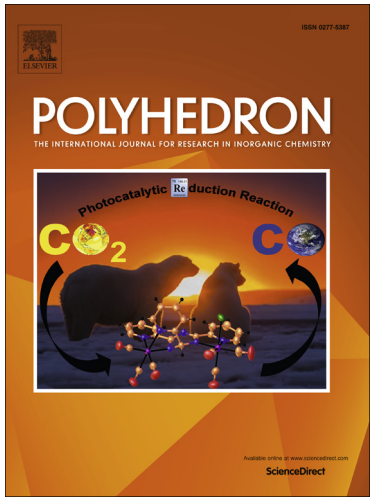


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# TAMEisoquin, a novel tripodal fluorescent zinc sensor with high Zn(II) affinity and Zn(II)/Cd(II) selective fluorescence response: Synthesis, coordination geometry, spectroscopy, and comparative response to biometal ions

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## Abstract

Herein we report a novel fluorescent zinc sensor 1,1,1-tris (aminomethylethane) N, N', N''-(2-methylisoquinoline) (TAMEisoquin), $[Zn(TAMEisoquin)]^{2+}$ complex and its structural and photophysical studies. TAMEisoquin was obtained from reduction of templated $[Ni(TAMEisoquin\text{-}trisimine)]^{2+}$ complex to give $[Ni(TAMEisoquin)]^{2+}$, which was subsequently decomposed with excess sodium cyanide to afford TAMEisoquin. A competition reaction between TAMEisoquin and the well-known Zn(II) chelator (N, N, N', N'-tetrakis(2-pyridymethyl)ethylenediame (TPEN) at pH 7.2 indicated a conditional dissociation constant for $[Zn(TAMEisoquin)]^{2+}$ of $K_d=1.4x10^{-15}$ M. The affinity of TAMEisoquin for Zn(II) is indicative of a ligand design exploiting ring size, preorganization and chelate effects to potentially detect picomolar and femtomolar zinc concentrations. X-ray crystallographic analysis of $[Zn(TAMEisoquin)]^{2+}$ showed four unique cations that preferred a distorted octahedral geometry with trigonal twist angle that range between 34(2)° and 43.1(8)°. Moreover, $[Zn(TAMEisoquin)]^{2+}$ complex showed Zn-N bond distance slightly similar to our previously reported $[Zn(TAMEpyr)]^{2+}$ complex, being Zn-$N_{heterocyclic}$, 2.160 (1)Å and Zn-$N_{aliphatic}$, 2.178(4)Å versus 2.128(2) Å and 2.169(2) Å respectively for $[Zn(TAMEpyr)]^{2+}$. The addition of Zn(II) to TAMEisoquin displayed 11-fold fluorescence enhancement, selective by comparison to divalent biometal ions and Cd(II) which induces only 16% of the Zn(II) response.

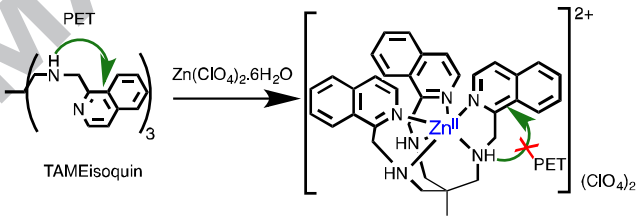


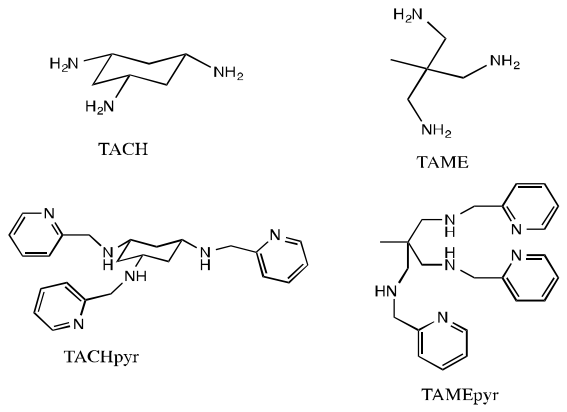

**Figure 1**: Synthesis of $[Zn(TAMEisoquin)]^{2+}$

## 1. Introduction

Zinc is the second most abundant element in the body with very diverse biological functions such as structural, enzymatic, catalytic, charge neutralization and signaling.[1] Although zinc proteins and metallothioneins regulate the concentration of zinc in the body, the disruption of zinc homeostasis is associated with various diseases such as diabetes,[2] Alzheimer's,[3] and prostate cancer.[4,5]This is critical because concentration of free zinc in the body generally vary between 1 nM and 1 mM[6,7] with particular high concentration in the brain, eyes, pancreas and spermatozoa.[7] For instance, numerous metalloneurochemistry studies showed that free zinc causes detrimental effects to central nervous system.[8-11] Therefore, the detection of zinc in living organisms, environment, and food samples is important to understand functions of free zinc in the cellular environment and its effects on human health.

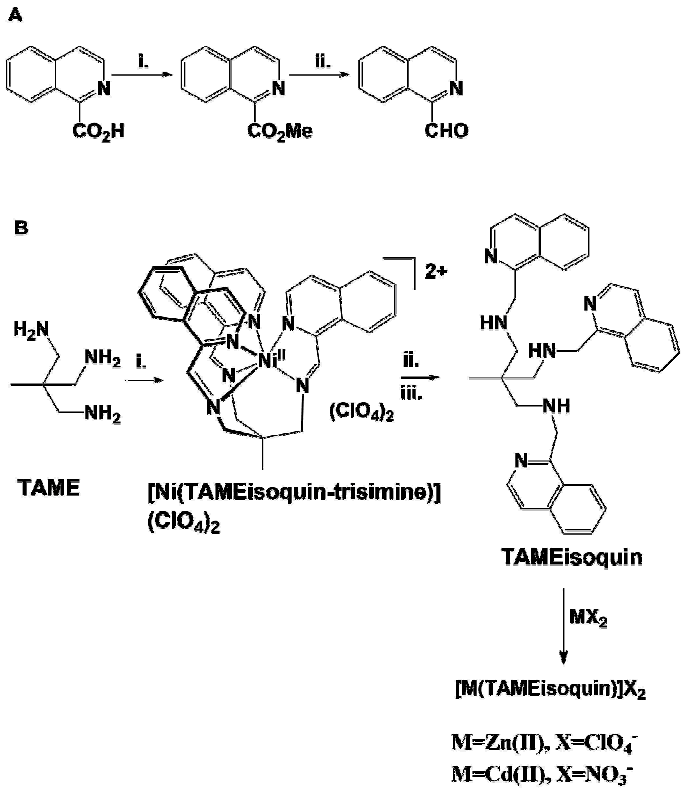
In recent years, a great number of fluorescent zinc sensors have become available to satisfy the criteria of metal ion readout, rapid response, selectivity, and biological stability.[12-14] However, one of the challenges associated with zinc sensors is their ability to induce similar fluorescence response for Zn(II) and Cd(II)[15,16] since they are both divalent $d^{10}$ metal ions, which possess similar coordination and photophysical properties. According to the U.S Environment Protection Agency (EPA), cadmium is listed as one of the 126 priority pollutants with a half-life between 15 and 20 years in humans.[17-19] Similar to zinc, excessive exposure to cadmium is linked with various health effects such as kidney dysfunction, pulmonary cancer, and lung diseases.[18,19] It is therefore important to discriminate these two metal ions in environmental and biological studies, such that we are studying effects of chelate ring size and metal-ion binding pocket size upon preference for Zn(II) relative to Cd(II).

In the past, we have developed the chemistry of two tripodal chelator frameworks, *cis,cis*-1,3,5-triaminocylohexane (TACH) [20] and 1,1,1-tris(aminomethyl)ethane (TAME) [21] by attaching suitable pendant groups to provide tripodal hexadentate chelators (**Scheme 1**). We are mainly interested in hexadentate tripodal ligands because of their size selective nature, formation of metal complexes with defined configurations, and high binding affinity due to six coordinating nitrogen atoms on the ligand.




**Scheme 1**: The TACH and TAME frameworks and their 2-methylpyridyl derivatives serving as basis for the Zn sensor TAMEisoquin described herein.

Our previous studies with hexadentate tripodal chelators such as tachpyr showed marked size selectivity attributed to the metal-binding cavity size. For example, the tachpyr complexes of Cd(II), Hg(II) or In(III) were weakly bound and distorted relative to Zn(II) or Ga(III) complexes.[22,23] In the present article we explore the chemistry of TAME framework by using isoquinoline as coordinating fluorophore. We use isoquinoline because our previous results showed that substituents ortho to the heterocyclic nitrogen create substantial steric hindrance upon metal complexation. [24] Therefore, using isoquinoline rather than quinoline prevents steric congestion at the $C_3$-apex of the complex. TAMEisoquin sensor operates through photoinduced electron transfer (PET) mechanism whereby Zn(II) binding prevents fluorescence quenching, and thus enhances emission intensity. In this paper, we report synthesis of a novel indicator, TAMEisoquin (**Scheme 2**), its structural and photophysical properties relative to Zn(II) sensing, and the selectivity of response to Zn(II) relative to Cd(II) and other divalent biometal ions.




**Scheme 2**. **A**. Preparation of isoquinoline-1-carboxaldehyde: i. $SOCl_2$, MeOH, reflux, 16 h. ii. a)0.5 equiv $LiAlH_4$, THF, -78°, 15 min. b)AcOH. **B**. Preparation of TAMEisoquin: i) 3.0 equiv 1-isoquinolinecarboxaldehyde, $Ni(ClO_4)_2 \cdot 6H_2O$, $H_2O$, EtOH, 12 h; ii) $NaBH_4$, $H_2O$, EtOH, 25 °C, 12 h; iii) NaCN(aq), $CH_2Cl_2$ extraction and evaporation

## 2. Experimental

### *2.1. General Considerations*

All the materials listed below were of research grade or a spectro-grade in the highest purity available and were generally used without further purification except diethyl ether, which was distilled from sodium benzophenone. Anhydrous methanol, acetonitrile, and the salts $Zn(ClO_4)_2.6H_2O$, $Cd(NO_3)_2.4H_2O$, $Ni(ClO_4)_2{\cdot}6H_2O$, $Co(BF_4)_2.6H_2O$, $Fe(ClO_4)_2.6H_2O$, $Mn(ClO_4)_2.6H_2O$, and $AgNO_3$ were purchased from Aldrich. The salts NaCl, KCl, and $CaCl_2$ were purchased from EM science. Anhydrous grade EtOH was purchased from Pharmco. 1,1,1-Tris(aminomethyl)ethane was synthesized as we previously reported.[21] (N,N,N'N'-Tetrakis-(2-pyridylmethyl)(ethylenediamine) "TPEN" was from TCI. DMSO-$d_6$, $CDCl_3$ and DMF-$d_6$ were obtained from Cambridge Isotope Laboratories. The UV-visible spectra were measured using Varian Cary 50 Bio spectrometer with 3 $cm^3$ quartz cuvettes (1 cm path-length). The fluorescence spectra were recorded using a Cary Eclipse fluorescence spectrophotometer with 3 $cm^3$ quartz cuvettes (1 cm path-length). $^1H$ and $^{13}C$ NMR were obtained using a Varian Mercury 400 MHz NMR instrument and chemical shifts are reported in ppm relative to the deuterated solvents used. Elemental analysis was performed by Atlantic Microlabs (Atlanta, Georgia). Drying was accomplished under a stream of nitrogen or under reduced pressure (*ca.* $10^{-2}$ Torr) with a standard Schlenk line.

### *2.2. Synthesis of compounds*

#### *2.2.1. Methyl 1-isoquinolinecarboxylate*

To a suspension of 1-isoquinoline carboxylic acid (3.00 g, 17.3 mmol) in MeOH (30 mL) was added $SOCl_2$ (6.18 g, 3.79 mL, 52.0 mmol) via pipet. The resulting pale yellow solution was refluxed under a stream of nitrogen for 16hr. The solvent was then removed under vacuum affording pale yellow solids. The residue was dissolved into $H_2O$ (20 mL), neutralized with excess $Na_2CO_3$, and extracted with $CH_2Cl_2$ (4x20 mL). The organic extracts were combined, dried over $Na_2SO_4$, filtered, and the solvent removed by vacuum leaving behind a yellow oil. The oil was distilled under reduced pressure affording methyl 1-quinolinecarboxylate as a colorless oil (1.59 g, 49%, bp=144-145°C, 700 mTorr). $^1H$ NMR ($CDCl_3$): 8.84 ppm (1H, d, J=8.8 Hz); 8.63 ppm (1H, d, 5.6 Hz); 7.883 ppm (1H, d, J=8.0 Hz); 7.83 ppm (1H, d, J=5.6 Hz); 7.71 ppm (2H, m); 4.10 ppm (3H, s). $^{13}C$ NMR ($CDCl_3$): 166.35, 148.24, 141.65, 137.01, 130.65, 128.89, 127.20, 126.97, 126.46, 124.40, 53.06 ppm. IR (liquid film): 3055, 2951, 2849, 1720 $cm^{-1}$.

*2.2.2. 1-Isoquinolinecarboxaldehyde.*

A solution of methyl 1-isoquinolinecarboxylate (1.00 g, 5.34 mmol) in dry THF (30mL) was cooled to -70°C in a dry ice/MeOH bath. To that solution was added a suspension of $LiAlH_4$ (0.10 g, 2.67 mmol) in THF (10 mL). The resulting brown reaction mixture was stirred for 15min followed by a quench with glacial acetic acid (1.3 mL). Once the mixture warmed to RT the aluminum salts were precipitated with a few drops 1N NaOH. The heterogeneous mixture was filtered, and the solvent was removed under vacuum. The residue was dissolved into a small portion of $CH_2Cl_2$ and chromatographed on silica (1:1hexanes:EtOAc , $R_f$=0.79) affording 1-isoquinolinecarboxaldehyde (0.28g, 33%) as a crystalline white solid. $^1H$ NMR ($CDCl_3$): 10.40 ppm (1H, s); 9.33 ppm (1H, m); 8.76 ppm (1H, d, J=5.6 Hz); 7.91 ppm (2H, m); 7.77 ppm (2H, m). $^{13}C$ NMR ($CDCl_3$): 195.63, 149.79, 142.41, 136.85, 130.74, 130.01, 126.91, 126.31, 125.70, 125.45 ppm. IR (KBr pellet): 3033, 2844, 1703 $cm^{-1}$.

*2.2.3. [Ni (TAMEisoquin-trisimine)] $(ClO_4)_2$·MeCN*

To a solution of TAME (30.9 mg, 264 mmol) in EtOH (2.3 mL) was added Ni $(ClO_4)_2$·$6H_2O$ (93.6 mg, 256 mmol) dissolved in $H_2O$ (4.0 mL). The resulting pale blue solution was titrated with 1-isoquinolinecarboxaldehyde (121 mg, 770 mmol) dissolved in EtOH (3.0 mL). A finely divided orange powder precipitated from solution immediately. The heterogeneous reaction mixture was allowed to stir at RT for 16hr. The solids were filtered on a glass frit, washed with portions of $Et_2O$ (3x10mL), and dried under vacuum. The crude trisimine complex was recrystallized from 1:1 MeOH: MeCN (v/v) via $Et_2O$ diffusion as large, lustrous orange needles (137 mg, 62%). IR (KBr pellet): 3256, 2964, 2933, 1623, 1589, 1458, 1322, 1093 $cm^{-1}$. (Found: C, 53.06; H, 3.99; N 11.54. $C_{35}H_{30}N_6NiCl_2O_8$·$C_2H_3N$ requires: C, 53.33; H, 3.99; N, 11.77 %.). Crystals suitable for X-ray crystallography were obtained by slow $Et_2O$ diffusion into a 1:1 MeCN: MeOH (v/v) solution of the complex.

*2.2.4. TAMEisoquin*

To a suspension of [Ni (TAMEisoquin)] $(ClO_4)_2$·2MeCN (50 mg, 63 mmol) in $H_2O$ (10 mL) was added $NaBH_4$ (151 mg, 4.00 mmol). The resulting mixture was sonicated and then shaken to afford copious amounts of a pale pink precipitate. The mix was stirred at RT for 3hr. The reaction was then quenched with the addition of conc. $HClO_4$ (pH 6). The mixture was centrifuged and the supernatant was decanted away from the solid plug. The solids were washed with $Et_2O$ (2x20 mL) and then dried under vacuum. The resulting pale pink triamine

complex was dissolved into MeOH (3 mL). To the solution was added excess NaCN (19.1 mg, 391 mmol) dissolved in $H_2O$ (1 mL). The mixture was shaken vigorously and then extracted with $CH_2Cl_2$ (3x3 mL). The organic fractions were combined, dried over $Na_2SO_4$, decanted, and the solvent was removed under vacuum to afford TAMEisoquin as a pale yellow oil (34.0 mg, 99%). $^1$H NMR($CDCl_3$): 8.39 ppm (3H, d, J=6.0 Hz); 8.21 ppm (3H, d, J=8.8 Hz); 7.77 ppm (3H, d, J=8.0 Hz); 7.60 ppm (3H, m); 7.50 ppm (3H, d, J=6.0 Hz); 7.46 ppm (3H, m); 4.36 ppm (6H, s); 2.77 ppm (6H, s); 2.53 ppm (3H, br.s); 1.02 ppm (3H, s). $^{13}$C NMR ($CDCl_3$): 159.54, 141.85, 136.31, 130.01, 127.35, 127.15, 127.06, 125.38, 120.06, 57.09, 54.04, 39.22, 21.95 ppm. The ligand was also prepared as a hydrochloride salt by dissolving the free amine into EtOH and adding conc. HCl dropwise. The resulting white precipitate was isolated, washed with $Et_2O$, and dried under vacuum. (Found: C, 54.31; H, 5.74; N, 10.86. $C_{35}H_{36}N_6 \cdot 6HCl \cdot H_2O$ requires: C, 54.07; H, 5.70; N, 10.81%).

*2.2.5. [Zn (TAMEisoquin)] $(ClO_4)_2.2H_2O.1/2MeCN$*

To a solution of TAMEisoquin (28.3mg, 52.3 mmol) in MeOH (3mL) was added $Zn(ClO_4)_2 \cdot 6H_2O$ (19.5mg, 52.3 mmol) dissolved in MeOH (1mL). The mixture was allowed to stand for 5min, and then $Et_2O$ (20mL) was added affording copious amounts of a white precipitate. The heterogeneous mixture was centrifuged and the supernatant was decanted. The solid plug was washed with $Et_2O$ and then dried under vacuum affording the desired complex as a white powder (38.4 mg, 91%). $^1$H NMR (DMSO-$d_6$): 8.44 ppm (3H, d, J=8.8 Hz); 8.15 ppm (3H, d, J=8.0 Hz); 8.01 ppm (3H, t, J=6.8 Hz); 7.95 ppm (3H, d, J=6.0 Hz); 7.89 ppm (3H, t, J=7.2Hz); 7.75 ppm (3H, d, J=6.0 Hz); 4.83 ppm (3H, m); 4.75 ppm (3H, m); 4.58 ppm (3H, m); 3.28 ppm (3H, m); 3.05 ppm (3H, m); 0.76 ppm (3H, s). IR (KBr): 3280, 3070, 2920, 1627, 1598, 1101 cm$^{-1}$. (Found: C, 50.83; H, 4.59; N, 10.10. $C_{35}H_{36}N_6ZnCl_2O_8.2H_2O.1/2MeCN$ requires: C, 50.19; H, 4.85; N, 10.57%). Crystals suitable for X-ray analysis were obtained via $Et_2O$ diffusion into a 1:1 MeOH: MeCN (v/v) solution of the complex.

*2.2.6. [Cd (TAMEisoquin)] $(NO_3)_2.2H_2O$*

To a solution of TAMEisoquin (43.7mg, 80.8 mmol) in MeOH (1.5 mL) was added $Cd(NO_3)_2 \cdot 4H_2O$ (22.9 mg, 74.2 mmol) dissolved MeOH (1.5 mL). The mixture was allowed to stand for 5min, and then $Et_2O$ (20 mL) was added affording copious amounts of a white precipitate. The heterogeneous mixture was centrifuged and the supernatant was decanted. The solid plug was washed with $Et_2O$ and then dried under vacuum affording the desired complex as a white powder (55.9 mg, 96%). Microcrystals were obtained via $Et_2O$ diffusion into

a 1:1 $MeNO_2$: MeCN (v/v) solution of the complex. $^1$H NMR(DMSO-$d_6$): 8.69 ppm (3H, d, J=6.0 Hz); 8.43 ppm (3H, d, J=8.4 Hz); 8.14 ppm (3H, d, J=8.0 Hz); 8.05 ppm (3H, d, J=6.0 Hz); 7.96 ppm (3H, t, J=7.6 Hz); 7.84 ppm (3H, t, J=7.2 Hz); 4.72 ppm (6H, s); 4.55 ppm (3H, m); 3.24 ppm (6H, m); 0.89 ppm (3H, s). IR (KBr pellet): 3219, 3065, 2919, 2869, 1625, 1381, 1346, 1312 cm$^{-1}$. (Found: C, 51.83; H, 4.64; N, 13.93. $C_{35}H_{36}N_6CdN_2O_6$ .2$H_2O$ requires: C, 51.70; H, 4.96; N, 13.78%).

*2.2.7. Photophysical studies*

Stock solutions of the ligand "TAMEisoquin" and metal ions were prepared in 1:1 DMSO: H2O (v/v) and 100% water respectively. The stock solution of the ligand was used to make 14 µM in 0.1 M HEPES buffer pH 7.2 and titrated with various metal ions aliquots. The samples used for photophysical studies contained 0.4% (v/v) DMF/$H_2O$ except for quantum yield determination where 1:1 DMF: $H_2O$ was used. All spectral measurements were carried out in a 3cm$^3$ cuvette with a 1 cm path-length. The fluorescence spectra were conducted with an excitation wavelength ($\lambda_{ex}$) of 321 nm.

Recrystallized [Zn (TAMEisoquin)]$(ClO_4)_2$ was used to prepare serial dilutions of the complex and its quantum yield of $[Zn(TAMEisoquin)]^{2+}$ was determined by comparing with a quinine sulfate standard[25] measured in 1N $H_2SO_4$. The formula used to calculate the quantum yield of the Zn(II) complex is:

$$\Phi_x = \Phi_{st}\left(\frac{Grad_x}{Grad_{st}}\right)\left(\frac{\eta_x^2}{\eta_{st}^2}\right)$$

The subscripts 'x' and 'st' refer to the experimental sample and the standard, respectively, and 'Grad' refers to the slope of a least-squares straight line through a plot of integrated fluorescence intensity vs. absorbance.[26] The refractive indices, $\eta$, were neglected in the computation and may introduce a small amount of error in the quantum yield reported herein.

*2.2.8. Binding constant studies*

In order to further investigate the affinity of Zn(II) for TAMEisoquin, we carried out a ligand exchange reaction with the strongly binding chelator TPEN (N, N, N', N'-Tetrakis- (2-pyridylmethyl)-ethylenediamine. Stock solutions of $[Zn(TAMEisoquin)]^{2+}$ and TPEN were prepared in 0.1 M HEPES buffered at pH 7.2 with 0.1 M $KNO_3$ as ionic strength adjuster. A 14 µM solution of $[Zn(TAMEisoquin)]^{2+}$ with 1.0 equiv of TPEN was prepared in the HEPES buffer pH 7.2. A decrease in the emission intensity at 350 nm was monitored over the course of three

weeks (T=25°C). A 14 μM solution of $[Zn(TAMEisoquin)]^{2+}$ without the competing ligand was also monitored as a control for photobleaching.

## 3. Results and Discussion

### *3.1. Synthesis of TAMEisoquin and its Ni(II), Zn(II) and Cd(II) complexes*

To prepare TAMEisoquin, we rely on the Ni-templated condensation of isoquinoline aldehyde analogous to the previously reported synthesis of TAMEpyr (N,N',N''-tris(2-pyridylmethyl)-tame) from TAME and pyridine-2-carboxaldehyde.[21] The required aldehyde is prepared from 1-isoquinolinecarboxylic acid via reduction of its methyl ester (**Scheme 2a**). The reaction of TAME, $Ni(ClO_4)_2.6H_2O$ and 3.0 equiv of 1-isoquinolinecarboxaldehyde gave the orange complex $[Ni(TAMEisoquin\text{-}trisimine)]^{2+}$ in high yields. The ligand is prepared by treating $[Ni(TAMEisoquin\text{-}trisimine)]^{2+}$ with excess $NaBH_4$ and liberating TAMEisoquin from the metal center via 6.0 equiv of NaCN (**Scheme 2b**). TAMEisoquin ligand is also prepared as a hydrochloride salt by treating an ethanolic solution of the free ligand with concentrated HCl and isolating the resulting white powder under vacuum. Zn(II) and Cd(II) TAMEisoquin complexes are isolated by reactions of the corresponding metal salts and the ligand in MeOH with $Et_2O$ cosolvent to improve precipitation and yields. A 1:1 M:L composition of all metal complexes was verified by elemental analysis.

### *3.2. Structural studies*

Crystallographic studies reveal the coordination sphere of the TAMEisoquin ligand and particularly the fit of Zn(II) within the TAME-heterocycle framework. To assess the flexibility of TAMEisoquin, an X-ray study of $[Zn(TAMEisoquin)](ClO_4)_2.1/2MeCN$ (**Figure 2**) was carried out which showed four unique $[Zn(TAMEisoquin)]^{2+}$ cations in the asymmetric unit, labeled A,B,C, and D respectively. All four cations can be described as distorted octahedral complexes with twist angles that range between 34(2)º and 43.1(8)º, as viewed along the $C_3$ symmetry axis. The three C–N bonds of TAME restrict ligand twisting such that a structure between trigonal prismatic (α=0º) and octahedral (α=60º) is obtained.[27] The average distance $Zn\text{-}N_{aliphatic}$ is slightly longer than the average $Zn\text{-}N_{heterocyclic}$, at 2.178(4) vs. 2.16(1) Å for cation A. This behavior agrees with our previously published structure of $[Zn(TAMEpyr)]^{2+}$ where the distance of $Zn\text{-}N_{aliphatic}$ and $Zn\text{-}N_{heterocyclic}$ were 2.169(2) and 2.128(2) Å respectively.[21] Analogous to the $[Fe(tptMetame)]^{2+}$ complex reported by Al-Obaidi[28] (where tptMetame = the pyridylmethyl TAME-based chelator, 1,1,1-tris{[N-(2-pyridylmethyl)-N-methylamino]-methyl}ethane), each

cation has six chelate rings and three stereogenic nitrogen atoms, potentially giving rise to a large number of isomers. The conformation of the three six membered chelate rings with reference to the $C_3$ axis in molecule A is $\delta^{cap}$. The absolute configuration of the cation in molecule A as defined by the arrangement of the five membered chelate rings is assigned as Δ. The chiral aliphatic nitrogen donors are designated ***S,S,S***. Lastly, the conformation of the five membered chelate rings is slightly puckered and has been assigned as λλλ. Thus stereochemistry for molecule A follows as Δ $\delta^{cap}$ λλλ (*SSS)*, and molecules B-D are similarly assigned (**Table 1**). In summary, $[Zn(TAMEisoquin)]^{2+}$ $(ClO_4)_2$ assumes a number of conformational modes in the solid state, which are attributed to the flexibility of TAME framework.

We attempted synthesis of $[Cd(TAMEisoquin)]^{2+}$; unfortunately crystals suitable for X-ray crystallographic analysis were not isolated. We were able to perform computational studies on $[Cd(TAMEisoquin)]^{2+}$ complex (**Figure S9**) which revealed that the Cd complex is more distorted than the Zn complex. The computational data showed that average bond length for Cd-N(amine) (2.43Å) is longer than the average bond length for Cd-N (heterocyclic) (2.36 Å). Known $CdN_6$ complexes have crystallographically measured bonds that vary between ca. 2.3-2.4Å which support the validity of the computational results.[29] However, comparison between Zn and Cd complexes shows that the Cd complex is more distorted. For example, the largest deviation from $180^0$ for the Cd complex was observed in N5-Cd-N3 ($152.70^0$) which is significantly higher compared to the largest deviation for Zn complex, N5-Zn-N3 ($160.84^0$).

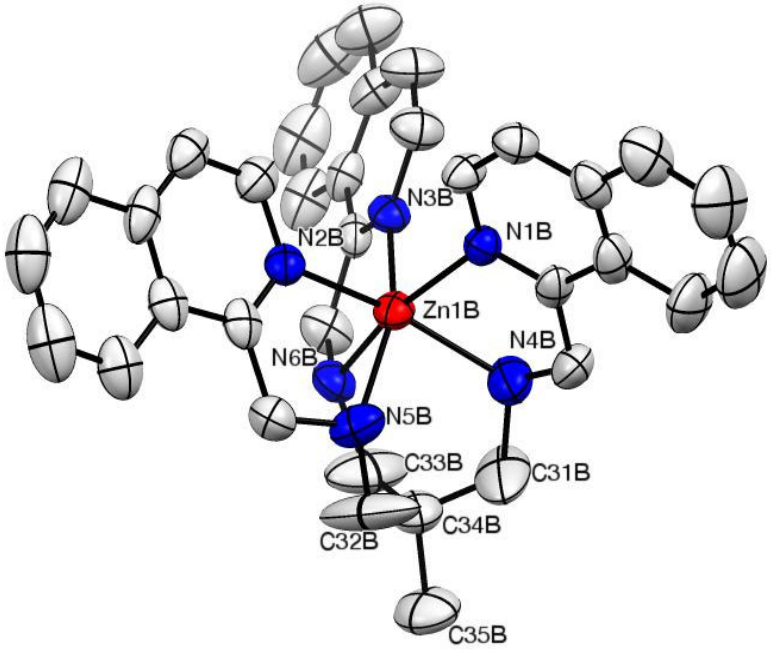



**Figure 2**: ORTEP plot (50% probability) for [Zn(TAMEisoquin](ClO4)2.1/2MeCN. Coun solvents and hydrogens were omitted for clarity. Color code: C, gray; Zn, Red; N, blue. Sele lengths (Å): Zn1B-N1B, 2.173(4); Zn1B-N2B, 2.164(5), Zn1B-N3B, 2.142(5); Zn1B-N4B Zn1B-N5B, 2.168(6) and Zn1B-N6B, 2.168(5)

| Complex | Zn-N (heterocyclic) Average bond length (Å) | Zn-N (amine) Average bond length (Å) | Twist angle α (°) | Stereochemical configuration |
|---|---|---|---|---|
| Cation A | 2.160(1) | 2.178(4) | 34(2) | $\Delta\delta^{CAP}\lambda\lambda\lambda$(SSS) |
| Cation B | 2.160(1) | 2.164(6) | 38(2) | $\Delta\lambda^{CAP}\delta\delta\delta$(RRR) |
| Cation C | 2.150(1) | 2.158(1) | 43.1(8) | $\Delta\lambda^{CAP}\lambda\lambda\lambda$(SSS) |
| Cation D | 2.154(1) | 2.163(1) | 40(3) | $\Delta\lambda^{CAP}\lambda\lambda\lambda$(SSS) |

**Table 1:** Selected structural data of four cations of $[ZnTAMEisoquin]^{2+}$: Average bond lengths (Å), twist angles(°), and stereochemical configurations are reported.

*3.3. Photophysical studies*

Spectroscopic study of products from reaction of Zn(II) with TAMEisoquin is consistent with weakened π bonding in isoquinoline upon coordination and a 1:1 stoichiometry of Zn(II)-TAMEisoquin interaction. TAMEisoquin in aqueous DMF exhibits four maxima at 322nm ($\log\varepsilon_1$ = 3.96±0.01), 311nm ($\log\varepsilon_2$ = 3.91±0.01), 283 nm ($\log\varepsilon_3$ = 4.00±0.01), and 272 nm ($\log\varepsilon_4$ = 4.16±0.01) (**Figure 3**). When TAMEisoquin is exposed to 1.0 equiv of Zn(II) ion there is a slight red shift of the four maxima with a concomitant enhancement of intensity at 325 nm and 313 nm ($\log\varepsilon_{1'}$ = 4.05±0.01 and $\log\varepsilon_{2'}$ = 4.00±0.01 respectively) and attenuation of intensity at 284 nm and 273 nm ($\log\varepsilon_{3'}$ = 3.99±0.01 and $\log\varepsilon_{4'}$ = 4.19±0.01 respectively. There is no further change when TAMEisoquin is exposed to >1.0 equivalent of metal ion, supporting 1:1 M:L stoichiometry as confirmed by Job's plot analysis (**Figure 4**)

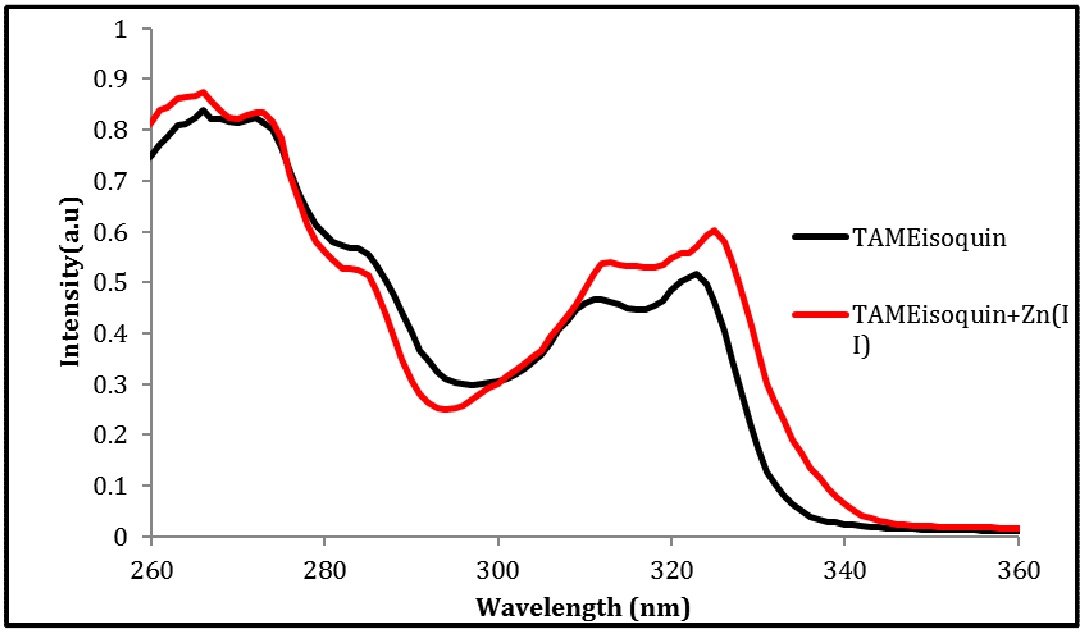



**Figure 3**: UV-Vis absorbance spectra of 56 μM TAMEisoquin in 0.1M HEPES buffer, pH 7.2. The black line represents TAMEisoquin alone and the red line represents TAMEisoquin and 1 equivalent Zn(II).

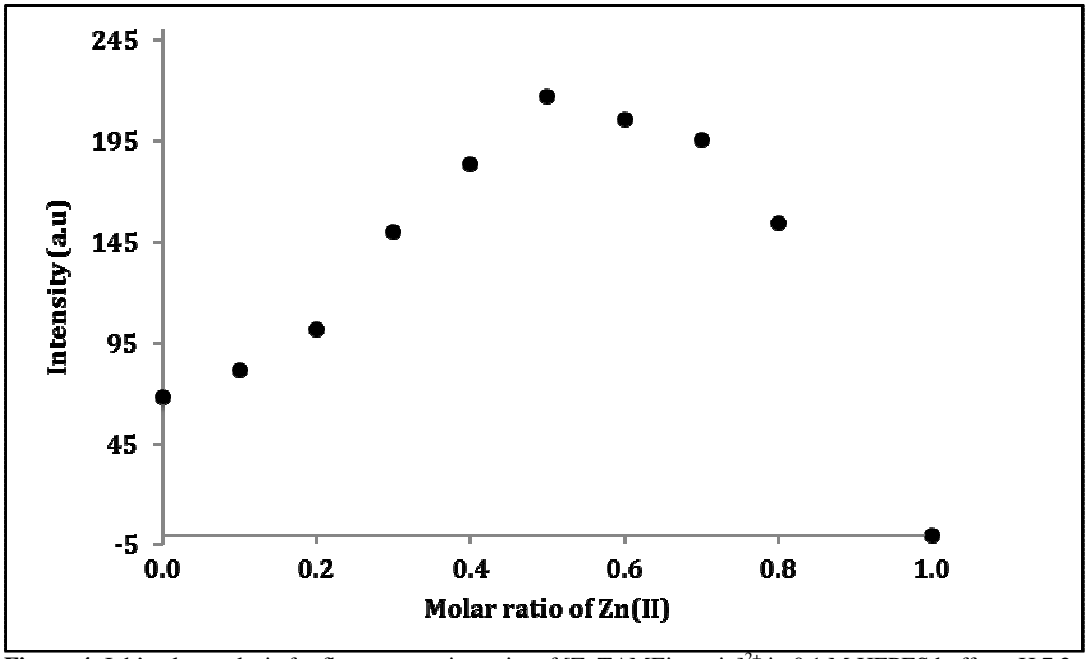



**Figure 4**: Job's plot analysis for fluorescence intensity of $[ZnTAMEisoquin]^{2+}$ in 0.1 M HEPES buffer, pH 7.2 at 350 nm. Fluorescence intensity at 0 molar ratio of Zn(II) is due to background fluorescence of TAMEisoquin.

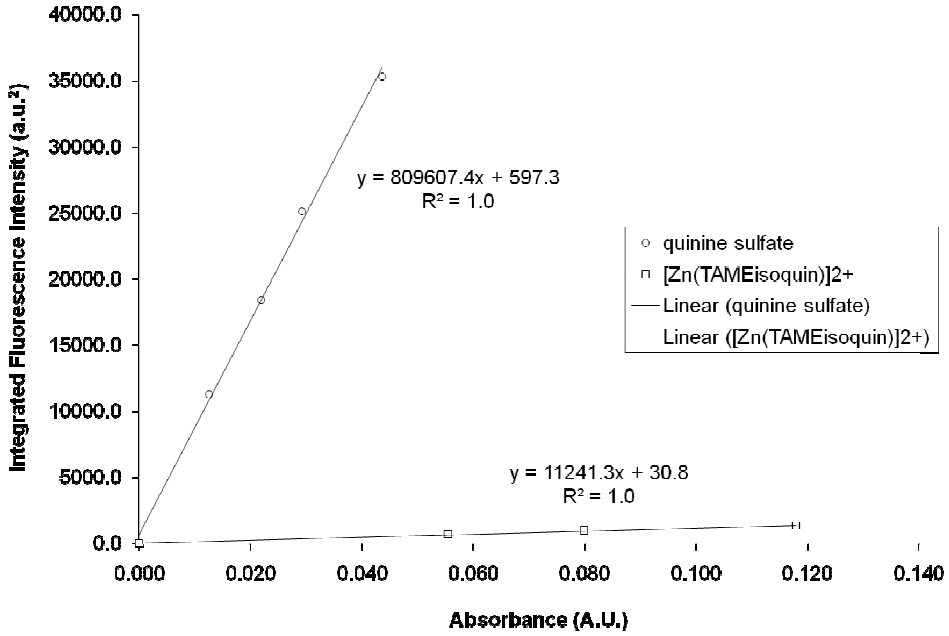



**Figure 5:** Quantum yield determination of $[Zn(TAMEisoquin)]^{2+}$ in DMF:Water (1:1).

To determine emission efficiency, we measured the quantum yield of $[Zn(TAMEisoquin)]^{2+}$ by using quinine sulfate as a standard (1N $H_2SO_4$, $\Phi_f$=0.54).[29] A quantum yield of 0.0077 is obtained for $[Zn(TAMEisoquin)]^{2+}$ with a linear emission intensity up to a concentration of *ca.* 20 mM (log ***K*** = 6.32±0.02 where $F=K\cdot[Zn(TAMEisoquin)]^{2+}$) (**Figure 5**).

TAMEisoquin shows nearly no fluorescence when excited with 321 nm light while the addition of an equimolar amount of Zn(II) causes an 11-fold enhancement of emission intensity ($\lambda_{em}$=350nm) (**Figure 6**). The emission intensities are detected at 350 nm and 480 nm respectively. The Zn(II)-cation binds to both the secondary amines and the heterocyclic nitrogens of TAMEisoquin preventing the PET-mediated quenching of the isoquinoline groups. The sensor is then turned-on and the resulting luminescent response amplified via the chelation-enhanced fluorescence (CHEF) mechanism.[30,31] The emission intensity at 480nm is due to excimer interaction between two isoquinoline molecules.[32] Consequently, the decrease in emission intensity at 480 nm is due to the breaking up of isoquinoline excimers upon $[Zn(TAMEisoquin)]^{2+}$ formation.

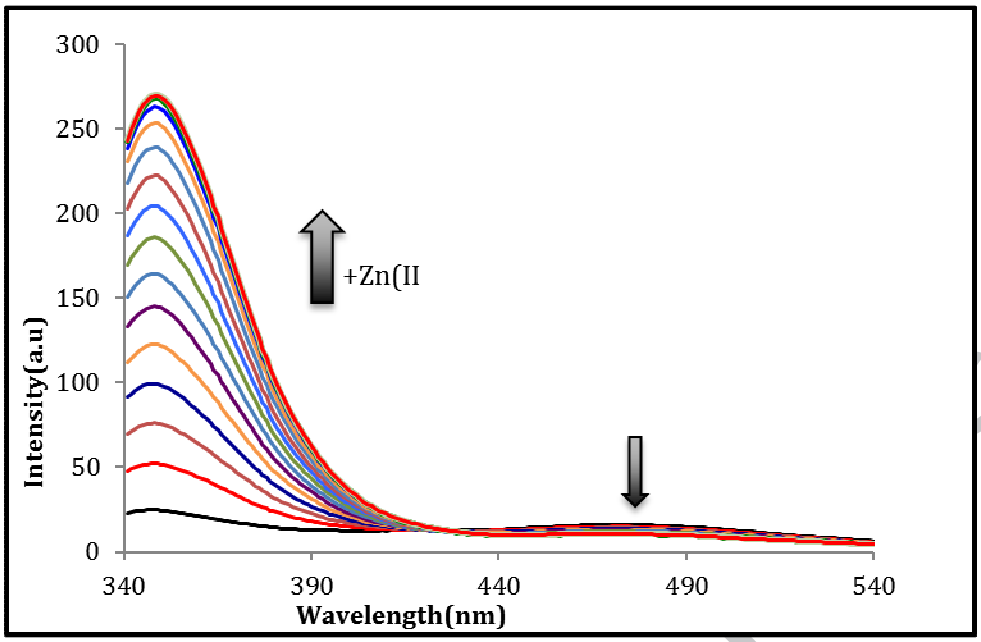



**Figure 6**: Fluorescence spectra of 14 μM TAMEisoquin ($\lambda_{ex}$=321 nm) in the presence of an increasing concentration of Zn(II) in 0.1 M HEPES buffer pH 7.2.

*3.4. Binding constant determination*

The binding affinity of Zn(II) to TAMEisoquin was determined by using a ligand exchange method at pH 7.2 and ionic strength μ = 0.1 ***M***. TPEN (N,N,N',N'-Tetrakis-(2-pyridylmethyl)-ethylenediamine), a well-known zinc chelator, was added to a solution of [ZnTAMEisoquin] $^{2+}$ and a decrease of approximately 50% of the original emission intensity remained constant after 1 week (**Figure 7**). From this data, a conditional dissociation constant $K_d$ of 1.4 x $10^{-15}$ M was calculated. The reaction was repeated with 2.0 eq of competing TPEN with the same outcome (not shown). Thus, although timeframe of response is an issue for Zn(II) sensing in a situation of changing Zn(II) concentration, the initial uptake of Zn(II) is quite rapid as shown by Job's plot studies (Figure 4).

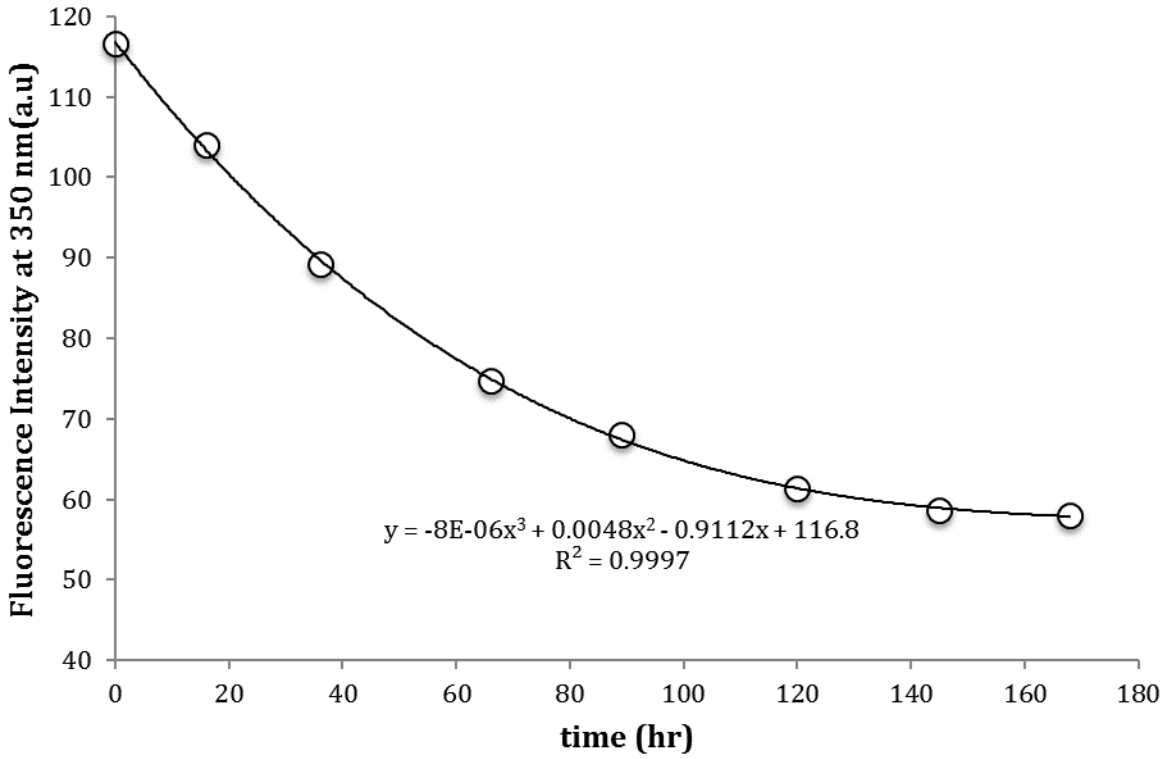



**Figure 7**: Binding constant of $[ZnTAMEisoquin]^{2+}$ in 0.1 M HEPES buffer, pH 7.2, at 350 nm. TPEN is used as a competing ligand and equilibrium is reached after one week.

*3.5. Metal-binding preference*

A screen of comparative response of Zn(II) to other mono- and divalent metal ions indicates the preference of TAMEisoquin toward Zn(II). The responses of TAMEisoquin to the metal ions alone were first determined (**Figure 8, black bars**). TAMEisoquin is used as a reference and the low fluorescence responses to dicationic metal ions of the first *d*-series, Mn(II) to Cu(II), are expected based on their quenching behavior. The weak responses of Na(I), K(I), and Ca(II) may be attributed to their very weak interactions with TAMEisoquin in accordance with hard-soft acid-base theory and therefore an inability to engage the CHEF mechanism. Although probes based on the PET/CHEF mechanism readily detect Cd(II), TAMEisoquin only induces a small enhancement (16% of the Zn(II) response) of emission intensity at 350 nm. In comparison, the emission intensity of cadmium complexes of N,N,N',N'-tetrakis(8-quinolylmethyl)ethylenediamine (TQEN), and the 1– or 3–isoquinolyl analogues are approximately 60% and <15% respectively of their corresponding zinc complexes.[15,33] In order to determine relative preferences of TAMEisoquin for Zn(II) against these metal ions, we introduced 1.0 equiv of Zn(II) to the solution containing TAMEisoquin and selected metal ions

(**Figure 8, red bars**). TAMEisoquin detects Zn(II) in the presence of one equivalent of Na(I), K(I), Ca(II) and Mn(II). The addition of Zn(II) to a solution containing 1.0 equiv of Fe(II), Cu(II), Co(II) or Ni(II) showed slight fluorescence enhancement, which can be ascribed to a small amount of displacement of these fluorescence–quenching *d*-metal ions by Zn(II). Moreover, with reference to TPEN, the relative affinity of Zn(II) for TAMEisoquin against these metal ions is as expected. Thus, the analysis of the fluorescence spectra (**Figure 8**) revealed that the order of binding affinity of TAMEisoquin to 3d-metal ions agrees with Irving-Williams series (Mn<Fe<Co<Cu>Zn).[34] The low emission intensity for the Cd(II)–TAMEisoquin interaction

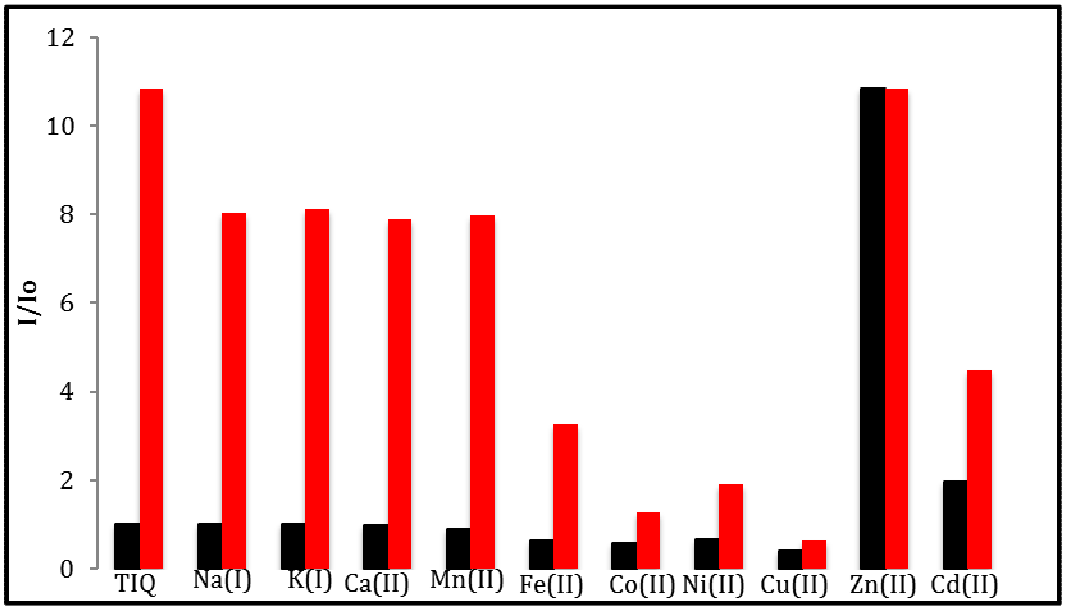



**Figure 8:** Preference of TAMEisoquin (TIQ) for Zn(II) over selected divalent metal ions in 0.1 M HEPES buffer at pH 7.2. The black bars represent the emission of TAMEisoquin in the presence of 1eq of divalent metal ions of interest. The red bars represent the emission when 1 eq of Zn(II) is added to each solution. The response I is normalized in reference to the free emission of the free ligand "TIQ" ($I_0$).

indicates that TAMEisoquin will detect Zn(II) with greater sensitivity than Cd(II).

Binding preference of TAMEisoquin for Zn(II) over Cd(II) is not definitively established in the present studies, however the response of $[Cd(II)(TAMEisoquin)]^{2+}$ challenged by $Zn^{2+}(aq)$ indicates that Cd(II) is partially displaced. This result in concert with the computational study of $[Cd(II)(TAMEisoquin)]^{2+}$ indicating considerable distortions in CdN6 coordination sphere, suggests that the poor fit of Cd(II) in TAMEisoquin leads to a decrease in the stability of

[Cd(II)(TAMEisoquin)]$^{2+}$ relative to complexes without the binding-pocket constraints of TAMEisoquin. It has been noted in the case of ligands that are sterically hindered near the metal coordination site, such as 2-methyl-1,10-phenanthroline, that the formation constants for Cd(II) are slightly larger than for Zn(II) (log $\beta_2$ (Cd(II)) = 9.68 vs. log $\beta_2$ (Zn(II)) = 9.35).[35] The present results suggest the formation constants of Zn(II) and Cd(II) for TAMEisoquin are on par, but further study is necessary to verify this and to elucidate factors that will improve Zn/Cd selectivity.

## 4. Conclusion

In this study, a novel zinc fluorescent sensor, TAMEisoquin was successfully synthesized. The X-ray crystallographic analysis of [Zn(TAMEisoquin)]2+ reveals four unique cations attributed to the flexibility of TAME framework. All four cations in the asymmetric unit show a distorted octahedral geometry. Photophysical and metal ion competition studies demonstrate Zn-binding stoichiometry of 1:1 and preference of Zn(II) over selected bioavailable metal ions. The hexadentate, preorganized design of TAMEisoquin confers a high stability for Zn(II) upon the sensor. The comparison of Zn(II) and Cd(II) responses confirms that TAMEisoquin exhibits a stronger fluorescence response to Zn(II) while a computational study showed that Cd(II) complex is more distorted than the Zn(II) complex but size selectivity for Zn(II) over Cd(II) is not yet definitely shown. Further studies will be directed to improving the aqueous solubility of the sensor and further modifying the ligand to improve preferences of Zn(II) relative to Cd(II).

## Acknowledgment

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## Appendix A. Supplementary data

CCDC No 1416945 contains the supplementary crystallographic data for [Zn(TAMEisoquin)]$(ClO_4)_2$. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data

Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or email: deposit@ccdc.can.ac.uk

**ASSOCIATED CONTENT**

Supporting information

1H NMR, 13C NMR and single crystal X-ray data

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**Notes**

**The authors declare no competing for financial interest**

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**Abstract**

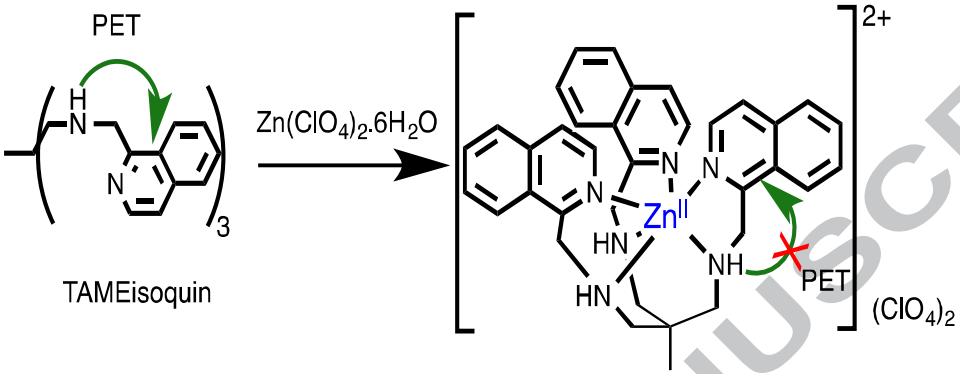


**Figure 1**: Synthesis of [Zn (TAMEisoquin)] $^{2+}$