# Highly Sensitive Fluorescent Probes for Zinc Ion Based on Triazolyl-Containing Tetradentate Coordination Motifs
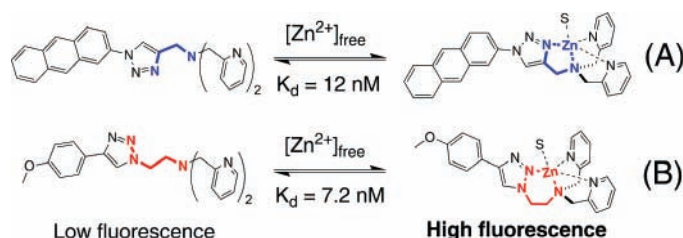
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**ABSTRACT**



Two new 1,2,3-triazolyl-containing N4-tetradentate ligands show nanomolar affinity for $Zn^{2+}$ under physiological conditions. Furthermore, they are easily derivatizable to afford fluorescent probes suitable for sensitive $Zn^{2+}$ detection. Structural characterizations in both solid and solution states suggest that N3 and N2 in the 1,2,3-triazolyl moiety coordinate with $Zn^{2+}$ in A and B to afford five-membered and six-membered coordination rings, respectively. The probes show sensitive fluorescence enhancement and ratiometric responses to $Zn^{2+}$.

Zinc ion ($Zn^{2+}$) is involved in many biochemical processes.[1–4] However, the exact roles, either structural or functional, of $Zn^{2+}$ are not entirely clear due to the lack of means for the accurate determination of spatiotemporal distributions of $Zn^{2+}$ in physiological events.[5] Three major challenges faced by $Zn^{2+}$ imaging in a physiological context are (1) the lack of spectroscopic signatures of $Zn^{2+}$ due to its $d^{10}$ full valence shell configuration;[6] (2) a very low abundance (below nanomolar) in the resting states of most cell types;[7] and (3) large spatiotemporal variations during physiological, especially neural, events.[8]

During the past decade, fluorescent probes for $Zn^{2+}$ have been developed to address the first challenge where the concentration-dependent fluorescence modulations of the probe molecules are used in the detection and quantification of $Zn^{2+}$ in physiological settings.[9–14] We initiated a research program to address the challenges imparted by low abundance and large dynamic range of physiological $Zn^{2+}$.[15,16] Herein, we report two new coordination motifs with nanomolar affinity for $Zn^{2+}$ that are easily derivatizable to afford sensitive fluorescent probes for $Zn^{2+}$ detection.

Nitrogen-rich tri- and tetradentate ligands such as tris(pyrazolyl)borate[17] and tris(2-pyridylmethyl)amine (TPA)[18] coordinate with $Zn^{2+}$ with high affinity. However, both tris(pyrazolyl)borate and TPA scaffolds are not highly adaptable

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to synthetic variations as evidenced by the few probe molecules derived from them.[19−21] In this study, the application of the versatile Cu(I)-catalyzed Huisgen cycloaddition reaction, also widely known as the "click" reaction,[22,23] offers easy synthetic access to highly sensitive fluorescent probes for $Zn^{2+}$ featuring coordinating 1,2,3-triazolyl moieties.

1,2,3-Triazolyl is a structural mimic of imidazolyl in histidine that binds $Zn^{2+}$ in proteins.[24] The 1,2,3-triazolyls resulting from the Cu(I)-catalyzed click reaction have been used extensively as linking units in projects ranging from bioconjugation[25] to surface sciences.[26] Recently, the coordination potential of 1,2,3-triazolyl has begun to be exploited.[27−30]

In this study, two tetradentate $Zn^{2+}$ coordination motifs were designed which were expected to chelate with $Zn^{2+}$ to afford a five-membered (Figure 1A)[31] and a six-membered
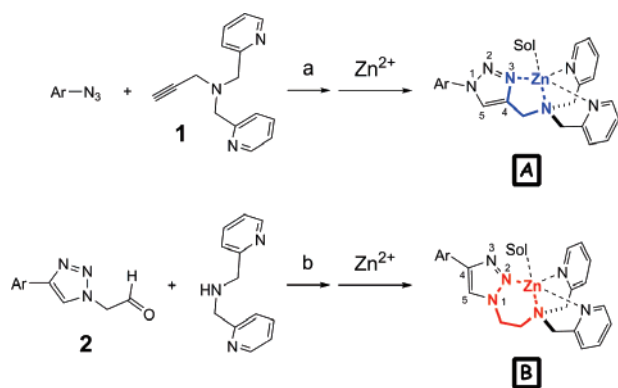


**Figure 1.** (a) *t*-BuOH, L-sodium ascorbate, $Cu(OAc)_2$, rt, 16 h; (b) i. 1,2-dichloroethane, 16 h, rt; ii. $NaBH(OAc)_3$, 4 h, Sol: solvent.

ring (Figure 1B), where N3 and N2 participate in binding, respectively. In addition to the conventional functions of 1,2,3-triazolyls as covalent linkers, in both designs, they serve as both chelating ligands to $Zn^{2+}$ and transmitters of a coordination event to the fluorophores (Ar in Figure 1).

The preparations of both motifs are outlined in Figure 1. Briefly, diazotization of arylamines affords arylazides which undergo the click reaction with **1** to afford molecules

containing motif A. Cu(I) and oxidized Cu(II) species in the reaction mixture can be easily separated from the chelating products by treating with EDTA during workup and subsequent alumina column chromatography. The click reaction between arylacetylene and 1-azidoacetaldehyde dimethylacetal furnishes 4-aryl-1,2,3-triazolyl acetaldehyde **2**. Reductive amination between **2** and di(2-picolyl)amine gives rise to probes featuring motif B.
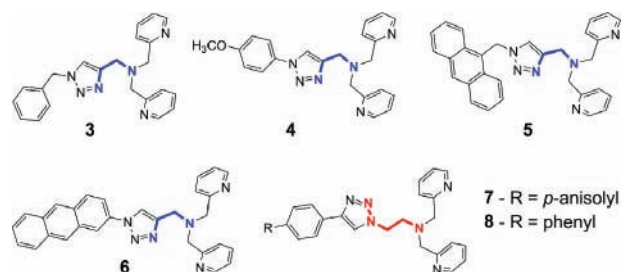
Compounds **3−6** and **7−8** (Figure 2) were expected to



**Figure 2.** Synthesized ligands **3−8**.

form five-membered and six-membered coordination rings with $Zn^{2+}$, respectively. The X-ray crystal structures of [Zn-(L)(AN)]$(ClO_4)_2$ (Figure 3, L: **3** or **4**, AN: acetonitrile)
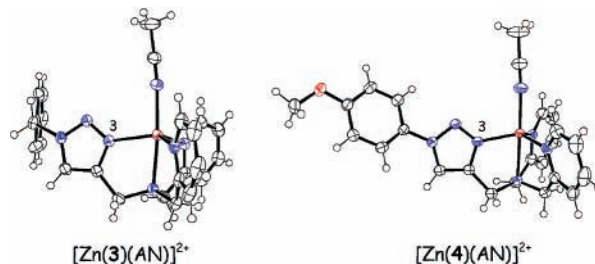


**Figure 3.** ORTEP diagrams (50% probability ellipsoids) of $Zn^{2+}$ complexes of **3** and **4**. Perchlorate counterions are omitted for clarity.

confirmed the N3-anchored five-membered coordination rings. The bond lengths of the triazolyl-N3 and pyridyl nitrogens to $Zn^{2+}$ are in the range of 2.02−2.04 Å, similar to those observed in the TPA-based $Zn^{2+}$ complexes.[18,32]

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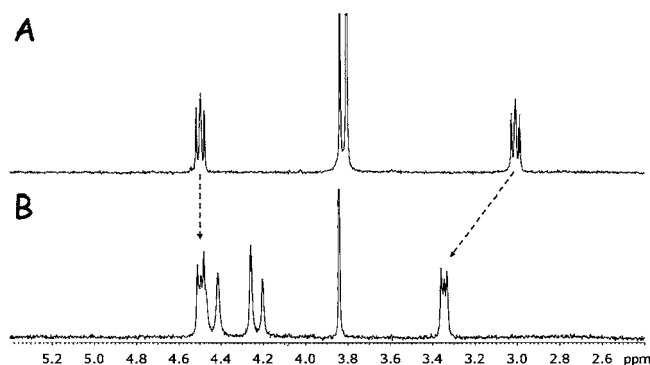
The ^{1}H NMR binding study of **7** (Figure 4) reveals that,



**Figure 4.** ^{1}H NMR (acetonitrile-$d_3$, 300 MHz) of (A) **7** and (B) Zn(**7**)(OTf)$_2$ from 2.4 to 5.4 ppm.

upon coordinating Zn$^{2+}$ in acetonitrile-$d_3$, the two triplets assigned to the CH$_2$ adjacent to the tertiary nitrogen and the CH$_2$ adjacent to N1 of the triazolyl ring (4.50 and 3.01 ppm, Figure 4A), respectively, transform into two multiplets (4.50 and 3.35 ppm, Figure 4B), an indication of restricted rotation as a result of the triazolyl coordination to Zn$^{2+}$. The 3.01 ppm triplet of the free ligand of **7** undergoes downfield shift to 3.35 ppm upon Zn$^{2+}$ coordination due to deshielding from the coordinated tertiary nitrogen. The triplet at 4.50 ppm is not affected. Similar ^{1}H NMR response was obtained for **8**. Furthermore, the affinity of **7** for Zn$^{2+}$ (nanomolar, see below) is much higher than that of ligands containing the tridentate di(2-picolyl)amino group (micromolar).[33] These observations are consistent with the coordination between the triazolyl group and Zn$^{2+}$ in motif B. Further structural studies including X-ray crystallography are underway to confirm that the exact loci of the association are the N2 of the triazolyl groups.

The fluorescence quantum yields ($\Phi_F$, Table 1) of **4**−**7**

**Table 1.** Dissociation Constants ($K_d$) between Ligands **4**−**7** and Zn$^{2+}$ and Fluorescence Quantum Yields ($\Phi_F$)

| ligand | 4 | 5 | 6 | 7 |
|---|---|---|---|---|
| $K_d$/nM[a] | $3.5 \pm 0.2$ | $5.7 \pm 1.1$ | $12 \pm 1.7$ | $7.2 \pm 0.4$ |
| $\lambda_{em}$/nm[b] | 404 | 418, 440 | 462 | 450 |
| $\Phi_F$ (ligand)[c] | 0.0026 | 0.29 | 0.018 | 0.0013 |
| $\Phi_F$ (complex)[c] | 0.017 | 0.45 | 0.14 | 0.0036 |

[a] Dissociation constants ($K_d$) were determined in water (HEPES: 50 mM, pH 7.2; KNO$_3$: 100 mM, EGTA: 10 mM). [b] $\lambda_{em}$: emission maxima. [c] Fluorescence quantum yields ($\Phi_F$) were determined in water (HEPES: 50 mM, pH 7.2; KNO$_3$: 100 mM).

were enhanced upon coordinating Zn$^{2+}$ under simulated physiological conditions (pH 7.2, $I = 0.1$). Compounds **4**

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and **6**, whose aromatic groups are directly attached to the triazolyl groups, show larger enhancements (6.5- and 7.8-fold in $\Phi_F$, respectively) upon Zn$^{2+}$ coordination than **5** (1.6-fold in $\Phi_F$), where the 9-anthryl group is one methylene away from the triazolyl group. **7** underwent both fluorescence enhancement (2.8-fold in $\Phi_F$) and emission band bathochromic shift upon Zn$^{2+}$ association. The fluorescence enhancements of **4**−**6** may be due to chelation-enhanced fluorescence by shutting down the photoinduced electron-transfer pathway of the excited free ligand upon Zn$^{2+}$ coordination.[34]

The dissociation constants ($K_d$) of the Zn$^{2+}$ complexes of **4**−**7** were determined by fitting the fluorescence titration data with a 1:1 association equation (Supporting Information), as shown in Figure 5B and D for **6** and **7**, which
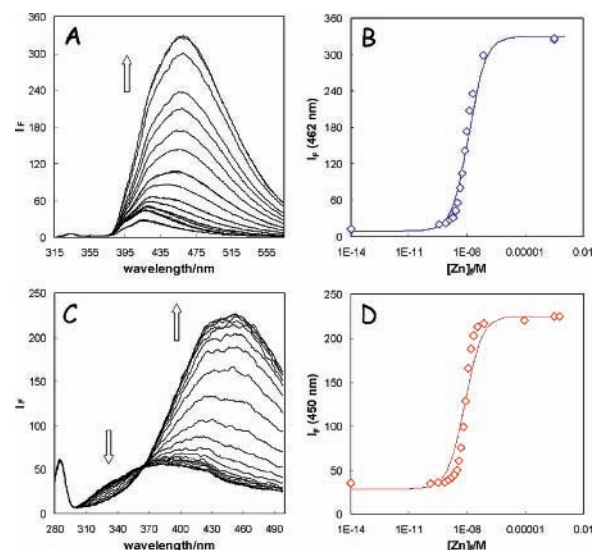


**Figure 5.** (A) Fluorescence spectra ($\lambda_{ex} = 300$ nm) of **6** (4.9 μM) in H$_2$O (HEPES: 50 mM, pH 7.2; KNO$_3$: 100 mM; EGTA: 10 mM) with incremental addition of zinc triflate (0−10 mM) at 25 °C. (B) Diamonds: Fluorescence intensity at 462 nm vs free Zn$^{2+}$ concentration ([Zn]$_f$); line: curve fit using a 1:1 association equation. (C) Fluorescence spectra ($\lambda_{ex} = 260$ nm) of **7** (4.6 μM) in H$_2$O (HEPES: 50 mM, pH 7.2; KNO$_3$: 100 mM; EGTA: 10 mM) with incremental addition of zinc triflate (0−10 mM) at 25 °C. (D) Diamonds: Fluorescence intensity at 450 nm vs [Zn]$_f$; line: curve fit using a 1:1 association equation. $I_F$: fluorescence intensity in arbitrary units.

represent probes containing motifs A and B, respectively. The affinities, hence the sensitivity, of all the compounds fall into the nanomolar range (Table 1),[35] which is within the basal level of intracellular free Zn$^{2+}$ concentrations.[36] Not only was the fluorescence intensity of both probes

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enhanced upon binding but their emission maxima underwent bathochromic shifts (from 416 to 462 nm for **6**, and from 380 to 450 nm for **7**). Therefore, they can also be used in ratiometric modes.[14]

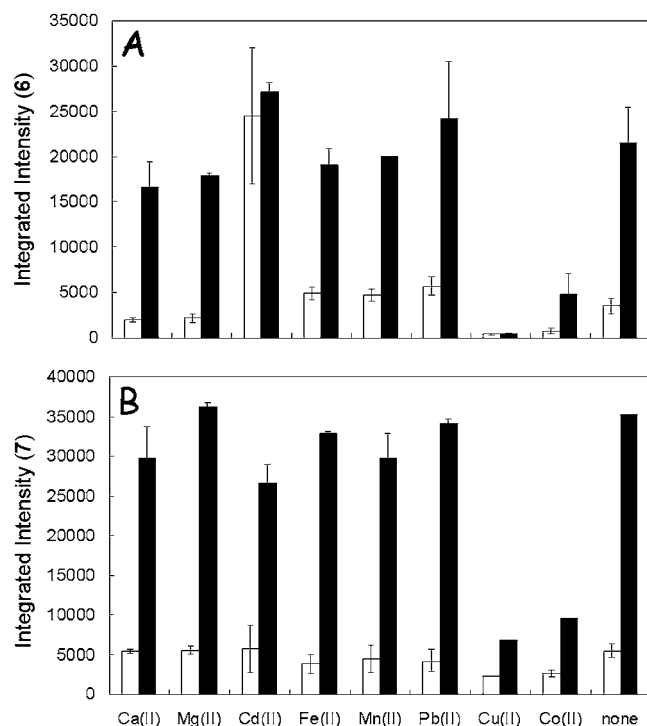The metal ion selectivity of **6** and **7** is shown in Figure 6



**Figure 6.** Fluorescence response of (A) **6** (2.5 $\mu$M, $\lambda_{ex}$ = 300 nm) and (B) **7** (2.3 $\mu$M, $\lambda_{ex}$ = 263 nm) to divalent metal ions (4.5 $\mu$M) in the absence of $Zn^{2+}$ (white bars) and in the presence of $Zn^{2+}$ (5.0 $\mu$M, black bars). All samples were measured in $H_2O$ (HEPES: 50 mM, pH 7.2; $KNO_3$: 100 mM) at 25 °C. *Y*-axes represent the integrated fluorescence intensity in arbitrary units from (A) 360 to 575 nm and (B) 300 to 500 nm, respectively.

as the dependence of integrated fluorescence intensity of the major emission bands upon $Zn^{2+}$ coordination. **6** shows selectivity toward $Zn^{2+}$ over other divalent ions such as $Ca^{2+}$, $Mg^{2+}$, $Fe^{2+}$, $Mn^{2+}$, and $Pb^{2+}$ (Figure 6A). Cadmium ($Cd^{2+}$) coordinates with **6** strongly to result in a large fluorescence enhancement. Due to the large abundance of $Ca^{2+}$ and $Mg^{2+}$ in physiological contexts, fluorescence enhancement of **6** upon $Zn^{2+}$ coordination was studied in the presence of 5.0 mM $Ca^{2+}$ and $Mg^{2+}$ where $Ca^{2+}$ and $Mg^{2+}$ again showed

negligible interference (Figure S8). $Cu^{2+}$ and $Co^{2+}$ quench the fluorescence of **6**, likely due to the metal-to-ligand electron transfer upon excitation.[37,38] Monovalent $Na^+$ and $K^+$ were present in the buffer.[39] They did not interfere with the association of the probes with divalent metal ions and the subsequent fluorescence modulations.

Compound **7**, which coordinates $Zn^{2+}$ to form a six-membered ring, shows improved selectivity over that of **6** toward $Zn^{2+}$ (Figure 6B). For all the divalent metal ions tested, only $Cu^{2+}$ and $Co^{2+}$ are able to coordinate with **7** *and* quench its fluorescence. Notably, **7** shows selectivity to $Zn^{2+}$ over $Cd^{2+}$—a known stereoelectronic isostere of $Zn^{2+}$. This observation is consistent with the theoretical prediction that large ions are disfavored to form six-membered coordination rings due to increased ring strain energy.[40]

In summary, (1) 1,2,3-triazolyl-containing fluorescent probes to $Zn^{2+}$ with nanomolar sensitivity were designed and synthesized. (2) Structural characterizations in both solid and solution states support the hypothesis that N3 and N2 in the 1,2,3-triazolyl coordinate with $Zn^{2+}$ in motifs A and B, respectively. (3) Both ligand motifs show nanomolar affinity to $Zn^{2+}$. (4) **6** and **7** show both fluorogenic and ratiometric responses upon binding $Zn^{2+}$ under physiological conditions. (5) Both ligand motifs show coordination selectivity for $Zn^{2+}$ over most other divalent metal ions. Further studies including (a) the elucidation of the photophysical origins of the coordination-dependent fluorescence modulations and (b) the construction of probes incorporating motifs A and B with both excitation and emission wavelengths in the visible region to protect biological sample from photodamage upon UV irradiation are underway.

**Supporting Information Available:** Experimental details of syntheses and spectroscopic studies. Additional spectroscopic data and .cif file of $[Zn(L)(AN)](ClO_4)_2$ (L = **3** and **4**). This material is available free of charge via the Internet at http://pubs.acs.org.

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