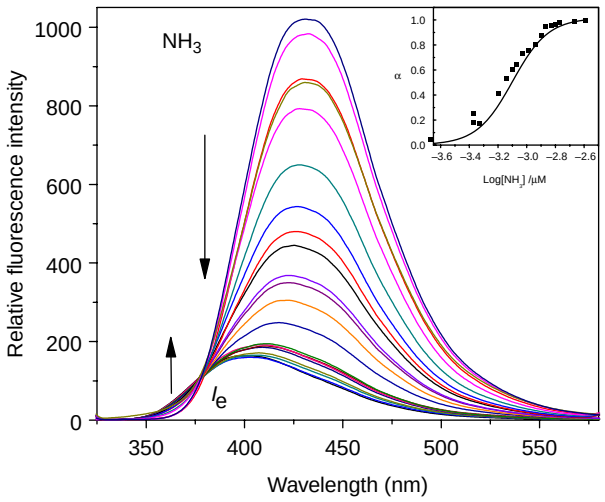


Figure 6. Fluorescence spectra of ligand **1**–Zn^{2+} complex in HEPES–CH_3CN (1:9) upon adding 0, 50, 100, 110, 110, 130, 170, 180, 190, 195, 200, 220, 240, 260, 300, 320, 370, 400, 500 and 600 μl of ammonia (8.5 mM). Inset: Response parameter (α) as a function of added ammonia.

scopic analysis. MALDI/TOF–MS showed the formation of ligand **1**–$2Zn^{2+}{\cdot}3H_2O$ complex (MW = 737.31; calcd for $C_{31}H_{31}N_9O_5Zn_2$, 737.10) (see Supplementary Information, available online).

Ligand **1** functions as a fluorescence chemosensor for Zn^{2+} based on the CHEF mechanism. CHEF mainly depends on photoinduced electron transfer (PET) process. Chelation of Zn^{2+} to ligand **1** enhances the fluorescence intensity by suppressing PET quenching through CHEF mechanism (*28*). In contrast, addition of ammonia leads decrease in fluorescence intensity due to the displacement of Zn^{2+} as $[Zn(NH_3)_4]^{2+}$, regenerating the free ligand **1** from the coordination complex disrupting the CHEF mechanism.

Conclusions

In conclusion, we have developed a novel pyridine-constrained *bis*(triazole-linked hydroxyquinoline) fluorescence chemosensor for Zn^{2+}. The ligand showed high selectivity with enhanced fluorescence emission for Zn^{2+} in presence of competing metal ions based on CHEF mechanism. An effective route for the regeneration of free ligand from Zn^{2+} complex was developed using ammonia. Thus the chemosensor reported here can be recycled for the detection of Zn^{2+} in a cost-effective manner.

Experimental

General experimental procedure

All reagents were purchased from Sigma-Aldrich, St. Louis, USA or Steinheim, Germany and used as received unless otherwise mentioned. The metal ion solutions of $NaClO_4$, $Mg(ClO_4)_2$, $Ba(ClO_4)_2$, $Mn(ClO_4)_2{\cdot}6H_2O$, $LiClO_4{\cdot}3H_2O$, $Cu(ClO_4)_2{\cdot}6H_2O$, $Pb(ClO_4)_2$, $Zn(ClO_4)_2{\cdot}6H_2O$, $Cd(ClO_4)_2{\cdot}H_2O$, $HgCl_2$, $AgClO_4$, $Fe(ClO_4)_2$, $Fe(ClO_4)_3$ and $Al(ClO_4)_3{\cdot}9H_2O$ were prepared in CH_3CN, and $Sr(NO_3)_2$ and $In(NO_3)_3$ were prepared in deionised water. HEPES buffer solution (10 mM, pH 7.2) was prepared in deionised water. Elemental analysis was carried out on Thermo Scientific FLASH 2000 Organic Element Analyzer. UV–vis spectra were recorded on a Perkin Elmer Model Lambda 900 spectrophotometer and fluorescence spectra were recorded on a Perkin Elmer model LS 55 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker AV-400 spectrometer with chemical shifts reported as ppm (in $CDCl_3/CD_3CN$, tetramethylsilane as internal standard). Mass spectra were obtained on Shimadzu GC–MS 2010 and Bruker Ultraflex II MALDI/TOF spectrometers. IR spectra were recorded on a Bruker IFS 66/V spectrometer, using KBr discs.

Synthesis and characterisation of compounds

*Preparation of 2,6-bis(azidomethyl)pyridine (**2**)*

To a solution of 2,6-*bis*(bromomethyl) pyridine (1.0 g, 3.77 mmol) in dimethyl sulfoxide (DMSO) (20 ml), sodium azide (0.614 g, 9.43 mmol) was added and the reaction mixture was stirred for 2 h at room temperature. The reaction was quenched by adding ice cold water (20 ml). The reaction mixture was extracted with ethyl acetate (30 ml × 3), washed with water (50 × 3 ml) and brine solution. Organic layer was dried over anhydrous sodium sulphate and concentrated under vacuo to obtain yellow oily product **2** in good yield (0.698 g, 98%). The azido compound **2** was used without purification for further reaction. IR: 2103 cm^{-1} (—N_3). ^1H NMR (400 MHz, $CDCl_3$) δ 4.48 (4H, s), 7.29 (2H, d, J = 8.0 Hz), 7.53 (1H, t, J = 8.0 Hz). ^{13}C NMR (400 MHz, $CDCl_3$) δ 55.4, 121.1, 138.0, 155.9. MS (EI): m/z = 189.87 [M^+] for $C_7H_7N_7$. Elemental analysis. Found: C, 44.38; H, 3.76; N, 51.86. Calcd: C, 44.44; H, 3.73; N, 51.83 for $C_7H_7N_7$ (*29*).

*Preparation of 8-(prop-2-ynyloxy)quinoline (**3**)*

To a solution of 8-hydroxyquinoline (1.5 g, 10.2 mmol) in dry acetone (60 ml), excess anhydrous K_2CO_3 (8.45 g, 61.20 mmol) was added and the mixture was refluxed for 30 min. Propargyl bromide (1.22 g, 10.2 mmol) was added slowly over a period of 5 h to the above reaction mixture using a pressure equalising funnel. The resulting mixture was refluxed over a period of 17 h and completion of the reaction was monitored by TLC. The reaction mixture was allowed to reach room temperature, filtered and the filtrate was evaporated to yield brown oily residue. The residue was re-dissolved in $CHCl_3$ (35 ml) and washed with water

(50 ml × 2) and saturated brine solution. Organic layer was dried over anhydrous sodium sulphate and concentrated under vacuo. The crude product was purified using column chromatography on silica gel using EtOAc/petroleum ether (40:60) as an eluent to afford brown solid product **3** in good yield (1.55 g, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.54 (1H, t, $J = 2.4$ Hz), 5.05 (2H, d, $J = 2.4$ Hz), 7.27 (1H, dd, $J = 5.6$, 1.4 Hz), 7.42–7.51 (3H, m), 8.14 (1H, dd, $J = 6.8$, 1.4 Hz), 8.94 (1H, dd, $J = 2.4$, 1.4 Hz). ^{13}C NMR (400 MHz, $CDCl_3$) δ 56.5, 76.1, 78.3, 110.0, 120.7, 121.7, 126.4, 129.5, 135.9, 140.3, 149.4, 153.1. MS (EI): $m/z = 183.87$ [M^+] for $C_{12}H_9NO$ Elemental analysis. Found: C, 78.62; H, 4.99; N, 7.63. Calcd C, 78.67; H, 4.95; N, 7.65 for $C_{12}H_9NO$.

*Preparation of 2,6-*bis*((4-((quinolin-8-yloxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)pyridine (ligand **1**)*

To a stirred solution of 2,6-*bis*(azidomethyl)pyridine **2** (0.5 g, 2.64 mmol) in toluene/*t*-butanol (4:1, 15 ml), 8-(prop-2-ynyloxy)quinoline **3** (0.96 g, 5.28 mmol), CuI (50.0 mg, 0.26 mmol) and *N,N′*-diisopropylethylamine (1.83 ml, 10.57 mmol) were added and stirring continued at room temperature for 38 h. The reaction mixture was concentrated under vacuo and the residue was extracted with dichloromethane (20 ml × 3). The combined dichloromethane extracts was washed with water (30 ml × 2) and brine solution. The organic layer was dried over sodium sulphate and the solvent was removed under vacuo. The crude product was purified on silica gel using methanol:dichloromethane (3:97) as an eluent to afford brown solid of ligand **1** in good yield (1.25 g, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.51 (4H, s), 5.55 (4H, s), 7.08 (2H, d, $J = 8.0$ Hz), 7.26 (2H, dd, $J = 7.2$, 2.0 Hz), 7.37–7.41 (6H, m), 7.62 (1H, t, $J = 7.6$ Hz), 7.89 (2H, s), 8.06 (2H, dd, $J = 5.6$, 1.6 Hz), 8.87 (2H, dd, $J = 2.4$, 1.6 Hz). ^{13}C NMR (400 MHz, *CDCl*$_3$) δ 53.8, 61.5, 108.7, 115.1, 119.0, 120.4, 123.1, 125.5, 128.2, 134.8, 137.2, 138.8, 142.8, 147.8, 152.4, 153.0. MS (EI): $m/z = 556.295$ [M^+] for $C_{31}H_{25}N_9O_2$. Elemental analysis. Found: C, 64.69; H, 4.50; N, 25.63. Calcd: C, 64.71; H, 4.45; N, 25.66 for $C_{31}H_{25}N_9O_2$.

Measurements of photophysical properties

For measuring the absorption and emission properties, the stock solution of ligand **1** was prepared (20 mM) in CH_3CN. The metal ion stock solutions were prepared in CH_3CN except for $Sr(NO_3)_2$ and $In(NO_3)_3$ which were prepared in deionised water in the order of 10^{-3} M. For all the measurements, ligand **1** and metal ion solutions were freshly prepared from the stock solutions. For emission studies, excitation of 300 nm was used with all excitation and emission slit widths at 15 and 5 nm, respectively.

Job's plot

We prepared a series of solutions such that the concentration of ligand **1** and Zn^{2+} varies, but the total concentration of the solution remaining constant (100 μM). The fluorescence intensity at 412 nm was plotted against the mole fraction of ligand **1** (shown in Figure 3) (*25*).

Determination of binding constant (log K)

By using the following Li's equation (*26*), we calculated the binding constant.

$$\left[M^{n+}\right]^m = \frac{1}{n \cdot K} \cdot \frac{1}{[\mathrm{L}]_T^{n-1}} \cdot \frac{1-\alpha}{\alpha^n}$$

where K is the equilibrium constant, α is the response parameter, [L] is ligand concentration, [M^{n+}] is the metal ion concentration, M_mL_n is the metal–ligand complex. In our case, ligand and Zn^{2+} are in 1:2 stoichiometry, then the equation is given as follows:

$$\left[\mathrm{Zn}^{2+}\right]^2 = \frac{1}{2K} \cdot \frac{1}{[\mathrm{L}]} \cdot \frac{(1-\alpha)}{\alpha^2}.$$

The curve fitting experimental data points were calculated from this equation with $\log K = 11.18$.

Acknowledgements

We thank Prof. C.N.R. Rao, FRS, for constant support, JNCASR and Department of Science & Technology (DST), India, for financial support, and CSIR, New Delhi, India, for awarding a JRF to NN and DM.

References

(1) (a) Emsley, J. In *Zinc, Nature's Building Blocks: An A–Z Guide to the Elements*; Oxford University Press: Oxford, 2001, pp 499–505. (b) Frederickson, C.J. *Int. Rev. Neurobiol.* **1989**, *31*, 145–238.
(2) (a) Cousins, R.I. In *Present Knowledge in Nutrition*; Zeigler, E.E., Filer, L.J., Eds.; ILSI Press: Washington, DC, 1996. (b) Cuajungco, M.P.; Lees, G.J. *Neurobiol. Disease* **1997**, *4*, 137–169. (c) Wagner, S.; Hess, M.A.; Hanson, P.O.; Malandro, J.; Hu, H.; Chen, M.; Kehrer, R.; Frodsham, M.; Schumacher, C.; Beluch, M.; Honer, C.; Skolnick, M.; Ballinger, D.; Bowen, B.R. *J. Biol. Chem.* **2000**, *275*, 15685–15690.
(3) Vallee, B.L.; Falchuk, K.H. *Physiol. Rev.* **1993**, *73*, 79–118.
(4) Walker, C.F.; Black, R.E. *Annu. Rev. Nutr.* **2004**, *24*, 255–275.
(5) Van Wouwe, J.P. *Eur. J. Pediatr.* **1989**, *149*, 2–8.
(6) Truong-Tran, A.Q.; Carter, J.; Ruffin, R.E.; Zalewski, P.D. *Biometals* **2001**, *14*, 315–330.
(7) Manzerra, P.; Behrens, M.M.; Canzoniero, L.M.T.; Wang, X.Q.; Heidinger, V.; Ichinose, T.; Yu, S.P.;

Choi, D.W. *Proc. Natl Acad. Sci. USA* **2001**, *98*, 11055–11061.
(8) Berdanier, C.D.; Dwyer, J.T.; Feldman, E.B. In *Handbook of Nutrition and Food*, 2nd ed. Nielsen, F.H., Ed.; CRC Press: Boca Raton, FL, 2007; Chapter 8, pp 166.
(9) Fosmire, G.J. *Am. J. Clin. Nutr.* **1990**, *51*, 225–227.
(10) (a) Bush, A.I.; Pettingell, W.H.; Multhaup, G.; Paradis, M.D.; Vonsattel, J.P.; Gusella, J.F.; Beyreuther, K.; Masters, C.L.; Tanzi, R.E. *Science* **1994**, *265*, 1464–1467. (b) Cherny, R.A.; Legg, J.T.; McLean, C.A.; Fairlie, D.P.; Huang, X.; Atwood, C.S.; Beyreuther, K.; Tanzi, R.E.; Masters, C.L.; Bush, A.I. *J. Biol. Chem*. **1999**, *274*, 23223–23228. (c) Tõugu, V.; Karafin, A.; Palumaa, P. *J. Neurochem*. **2008**, *104*, 1249–1259. (d) Noy, D.; Solomonov, I.; Sinkevich, O.; Arad, T.; Kjaer, K.; Sagi, I. *J. Am. Chem. Soc*. **2008**, *130*, 1376–1383. (e) Faller, P.; Hureau, C. *Dalton Trans*. **2009**, *21*, 1080–1094.
(11) Taylor, C.G. *Biometals* **2005**, *18*, 305–312.
(12) Xu, Z.; Yoon, J.; Spring, D.R. *Chem. Soc. Rev.* **2010**, *39*, 1996–2006.
(13) (a) Woodroofe, C.C.; Lippard, S.J. *J. Am. Chem. Soc*. **2003**, *125*, 11458–11459. (b) Komatsu, K.; Kikuchi, K.; Kojima, H.; Urano, Y.; Nagano, T. *J. Am. Chem. Soc*. **2005**, *127*, 10197–10204. (c) Royzen, M.; Durandin, A.; Young, V.G.; Geacintov, N.E.; Canary, J.W. *J. Am. Chem. Soc*. **2006**, *128*, 3854–3855. (d) Lu, C.; Xu, Z.; Cui, J.; Zhang, R.; Qian, X. *J. Org. Chem*. **2007**, *72*, 3554–3557. (e) Xue, L.; Liu, Q.; Jiang, H. *Org. Lett*. **2009**, *11*, 3454–3457. (f) Xue, L.; Liu, C.; Jiang, H. *Org. Lett*. **2009**, *11*, 1655–1658.
(14) (a) Divya, K.P.; Sreejith, S.; Balakrishna, B.; Jayamurthy, P.; Anees, P.; Ajayaghosh, A. *Chem. Commun*. **2010**, *46*, 6069–6071. (b) Carol, P.; Sreejith, S.; Ajayaghosh, A. *Chem. Asian J*. **2007**, *2*, 338–348. (c) Sreejith, S.; Divya, K.P.; Ajayaghosh, A. *Chem. Commun*. **2008**, 2903–2905. (d) Ajayaghosh, A.; Carol, P.; Sreejith, S. *J. Am. Chem. Soc*. **2005**, *127*, 14962–14963.
(15) (a) Wu, J.; Liu, W.; Zhuang, X.; Wang, F.; Wang, P.; Tao, S.; Zhang, X.; Wu, S.; Lee, S. *Org. Lett*. **2007**, *9*, 33–36. (b) Jiang, W.; Fu, Q.; Fan, H.; Wang, W. *Chem. Commun*. **2008**, 259–261. (c) Williams, N.J.; Gan, W.; Reibenspies, J.H.; Hancock, R.D. *Inorg. Chem*. **2009**, *48*, 1407–1415. (d) Xu, Z.; Baek, K.; Kim, H.N.; Cui, J.; Quin, X.; Spring, D.R.; Shin, I.; Yoon, J. *J. Am. Chem. Soc*. **2010**, *132*, 601–610. (e) Zhou, X.; Yu, B.; Guo, Y.; Tang, X.; Zhang, H.; Liu, W. *Inorg. Chem*. **2010**, *49*, 4002–4007.
(16) Jung, H.S.; Ko, K.C.; Lee, J.H.; Kim, S.H.; Bhuniya, S.; Lee, J.Y.; Kim, Y.; Kim, S.J.; Kim, J.S. *Inorg. Chem*. **2010**, *49*, 8552–8557; Zhang, J.F.; Bhuniya, S.; Lee, Y.H.; Bae, C.W.; Lee, J.H.; Kim, J.S. *Tetrahedron Lett*. **2010**, *51*, 3719–3723.
(17) (a) Tamanini, M.; Katewa, A.; Sedger, L.M.; Todd, M.H.; Watkinson, M. *Inorg. Chem*. **2009**, *48*, 319–324. (b) Tamanini, M.; Flavin, K.; Motevalli, M.; Piperno, S.; Gheber, L.A.; Todd, M.H.; Watkinson, M. *Inorg. Chem.* **2010**, *49*, 3789–3800. (c) Lau, Y.H.; Rutledge, P.J.; Watkinson, M.; Todd, M.H. *Chem. Soc. Rev.* **2011**, *40*, 2848–2866.
(18) (a) Bush, A.I. *Neurobiol. Aging* **2002**, *23*, 1031–1038. (b) Choia, J.-S.; Braymerb, J.J.; Nanga, R.P.R.; Ramamoorthy, A.; Lima, M.H. *Proc. Natl Acad. Sci. USA* **2010**, *107*, 21990–21995.
(19) (a) Su, N.; Bradshaw, J.S.; Zhang, X.X.; Song, H.; Savage, P.B.; Xue, G.; Krakowiak, K.E.; Izatt, R.M. *J. Org. Chem*. **1999**, *64*, 8855–8861. (b) Prodi, L.; Bargossi, C.; Montalti, M.; Zaccheroni, N.; Su, N.; Bradshaw, J.S.; Izatt, R.M.; Savage, P.B. *J. Am. Chem. Soc*. **2000**, *122*, 6769–6770. (c) Jotterand, N.; Pearce, D.A.; Imperiali, B. *J. Org. Chem*. **2001**, *66*, 3224–3228. (d) Moon, S.Y.; Cha, N.R.; Kim, Y.H.; Chang, S. *J. Org. Chem*. **2004**, *69*, 181–183. (e) Tang, X.; Peng, X.; Dou, W.; Mao, J.; Zheng, J.; Qin, W.; Liu, W.; Chang, J.; Yao, X. *Org. Lett.* **2008**, *10*, 3653–3656. (f) Zhao, Y.; Sun, Y.; Lv, X.; Liu, Y.; Chen, M.; Guo, W. *Org. Biomol. Chem*. **2010**, *8*, 4143–4147.
(20) (a) Li, Y.; Huffman, J.C.; Flood, A.H. *Chem. Commun*. **2007**, 2692–2694. (b) Li, Y.; Flood, A.H. *Angew. Chem. Int. Ed*. **2008**, *47*, 2649–2652. (c) Lee, S.; Hua, Y.; Park, H.; Flood, A.H. *Org. Lett*. **2010**, *12*, 2100–2102.
(21) (a) Urankar, D.; Pinter, B.; Pevec, A.; Proft, F.D.; Turel, I.; Kosmrlj, J. *Inorg. Chem*. **2010**, *49*, 4820–4829. (b) Kim, H.; Lee, S.; Lee, J.; Tae, J. *Org. Lett*. **2010**, *12*, 5342–5345.
(22) (a) Li, H.; Yao, Y.; Han, C.; Zhan, J. *Chem. Commun*. **2009**, 4812–4814. (b) Zhang, Y.M.; Chen, Y.; Li, Z.Q.; Li, N.; Liu, Y. *Bioorg. Med. Chem*. **2010**, *18*, 1415–1420.
(23) (a) Struthers, H.; Mindt, T.L.; Schibli, R. *Dalton Trans*. **2010**, *39*, 675–696. (b) Manbeck, G.F.; Brennessel, W.W.; Evans, C.M.; Eisenberg, R. *Inorg. Chem*. **2010**, *49*, 2834–2843.
(24) (a) Maity, D.; Govindaraju, T. *Chem. Commun*. **2010**, *46*, 4499–4501. (b) Maity, D.; Govindaraju, T. *Inorg. Chem*. **2010**, *49*, 7229–7231.
(25) Job, P. *Ann. Chim.* **1928**, *9*, 113–203.
(26) Grynkiewcz, G.; Poenie, M.; Tsein, R.Y. *J. Biol. Chem.* **1985**, *260*, 3440–3450.
(27) Sahoo, P.; Narasimhan, S.V.; Kannan, S.E. *J. Radioanal. Nucl. Chem.* **1996**, *204*, 3–14.
(28) 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.
(29) Alvarez, S.G.; Alvarez, M.T. *Synthesis* **1997**, 413–414.