Inorganic Chemistry

Article

pubs.acs.org/IC

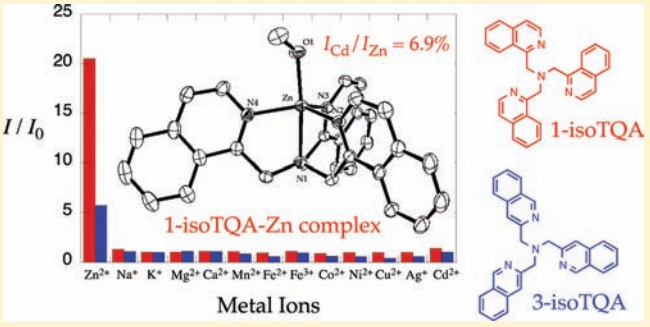
# Zinc-Specific Fluorescent Response of Tris(isoquinolylmethyl)amines (isoTQAs)

Yuji Mikata,[†,*] Keiko Kawata,[‡] Satoshi Iwatsuki,[§] and Hideo Konno[⊥]

[†]*KYOUSEI* Science Center and [‡]Department of Chemistry, Faculty of Science, Nara Women's University, Nara 630-8506, Japan,
[§]Department of Chemistry of Functional Molecules, Faculty of Science and Engineering, Konan University, 8-9-1 Okamoto, Higashinada, Kobe 658-8501, Japan, and
[⊥]National Institute of Advanced Industrial Science and Technology (AIST), 1-1-1 Higashi, Tsukuba, Ibaraki 305-8565, Japan

Supporting Information

**ABSTRACT:** Isoquinoline-based tetradentate ligands with $C_3$-symmetry, tris(1- or 3-isoquinolylmethyl)amine (1- or 3-isoTQA), have been prepared and their zinc-induced fluorescence enhancement was investigated. Upon excitation at 324 nm, 1-isoTQA shows very weak fluorescence ($\phi$ = ~0.003) in DMF/$H_2O$ (1/1) solution. In the presence of zinc ion, 1-isoTQA exhibits fluorescence increase ($\phi$ = 0.041) at 359 and 470 nm. This fluorescence enhancement at 470 nm is specific for zinc. However, 3-isoTQA exhibited a smaller fluorescence enhancement upon zinc complexation ($\phi$ = 0.017, $\lambda_{em}$ = 360 and 464 nm) compared with 1-isoTQA. Crystal structures of zinc complexes of isoTQAs demonstrate the diminished steric crowding and shorter Zn−$N_{aromatic}$ distances compared with isoTQENs (*N,N,N′,N′*-tetrakis(isoquinolylmethyl)ethylenediamines) leads to a higher fluorescent response toward zinc relative to cadmium.



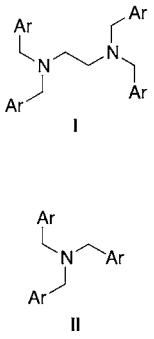

## INTRODUCTION

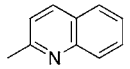
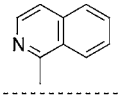
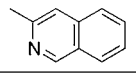
Zinc ions are indispensable metal ions in living systems. Zinc plays many important structural and catalytic roles in enzymes and is one of the key components in cellular processes including gene expression and signal transduction.[1−5] Mobile zinc pools that exist in living cells in many organisms but their exact roles are still not fully understood. Fluorescent zinc sensor molecules have been extensively developed in recent years, aiming at the visualization and/or quantification of the dynamic and transient zinc ion distribution inside the cell.[4−29] Although several well-known mechanisms altering the fluorescence properties of the probe molecules upon guest binding have been established,[30−32] there is still a need for rational design strategies for specific metal ions recognition in high selectivity and appropriate sensitivity.

We have previously reported that *N,N,N′,N′*-tetrakis(2-quinolylmethyl)ethylenediamine (TQEN) can act as a fluorescent zinc detection molecule (Chart 1).[33] The structure of TQEN is based on well-known heavy metal chelator, *N,N,N′,N′*-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN). The 1- and 3-isoTQEN, isoquinoline derivatives of TQEN (Chart 1), exhibited improved properties compared to TQEN.[34] In recent years, many other quinoline-based fluorescent zinc sensors have been developed;[34−58] however, very few isoquinoline-based fluorescent sensor molecules have been developed.[59]

TQA, tris(2-quinolylmethyl)amine, is an analogue of TPA, tris(2-pyridylmethyl)amine and was first prepared by Karlin and co-workers to investigate the dioxygen activation chemistry by its copper complex (Chart 1).[60,61] Subsequently, Hancock and co-workers reported the zinc specific fluorescent response of TQA and discussed its fluorescent intensity difference between zinc(II) and cadmium(II) complexes based on the differential CHEF (chelation enhanced fluorescence) effect that is mainly controlled by steric effects upon complexation.[54] TQA exhibits lower cadmium response ($I_{Cd}/I_{Zn}$) compared with TQEN because of reduced steric hindrance that allows efficient CHEF mechanism in TQA−Zn complex.

**Chart 1**




| —Ar | Structure | Compound |
|---|---|---|
|  | I | TPEN |
| | II | TPA |
|  | I | TQEN |
| | II | TQA |
|  | I | 1-isoTQEN |
| | II | 1-isoTQA |
|  | I | 3-isoTQEN |
| | II | 3-isoTQA |

**Received:** October 5, 2011
**Published:** January 19, 2012

   dx.doi.org/10.1021/ic202159v | *Inorg. Chem.* 2012, 51, 1859−1865

In this article, we report the zinc-induced fluorescence enhancement of isoquinoline derivatives of TQA, 1- and 3-isoTQA (tris(1- or 3-isoquinolylmethyl)amines) (Chart 1). 3-IsoTQA has been prepared by Canary and co-workers and its zinc-binding property was investigated by ESI-MS;[62] however, no fluorescent response toward zinc has been reported to date. This article reports the synthesis and evaluation of the fluorescent properties of 1- and 3-isoTQA as a new scaffold for fluorescent zinc sensor molecules. Reduced steric hindrance of isoTQA–Zn complexes suppresses the cadmium response ($I_{Cd}/I_{Zn}$) compared with isoTQENs, in parallel with the TQA/TQEN system.

## EXPERIMENTAL SECTION

**General.** All reagents and solvents used for synthesis were from commercial sources and used as received. *N*,*N*-Dimethylformamide (DMF, Dojin) was spectral grade (Spectrosol). All aqueous solution was prepared using Mili-Q water (Milipore). $^1$H NMR (300 Hz) and $^{13}$C NMR (75.5 Hz) spectra were recorded on a Varian GEMINI 2000 spectrometer and referenced to internal $Si(CH_3)_4$ or solvent signals. UV–vis and fluorescence spectra were measured on a Jasco V-660 spectrophotometer and Jasco FP-6300 spectrofluorometer, respectively. Fluorescence quantum yields were measured on a HAMAMATSU photonics C9920–02 absolute PL quantum yield measurement system. *CAUTION: Perchlorate salts of metal complexes with organic ligands are potentially explosive. All due precautions should be taken.*

***N*-(1-isoquinolylmethyl)phthalimide (2).** To the DMF solution (11 mL) of 1-chloromethylisoquinoline[34,63] (**1**) (265 mg, 1.49 mmol) was added potassium phthalimide (278 mg, 1.50 mmol) and stirred overnight at room temperature. After addition of chloroform, the organic layer was washed with water and 10% $NaOH_{aq}$. The organic layer was dried, evaporated, and washed with hot ethanol to give **2** as white powder. Yield, 303 mg (1.05 mmol, 70%).

$^1$H NMR ($CDCl_3$): $\delta$ 8.31 (d, $J$ = 5.7 Hz, 1H), 8.21 (d, $J$ = 7.8 Hz, 1H), 7.90–7.93 (m, 2H), 7.83 (d, $J$ = 8.4 Hz, 1H), 7.65–7.76 (m, 4H), 7.52 (d, $J$ = 5.7 Hz, 1H), 5.54 (s, 2H).

$^{13}$C NMR ($CDCl_3$): $\delta$ 168.5, 153.0, 141.9, 136.1, 133.9, 132.5, 130.1, 127.5, 125.9, 123.7, 123.5, 120.3, 40.7.

Anal. Calcd for $C_{18}H_{12}N_2O_2$ (**2**): H, 4.20; C, 74.99; N, 9.72. Found: H, 4.19; C, 74.85; N, 9.65.

**1-Aminomethylisoquinoline (3).** To the methanol solution (11 mL) of *N*-(1-isoquinolylmethyl)phthalimide (**2**) (132 mg, 0.46 mmol) was added hydrazine monohydrate (0.38 mL, 7.8 mmol) and refluxed for 1.5 h. After addition of water, the insoluble materials were filtered off. The filtrate was acidified with hydrochloric acid and filtered. The filtrate was neutralized with $NaOH_{aq}$ and extracted with ethyl acetate. The organic layer was dried and evaporated to give **3** as yellow oil. Yield, 46 mg (0.29 mmol, 63%).

$^1$H NMR ($CDCl_3$): $\delta$ 8.45 (d, $J$ = 5.4 Hz, 1H), 8.08 (d, $J$ = 8.1 Hz, 1H), 7.81 (d, $J$ = 7.8 Hz, 1H), 7.50–7.70 (m, 3H), 4.49 (s, 2H).

$^{13}$C NMR ($CDCl_3$): $\delta$ 159.9, 141.2, 135.7, 129.7, 127.1, 127.0, 125.7, 123.8, 119.6, 44.6.

**Tris(1-isoquinolylmethyl)amine (1-isoTQA).** To the acetonitrile solution (90 mL) of 1-chloromethylisoquinoline (**1**) (501 mg, 2.82 mmol) and 1-aminomethylisoquinoline (**3**) (223 mg, 1.41 mmol) was added potassium carbonate (1.05 g, 7.60 mmol) and stirred for 4 days under reflux. After removal of the solvent, the residue was extracted with chloroform/water. The organic layer was dried, evaporated, and washed with acetonitrile to give 1-isoTQA as white powder. Yield, 170 mg (0.39 mmol, 27%).

$^1$H NMR ($CDCl_3$): $\delta$ 8.49 (d, $J$ = 5.7 Hz, 3H), 7.75 (d, $J$ = 8.4 Hz, 3H), 7.58 (d, $J$ = 6.0 Hz, 3H), 7.47 (dd, $J$ = 6.9, 8.1 Hz, 3H), 6.98 (d, $J$ = 8.7 Hz, 3H), 6.58 (dd, $J$ = 6.9, 8.4 Hz, 3H), 4.35 (s, 6H).

$^{13}$C NMR ($CDCl_3$): $\delta$ 158.0, 141.4, 136.0, 129.5, 127.3, 126.5, 125.9, 120.6, 60.0.

Anal. Calcd for $C_{30}H_{24.4}N_4O_{0.2}$ (1-isoTQA·0.2$H_2O$): H, 5.54; C, 81.13; N, 12.61. Found: H, 5.33; C, 80.98; N, 12.53.

**[Zn(1-isoTQA)($H_2O$)]($ClO_4$)$_2$.** In a chloroform suspension of 1-isoTQA was added equimolar amount of $Zn(ClO_4)_2·6H_2O$ in methanol, and the solution was kept at room temperature afforded white powder. Yield, 40%.

$^1$H NMR (DMSO-$d_6$): $\delta$ 8.59 (d, $J$ = 6.3 Hz, 3H), 8.28 (d, $J$ = 8.4 Hz, 3H), 8.09 (d, $J$ = 8.1 Hz, 3H), 8.03 (d, $J$ = 6.0 Hz, 3H), 7.92 (dd, $J$ = 7.2, 7.8 Hz, 3H), 7.83 (dd, $J$ = 6.9, 7.5 Hz, 3H), 5.32 (s, 6H).

$^{13}$C NMR (DMSO-$d_6$): $\delta$ 155.6, 138.0, 136.0, 132.6, 128.8, 127.4, 125.3, 124.9, 122.3, 60.4.

Anal. Calcd for $C_{30}H_{27}Cl_2N_4O_{9.5}Zn$ ([Zn(1-isoTQA)($H_2O$)]-($ClO_4$)$_2$·0.5$H_2O$): H, 3.72; C, 49.23; N, 7.66. Found: H, 3.52; C, 48.98; N, 7.59.

**[Zn(3-isoTQA)($H_2O$)]($ClO_4$)$_2$.** In a chloroform suspension of 3-isoTQA was added equimolar amount of $Zn(ClO_4)_2·6H_2O$ in methanol, and the solution was kept at room temperature afforded white powder. Yield, 40%.

$^1$H NMR ($CD_3OD$): $\delta$ 9.50 (s, 3H), 8.21 (d, $J$ = 8.1 Hz, 3H), 7.99 (s, 3H), 7.95 (d, $J$ = 8.1 Hz, 3H), 7.88 (dd, $J$ = 6.6, 7.2 Hz, 3H), 7.74 (dd, $J$ = 7.2, 7.8 Hz, 3H), 4.56 (s, 6H).

$^{13}$C NMR ($CD_3OD$): $\delta$ 153.9, 147.3, 138.6, 135.1, 130.4, 130.0, 129.7, 127.8, 123.0, 59.5.

Anal. Calcd for $C_{30}H_{29}Cl_2N_4O_{10.5}Zn$ ([Zn(3-isoTQA)($H_2O$)]($ClO_4$)$_2$·1.5$H_2O$): C, 48.05; H, 3.90; N, 7.47. Found: C, 47.69; H, 3.56; N, 7.67.

**X-ray Crystallography.** Single crystals of 1-isoTQA were obtained from $CH_3OH$ at 4 °C. Single crystals of [Zn(1-isoTQA)($CH_3OH$)]-($ClO_4$)$_2$·2$CH_3OH$ and [Zn(3-isoTQA)($H_2O$)]($ClO_4$)$_2$·2$CHCl_3$ were obtained by recrystallization from $CHCl_3$–$CH_3OH$ (1:1) at 4 °C under ether diffusion condition. These crystals were covered by Paraton-N oil and mounted on a glass fiber. All data were collected at 123 K on a Rigaku Mercury CCD detector, with monochromatic MoK$\alpha$ radiation, operating at 50 kV/40 mA. Data were processed on a PC using *CrystalClear* software (Rigaku). Structures were solved by direct methods (SIR-92)[64] and refined by full-matrix least-squares methods on $F^2$ (SHELXL-97).[65] Crystal data are summarized in Table S1 of the Supporting Information. CCDC-846307–846309 contain the supplementary crystallographic data for this article. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/datarequest/cif.

## RESULTS AND DISCUSSION

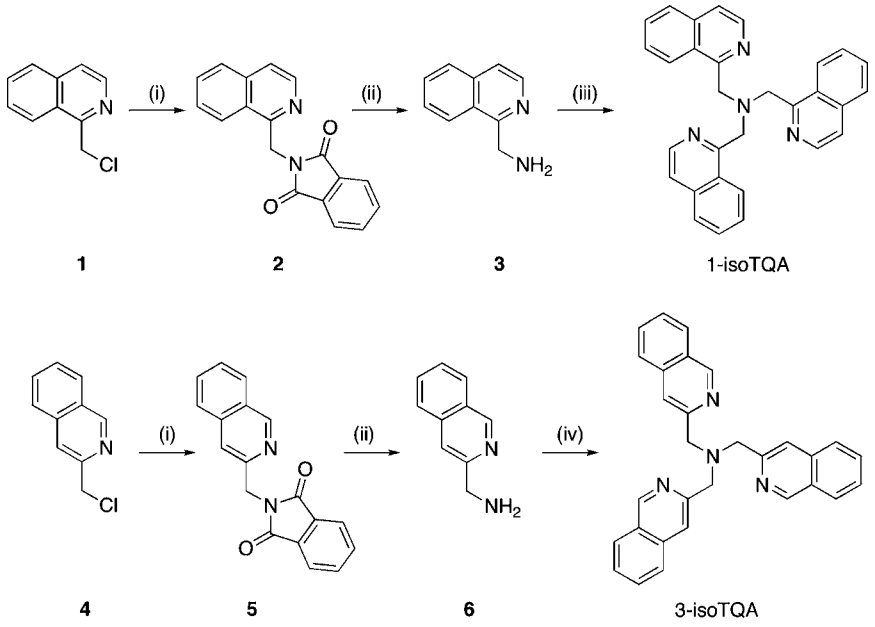
**Ligand Synthesis.** 1-IsoTQA and 3-isoTQA[62] were synthesized from corresponding chloromethylisoquinolines via Gabriel amine synthesis followed by N-alkylation with 2 equiv of chloromethylisoquinoline (Scheme 1). All new compounds were characterized by $^1$H and $^{13}$C NMR, and the purity of the final compounds was ensured by elemental analysis. X-ray crystallography further confirms the structure of 1-isoTQA in the crystalline state (Figure 1).

**UV–vis and Fluorescence Spectral Changes of 1-isoTQA Induced by Zinc.** A 34 $\mu$M solution in aqueous DMF (DMF/$H_2O$ = 1:1) at 25 °C was used for spectral measurements for 1-isoTQA. Upon addition of zinc ion, the UV–vis ligand absorption at 324 nm decreased and new peaks at 315 and 325 nm appeared (part a of Figure 2). Distinct isosbestic points were seen at 313, 318, and 327 nm during the titration and spectral changes stopped at the point where 1 eq of zinc ion was added, indicating the exclusive formation of 1:1 complex for 1-isoTQA and the zinc ion (part a of Figure S1 of the Supporting Information).

Part b of Figure 2 shows the fluorescence spectral change of 1-isoTQA with increasing amount of zinc ion added. Although 1-isoTQA emits negligible fluorescence in the absence of zinc ion upon excitation at 324 nm, the fluorescence increased at 359 (49-fold) and 470 nm (21-fold) respectively in the presence of 1 equiv of zinc ion. The fluorescence quantum yield of zinc complex of 1-isoTQA ($\phi$ = 0.041) is higher than that of 1-isoTQEN–Zn complex ($\phi$ = 0.034). On the bases of the number of aromatic rings in 1-isoTQA (*tris*isoquinoline) and


dx.doi.org/10.1021/ic202159v | *Inorg. Chem.* 2012, 51, 1859–1865

**Scheme 1. Synthesis of 1- and 3-isoTQA**[a]




[a]Reagents: (i) potassium phthalimidate; (ii) hydrazine; (iii) **1** (2 equiv), $K_2CO_3$; (iv) **4** (2 equiv), $NaHCO_3$.

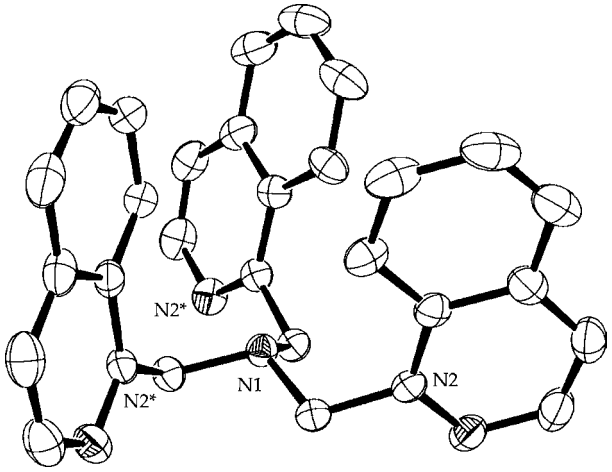



**Figure 1.** ORTEP plot for 1-isoTQA in 50% probability. Asterisks indicate the atoms generated by the symmetric operation. Hydrogen atoms were omitted for clarity.

1-isoTQEN (*tetrakis*isoquinoline), 1-isoTQA is a more efficient fluorophore (1.6-fold based on each isoquinoline ring) in comparison to 1-isoTQEN. The fluorescence increase stopped at the point where 1 eq of zinc ion was added, supporting the formation of fluorescent 1:1 ZnL complex (part b of Figure S1 of the Supporting Information).

**UV–vis and Fluorescence Spectral Changes of 3-isoTQA Induced by Zinc.** Addition of zinc ion to the solution of 3-isoTQA in DMF/$H_2O$ (1:1) changes UV–vis and fluorescence spectra (Figure 3). In the absorption spectra, $\lambda_{max}$ was changed from 314 and 324 nm to 318 and 328 nm and isosbestic points were observed at 313, 323, and 326 nm. Upon excitation at 324 nm, free ligand exhibited negligible fluorescence, and addition of zinc enhances the intensity at 360 and 464 nm, 9- and 6-fold respectively. The zinc-induced spectral changes in both spectra stopped after 1 eq of zinc was added (Figure S2 of the Supporting Information). The fluorescent quantum yield ($\phi$) of the 3-isoTQA–Zn complex (0.017) is less than half of the 1-isoTQA–Zn complex (0.041) (Figure S3 of the Supporting Information). This is in good agreement with our previous results in which 1-isoTQEN–Zn complex exhibited a higher quantum yield in comparison to the 3-isoTQEN–Zn complex.[34] This point is discussed further based on the crystal structure analysis (below).

**X-ray Crystallography of the Zinc Complexes of 1- and 3-isoTQA.** Single crystals of the zinc complexes of both 1- and 3-isoTQA were obtained from methanol/chloroform (1:1) as a perchlorate salt and analyzed by X-ray crystallography. Crystal data are summarized in Table S1 of the Supporting Information. Figures 4 and 5 show the crystal structures of the cations of $[Zn(1\text{-isoTQA})(CH_3OH)](ClO_4)_2{\cdot}2CH_3OH$ and $[Zn(3\text{-isoTQA})(H_2O)](ClO_4)_2{\cdot}2CHCl_3$. Table S2 of the Supporting Information lists the bond distances around zinc ion.

The zinc complexes of 1- and 3-isoTQA adopt trigonal bipyramidal geometry ($\tau$ = 0.76 for 1-isoTQA–Zn complex and 0.95 for 3-isoTQA–Zn complex) and no significant steric hindrance is found in the interatomic distances and angles around the zinc center. Between 1- and 3-isoTQA–Zn complexes, there are considerable differences in the coordination distances between the zinc ion and isoquinoline nitrogen atoms. The average $Zn-N_{aromatic}$ bond length is 2.045 Å for the 1-isoTQA–Zn complex and 2.080 Å for the 3-isoTQA–Zn complex. The former value is comparable with that of $[Zn(TPA)(CH_3CN)](ClO_4)_2$, 2.0475 Å.[66] This may account for the difference in the fluorescence intensity of the zinc complexes with 1- and 3-isoTQA. In addition, both isoTQA complexes have shorter $Zn-N_{aromatic}$ distances than 1-isoTQEN (2.1506 Å) and 3-isoTQEN (2.1590 Å).[34] These differences are well correlated with the fluorescence intensity of the zinc complexes, affording efficient CHEF in the more tightly bound complexes employing the isoTQA ligand framework (Table 1).

**Fluorescence Spectral Change of 1- and 3-isoTQA Induced by Other Metal Ions.** Figure 6 shows the metal ion specificity of the fluorescent response of 1- and 3-isoTQA


dx.doi.org/10.1021/ic202159v | *Inorg. Chem.* 2012, 51, 1859–1865

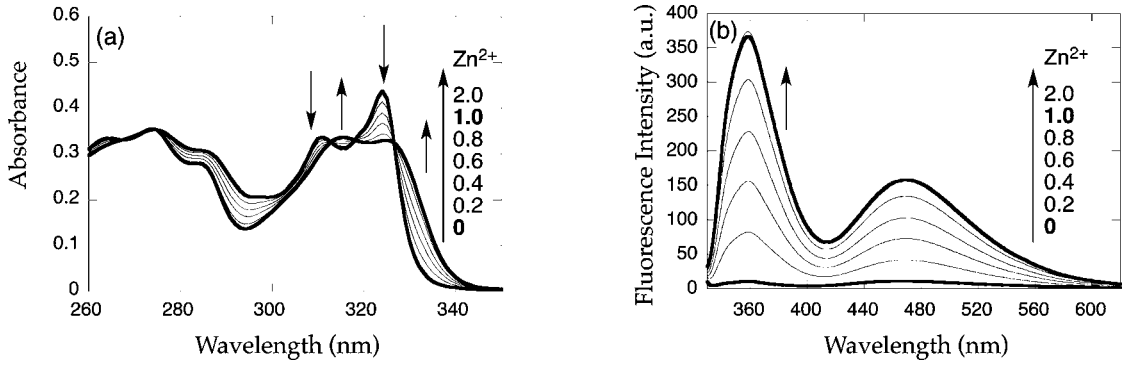



**Figure 2.** (a) UV−vis absorption and (b) fluorescence ($\lambda_{ex}$ = 324 nm) spectra of 34 $\mu$M 1-isoTQA in DMF/$H_2O$ (1:1) at 25 °C in the presence of various concentration of $Zn^{2+}$ ranging from 0 to 68 $\mu$M.

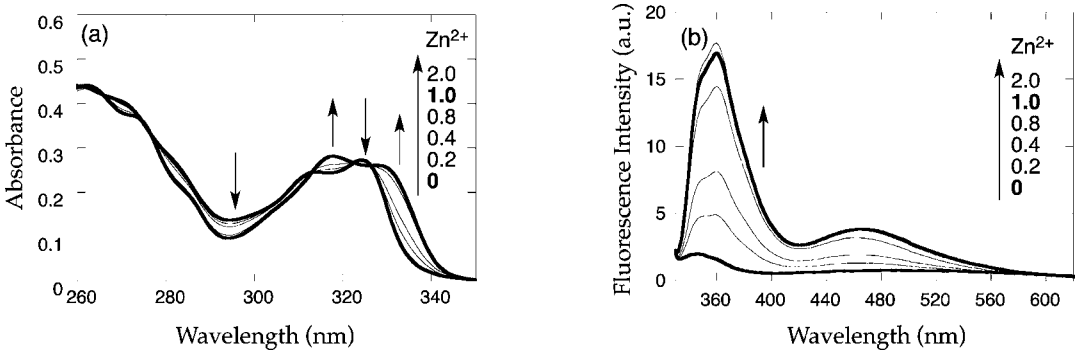



**Figure 3.** (a) UV−vis absorption and (b) fluorescence ($\lambda_{ex}$ = 324 nm) spectra of 34 $\mu$M 3-isoTQA in DMF/$H_2O$ (1:1) at 25 °C in the presence of various concentration of $Zn^{2+}$ ranging from 0 to 68 $\mu$M.

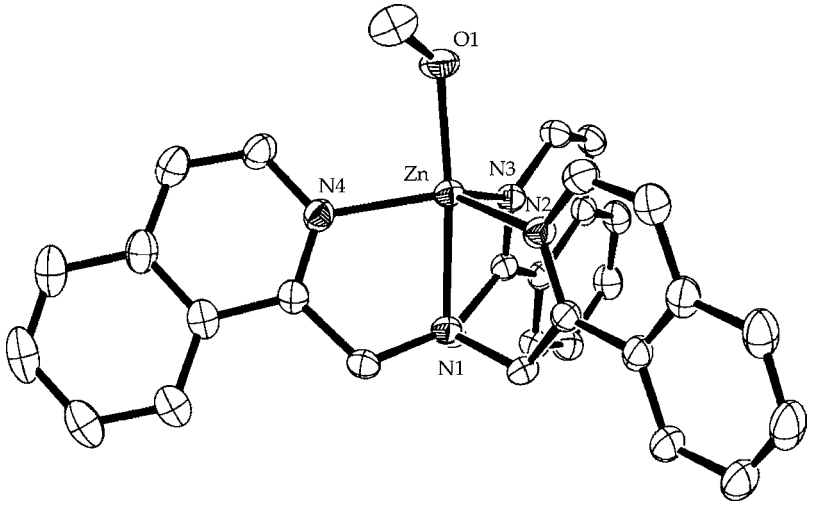



**Figure 4.** ORTEP plot for $[Zn(1\text{-isoTQA})(CH_3OH)](ClO_4)_2{\cdot}2CH_3OH$ in 50% probability. Atoms of counteranions, hydrogen, and solvents were omitted for clarity.

monitored at 470 and 464 nm, respectively. The fluorescence enhancement was specific for the zinc ion. Cadmium exhibited moderate fluorescence enhancement with 1-isoTQA at short wavelength (~360 nm), however, negligible response at lower energy emission (~470 nm) was observed (Figure S4 of the Supporting Information). The difference in radius between $Cd^{2+}$ (0.96 Å) and $Zn^{2+}$ (0.74 Å), and thus the overall structure, alters the CHEF effect on the isoTQA chromophores especially the isoquinoline−isoquinoline interaction that generates the emission at ca. 470 nm. Interestingly, the emission spectrum of the 1-isoTQA−Cd complex is similar to that of the 3-isoTQA−Zn complex except for the long-wavelength emission in the 3-isoTQA−Zn complex (Figures S3 and S4 of the Supporting Information). For the Zn and Cd complexes, the CHEF effect on the isoquinoline chromophore is similar, but the inter-isoquinoline interaction to generate the ~470 nm fluorescence is absent for cadmium complex.

In the presence of an equimolar amount of $Fe^{2+}$, $Co^{2+}$, $Ni^{2+}$, $Cu^{2+}$, and $Cd^{2+}$, the afterward addition of zinc did not enhance the fluorescence of 1- and 3-isoTQA. On the other hand, the fluorescence of 1-isoTQA−Zn complex was scarcely affected by following addition of 1eq of any metals listed in Figure 6 in several minutes.


dx.doi.org/10.1021/ic202159v | Inorg. Chem. 2012, 51, 1859−1865

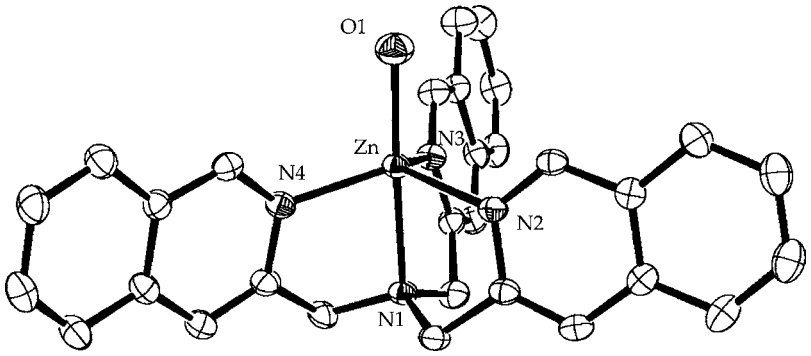



**Figure 5.** ORTEP plot for $[Zn(3\text{-isoTQA})(H_2O)](ClO_4)_2{\cdot}2CHCl_3$ in 50% probability. Atoms of counteranions, hydrogen, and solvents were omitted for clarity.

**Table 1. Structural and Fluorescent Properties for Zinc Complex of TPEN, 1-isoTQEN, 3-isoTQEN, TQEN, TPA, 1-isoTQA, 3-isoTQA, and TQA**

| ligand | mean Zn−$N_{aromatic}$ distance (Å) | fluorescent quantum yield for Zn complex | $I_{Cd}/I_{Zn}$ (%) |
|---|---|---|---|
| TPEN[a] | 2.15 | NA | NA |
| 1-isoTQEN[b] | 2.15 | 0.034 | 14 |
| 3-isoTQEN[b] | 2.16 | | 15 |
| TQEN[a] | 2.26 | 0.007 | 64 |
| TPA[c] | 2.05 | NA | NA |
| 1-isoTQA | 2.05 | 0.041 | 6.9 |
| 3-isoTQA | 2.08 | 0.017 | 18 |
| TQA[d] | 2.13 | | 22 |

[a]Ref 33. [b]Ref 34 [c]Ref 66. [d]Ref 54.

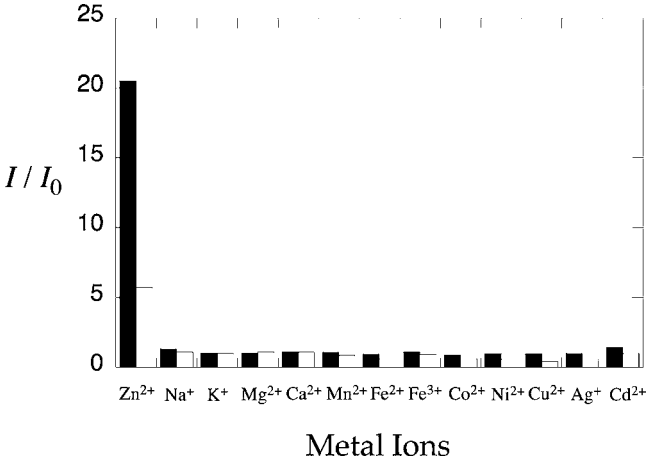



**Figure 6.** Relative fluorescence intensity of 1-isoTQA at 470 nm (filled bars) and 3-isoTQA at 464 nm (open bars) in the presence of 1 equivalent of metal ions in DMF/$H_2O$ (1:1) at 25 °C ($\lambda_{ex}$ = 324 nm). $I_0$ is the emission intensity of free ligand.

These observations indicate that the metal exchange process for isoTQA complexes is very slow, similarly to isoTQENs.[34,59] In the presence of $Cu^{2+}$, the fluorescence of 1-isoTQA−Zn complex was completely quenched after 1 day incubation at room temperature, due to higher affinity of $Cu^{2+}$with 1-isoTQA than $Zn^{2+}$.

**Relationship between Metal Selectivity, Fluorescent Response, and Complex Structure.** In these types of fluorescence probes, the steric hindrance and/or strength of metal binding reflected by the interatomic distances between zinc and coordinated aromatic nitrogen atoms were well correlated with the efficiency of CHEF (Table 1). The TQEN−Zn complex exhibits significant steric crowding arising from proximate quinoline rings to yield extremely long Zn−$N_{aromatic}$ distances (Zn−N = 2.1543, 2.4007, 2.1271, and 2.3711 Å).[33] In such a distorted coordination environment, CHEF induced by zinc coordination is far from maximal and the larger cadmium ion fits to the coordination cavity, resulting in poor fluorescent quantum yield of zinc complex ($\phi$ = 0.007) and zinc/cadmium selectivity ($I_{Cd}/I_{Zn}$ = 64%). Therefore, two structural modifications to remove this steric crowding from TQEN have been investigated: (1) reduction in the number of quinoline rings to afford bisquinoline derivatives, *N*,*N*′-bis(2-quinolylmethyl)-*N*,*N*′-dimethylethylenediamine (BQDMEN)[67] and (2) replacement of quinoline ring with the isoquinoline variant to afford 1- and 3-isoTQEN.[34] Both strategies worked well and the $I_{Cd}/I_{Zn}$ value was improved to 25% and 14% for BQDMEN and 1-isoTQEN, respectively.

In the present isoTQA system, decreasing the steric hindrance around the central zinc and also reducing the number of coordination atoms, strengthens the zinc-isoquinoline nitrogen interaction, enabling a more compact coordination geometry. As a result, the 1-isoTQA exhibited improved $I_{Cd}/I_{Zn}$ value up to 6.9% (free ligand is 5%). This is in good agreement with the steric control theory for $I_{Cd}/I_{Zn}$ selectivity that recently proposed by Hancock.[54]

**pH Effect on Fluorescence Intensity of 1- and 3-isoTQA.** Figure 7 demonstrates that the pH-dependent change in fluorescence intensity of 1- and 3-isoTQA is negligible. In the presence of 1 eq of zinc ion, fluorescence enhancement was observed in the range of pH = 3−9. The zinc-isoTQAs interaction is prevented by protonation of the nitrogen atoms

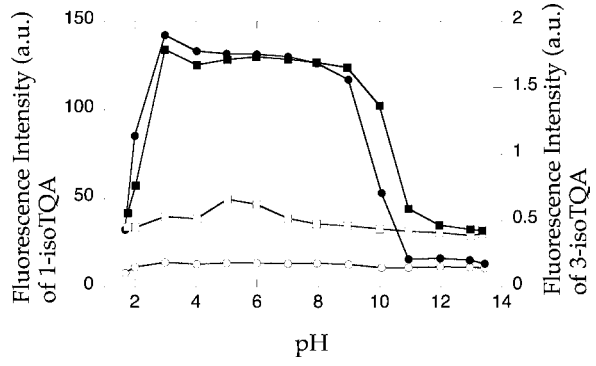



**Figure 7.** Effect of pH on fluorescence intensity of 34 $\mu$M 1-isoTQA at 470 nm (circles) and 3-isoTQA at 464 nm (squares) in the absence (open marks) and presence (filled marks) of 1 equivalent of zinc ion in DMF/$H_2O$ (1:1) at 25 °C ($\lambda_{ex}$ = 324 nm).


dx.doi.org/10.1021/ic202159v | *Inorg. Chem.* 2012, 51, 1859−1865

of the compound at low pH and by competing metal hydrolysis (formation of $Zn(OH)_2$) at high pH. From the pH-dependent spectral change in UV−vis spectra (0.1 M $NaClO_4$ in 1:1 DMF/$H_2O$, 25 °C), protonation constants of 1- and 3-isoTQA defined as the corresponding acid dissociation constants $K_{ai}$ = $[isoTQAH_{i-1}][H^+]/[isoTQAH_i]$ were determined to be $pK_{a1}$ = 5.35(2), $pK_{a2}$ = 3.38(3), and $pK_{a3}$ < 2 for 1-isoTQA and $pK_{a1}$ = 5.42(3), $pK_{a2}$ = 3.54(3), and $pK_{a3}$ = 2.14(4) for 3-isoTQA, respectively (Figures S5−S8). These values are parallel with those for TPA in different experimental conditions.[68,69]

**Zinc Binding Affinity of 1- and 3-isoTQA.** Since the zinc binding affinity of 1- and 3-isoTQA is significantly high as indicated by the sharp-edged titration curves shown in Figures S1 and S2 of the Supporting Information, it is impossible to determine the accurate binding constant of isoTQAs with metal ions by conventional titration. So, competition experiments using TPEN were conducted. Many fluorescent zinc sensor molecules release zinc upon addition of TPEN leading to fluorescence quenching because of the difference in zinc binding affinity. However, in the case of hexacoordinate zinc complexes of 1- and 3-isoTQEN, the zinc binding affinity is too high to observe transmetalation even in excess TPEN for a long period (more than 1 month).[34]

Upon addition of 1 equiv of TPEN to the zinc complex of 1-isoTQA in DMF/$H_2O$ (1:1), gradual (∼10 h to completion) decrease of fluorescence due to the slow zinc transfer from the 1-isoTQA−Zn complex to TPEN was observed. Thus, fairly strong zinc binding affinity of isoTQAs resist to immediate fluorescence quenching by TPEN.

## CONCLUSIONS

1- and 3-isoTQA exhibit zinc-specific fluorescence enhancement. No other metals studied, including $Cd^{2+}$, afford an emission response at ∼470 nm upon binding to the isoTQA ligand scaffold. The fluorescence intensity of 1-isoTQA is improved in comparison to the tetrakisquinoline derivatives (1-isoTQEN) due to a more efficient CHEF mechanism.

Quinoline ring interactions in the TQEN−Zn complex result in significant fluorescence quenching; however, the isoquinoline−isoquinoline interaction generates long wavelength emission that is specific for zinc binding for isoTQEN derivatives. Such excitonic interactions are well documented in phenyl-substituted tripodal systems based on TPA.[70] Similarly, the ∼470 nm emission of isoTQAs contain isoquinoline−isoquinoline interaction or energy transfer that is valid only for the associated zinc complexes.

Although the intensity of the low energy fluorescence for isoTQA−Zn complexes is weaker in comparison to the isoTQEN−Zn complexes because of reduced interisoquinoline interaction, it should be mentioned that such a strict structure-based photophysical requirement is still available for the tetradentate, trisisoquinoline derivatives studied in this work. Elucidation of the detailed fluorescent mechanism in these complexes is ongoing. In addition, preparation of water-soluble 1-isoTQA derivatives could serve as an important molecular design for high affinity intracellular zinc probes.

## ASSOCIATED CONTENT

### Supporting Information

Tables and figures of experimental procedure for synthesis of the compounds, and crystallographic data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

## AUTHOR INFORMATION

### Corresponding Author

*Phone/fax: +81-742-20-3095, e-mail: mikata@cc.nara-wu.ac.jp.

## ACKNOWLEDGMENTS

This work was supported by the Research for Promoting Technological Seeds, JST, Adaptable and Seamless Technology Transfer Program through Target-driven R&D, JST, Grant-in Aid for Scientific Research from the MEXT, Japan, and the Nara Women's University Intramural Grant for Project Research.

## REFERENCES

(1) Vallee, B. L.; Falchuk, K. H. *Physiol. Rev.* **1993**, *73*, 79−118.
(2) Vallee, B. L.; Auld, D. S. *Acc. Chem. Res.* **1993**, *26*, 543−551.
(3) Frederickson, C. J.; Suh, S. W.; Silva, D.; Frederickson, C. J.; Thompson, R. B. *J. Nutr.* **2000**, *130*, 1471S−1483S.
(4) Que, E. L.; Domaille, D. W.; Chang, C. J. *Chem. Rev.* **2008**, *108*, 1517−1549.
(5) Dai, Z.; Canary, J. W. *New J. Chem.* **2007**, *31*, 1708−1718.
(6) Burdette, S. C.; Walkup, G. K.; Spingler, B.; Tsien, R. Y.; Lippard, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 7831−7841.
(7) Burdette, S. C.; Frederickson, C. J.; Bu, W.; Lippard, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 1778−1787.
(8) Zhang, X.-A.; Hayes, D.; Smith, S. J.; Friedle, S.; Lippard, S. J. *J. Am. Chem. Soc.* **2008**, *130*, 15788−15789.
(9) Hirano, T.; Kikuchi, K.; Urano, Y.; Nagano, T. *J. Am. Chem. Soc.* **2002**, *124*, 6555−6562.
(10) Hanaoka, K.; Kikuchi, K.; Kojima, H.; Urano, Y.; Nagano, T. *J. Am. Chem. Soc.* **2004**, *126*, 12470−12476.
(11) Komatsu, K.; Kikuchi, K.; Kojima, H.; Urano, Y.; Nagano, T. *J. Am. Chem. Soc.* **2005**, *127*, 10197−10204.
(12) Komatsu, K.; Urano, Y.; Kojima, H.; Nagano, T. *J. Am. Chem. Soc.* **2007**, *129*, 13447−13454.
(13) Mizukami, S.; Okada, S.; Kimura, S.; Kikuchi, K. *Inorg. Chem.* **2009**, *48*, 7630−7638.
(14) Koike, T.; Watanabe, T.; Aoki, S.; Kimura, E.; Shiro, M. *J. Am. Chem. Soc.* **1996**, *118*, 12696−12703.
(15) Kimura, E.; Aoki, S.; Kikuta, E.; Koike, T. *Proc. Natl. Acad. Sci., USA* **2003**, *100*, 3731−3736.
(16) Qian, F.; Zhang, C.; Zhang, Y.; He, W.; Gao, X.; Hu, P.; Guo, Z. *J. Am. Chem. Soc.* **2009**, *131*, 1460−1468.
(17) Lim, N. C.; Schuster, J. V.; Porto, M. C.; Tanudra, M. A.; Yao, L.; Freake, H. C.; Brückner, C. *Inorg. Chem.* **2005**, *44*, 2018−2030.
(18) Taki, M.; Wolford, J. L.; O'Halloran, T. V. *J. Am. Chem. Soc.* **2004**, *126*, 712−713.
(19) Lu, C.; Xu, Z.; Cui, J.; Zhang, R.; Qian, X. *J. Org. Chem.* **2007**, *72*, 3554−3557.
(20) Tamanini, E.; Flavin, K.; Motevalli, M.; Piperno, S.; Gheber, L. A.; Todd, M. H.; Watkinson, M. *Inorg. Chem.* **2010**, *49*, 3789−3800.
(21) Hanaoka, K.; Muramatsu, Y.; Urano, Y.; Terai, T.; Nagano, T. *Chem.—Eur. J.* **2010**, *16*, 568−572.
(22) Xu, Z.; Baek, K.-H.; Kim, H. N.; Cui, J.; Qian, X.; Spring, D. R.; Shin, I.; Yoon, J. *J. Am. Chem. Soc.* **2010**, *132*, 601−610.
(23) Tomat, E.; Lippard, S. J. *Curr. Opi. Chem. Biol.* **2010**, *14*, 225−230.
(24) Ahmed, N.; Geronimo, I.; Hwang, I.-C.; Singh, N. J.; Kim, K. S. *Chem.—Eur. J.* **2011**, *17*, 8542−8548.
(25) Yang, X.-B.; Yang, B.-X.; Ge, J.-F.; Xu, Y.-J.; Xu, Q.-F.; Liang, J.; Lu, J.-M. *Org. Lett.* **2011**, *13*, 2710−2713.
(26) Buccella, D.; Horowitz, J. A.; Lippard, S. J. *J. Am. Chem. Soc.* **2011**, *133*, 4101−4114.
(27) Majzoub, A. E.; Cadiou, C.; Déchamps-Olivier, I.; Tinant, B.; Chuburu, F. *Inorg. Chem.* **2011**, *50*, 4029−4038.
(28) Jia, J.; Gu, Z.-Y.; Li, R.-C.; Huang, M.-H.; Xu, C.-S.; Wang, Y.-F.; Xing, G.-W.; Huang, Y.-S. *Eur. J. Org. Chem.* **2011**, 4609−4615.

(29) Xie, G.; Xi, P.; Wang, X.; Zhao, X.; Huang, L.; Chen, F.; Wu, Y.; Yao, X.; Zeng, Z. *Eur. J. Inorg. Chem.* **2011**, 2927−2931.
(30) 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.
(31) Urano, Y.; Kamiya, M.; Kanda, K.; Ueno, T.; Hirose, K.; Nagano, T. *J. Am. Chem. Soc.* **2005**, *127*, 4888−4894.
(32) Egawa, T.; Koide, Y.; Hanaoka, K.; Komatsu, T.; Terai, T.; Nagano, T. *Chem. Commun.* **2011**, *47*, 4162−4164.
(33) Mikata, Y.; Wakamatsu, M.; Yano, S. *Dalton Trans.* **2005**, 545−550.
(34) Mikata, Y.; Yamanaka, A.; Yamashita, A.; Yano, S. *Inorg. Chem.* **2008**, *47*, 7295−7301.
(35) Mikata, Y.; Wakamatsu, M.; Kawamura, A.; Yamanaka, N.; Yano, S.; Odani, A.; Morihiro, K.; Tamotsu, S. *Inorg. Chem.* **2006**, *45*, 9262−9268.
(36) Ichimura, C.; Shiraishi, Y.; Hirai, T. *Tetrahedron* **2010**, *66*, 5594−5601.
(37) Zhou, X.; Yu, B.; Guo, Y.; Tang, X.; Zhang, H.; Liu, W. *Inorg. Chem.* **2010**, *49*, 4002−4007.
(38) Zhu, J.-F.; Yuan, H.; Chan, W.-H.; Lee, A. W. M. *Tetrahedron Lett.* **2010**, *51*, 3550−3554.
(39) Liu, Z.-C.; Wang, B.-D.; Yang, Z.-Y.; Li, T.-R.; Li, Y. *Inorg. Chem. Commun.* **2010**, *13*, 606−608.
(40) Xue, L.; Liu, C.; Jiang, H. *Chem. Commun.* **2009**, 1061−1063.
(41) Xue, L.; Wang, H.-H.; Wang, X.-J.; Jiang, H. *Inorg. Chem.* **2008**, *47*, 4310−4318.
(42) Xue, L.; Liu, C.; Jiang, H. *Org. Lett.* **2009**, *11*, 1655−1658.
(43) Weng, Y.; Chen, Z.; Wang, F.; Xue, L.; Jiang, H. *Anal. Chim. Acta* **2009**, *647*, 215−218.
(44) Wang, H.-H.; Gan, Q.; Wang, X.-J.; Xue, L.; Liu, S.-H.; Jiang, H. *Org. Lett.* **2007**, *9*, 4995−4998.
(45) Zhang, Y.; Guo, X.; Si, W.; Jia, L.; Qian, X. *Org. Lett.* **2008**, *10*, 473−476.
(46) Shiraishi, Y.; Ichimura, C.; Hirai, T. *Tetrahedron Lett.* **2007**, *48*, 7769−7773.
(47) Royzen, M.; Durandin, A.; Young, J., V. G.; Geacintov, N. E.; Canary, J. W. *J. Am. Chem. Soc.* **2006**, *128*, 3854−3855.
(48) Chen, H.; Wu, Y.; Cheng, Y.; Yang, H.; Li, F.; Yang, P.; Huang, C. *Inorg. Chem. Commun.* **2007**, *10*, 1413−1415.
(49) Liu, Y.; Zhang, N.; Chen, Y.; Wang, L.-H. *Org. Lett.* **2007**, *9*, 315−318.
(50) Chen, Y.; Han, K.-Y.; Liu, Y. *BIoorg. Med. Chem.* **2007**, *15*, 4537−4542.
(51) Qiu, L.; Jiang, P.; He, W.; Tu, C.; Lin, J.; Li, Y.; Gao, X.; Guo, Z. *Inorg. Chim. Acta* **2007**, *360*, 431−438.
(52) Wu, D.-Y.; Xie, L.-X.; Zhang, C.-L.; Duan, C.-Y.; Zhao, Y.-G.; Guo, Z.-J. *Dalton Trans.* **2006**, 3528−3533.
(53) Aragoni, M. C.; Arca, M.; Bencini, A.; Blake, A. J.; Caltagirone, C.; De Filippo, G.; Devillanova, F. A.; Garau, A.; Gelbrich, T.; Hursthouse, M. B.; Isaia, F.; Lippolis, V.; Mameli, M.; Mariani, P.; Valtanconi, B.; Wilson, C. *Inorg. Chem.* **2007**, *46*, 4548−4559.
(54) Williams, N. J.; Gan, W.; Reibenspies, J. H.; Hancock, R. D. *Inorg. Chem.* **2009**, *48*, 1407−1415.
(55) Mameli, M.; Aragoni, M. C.; Arca, M.; Atzori, M.; Bencini, A.; Bazzicalupi, C.; Blake, A. J.; Caltagirone, C.; Devillanova, F. A.; Garau, A.; Hursthouse, M. B.; Isaia, F.; Lippolis, V.; Valtancoli, B. *Inorg. Chem.* **2009**, *48*, 9236−9249.
(56) Zhou, X.; Lu, Y.; Zhu, J.-F.; Chan, W.-H.; Lee, A. W. M.; Chan, P.-S.; Wong, R. N. S.; Mak, N. K. *Tetrahedron* **2011**, *67*, 3412−3419.
(57) Zhang, C.; Zhang, Y.; Chen, Y.; Xie, Z.; Liu, Z.; Dong, X.; He, W.; Shen, C.; Guo, Z. *Inorg. Chem. Commun.* **2011**, *14*, 304−307.
(58) Mikata, Y.; Yamashita, A.; Kawata, K.; Konno, H.; Itami, S.; Yasuda, K.; Tamotsu, S. *Dalton Trans.* **2011**, *40*, 4976−4981.
(59) Mikata, Y.; Yamashita, A.; Kawata, K.; Konno, H.; Itami, S.; Yasuda, K.; Tamotsu, S. *Dalton Trans.* **2011**, *40*, 4059−4066.
(60) Wei, N.; Murthy, N. N.; Chen, Q.; Zubieta, J.; Karlin, K. D. *Inorg. Chem.* **1994**, *33*, 1953−1965.
(61) Wei, N.; Murthy, N. N.; Karlin, K. D. *Inorg. Chem.* **1994**, *33*, 6093−6100.
(62) Xu, J.; Chuang, C.-L.; Canary, J. W. *Inorg. Chim. Acta* **1997**, *256*, 125−128.
(63) Newkome, G. R.; Kiefer, G. E.; Xia, Y.-J.; Gupta, V. K. *Synthesis* **1984**, 676−679.
(64) Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A.; Burla, M. C.; Polidori, G.; Camalli, M. *J. Appl. Crystallogr.* **1994**, *27*, 435.
(65) Sheldrick, G. M. *SHELXL-97, Program for Refinement of Crystal Structures*; University of Göttingen, Germany, 1997.
(66) Makowska-Grzyska, M. M.; Szajna, E.; Shipley, C.; Arif, A. M.; Mitchell, M. H.; Halfen, J. A.; Berreau, L. M. *Inorg. Chem.* **2003**, *42*, 7472−7488.
(67) Mikata, Y.; Yamashita, A.; Kawamura, A.; Konno, H.; Miyamoto, Y.; Tamotsu, S. *Dalton Trans.* **2009**, 3800−3806.
(68) Bravard, F.; Rosset, C.; Delangle, P. *Dalton Trans.* **2004**, 2012−2018.
(69) Ambundo, E. A.; Deydier, M.-V.; Grall, A. J.; Aguera-Vega, N.; Dressel, L. T.; Cooper, T. H.; Heeg, M. J.; Ochrymowycz, L. A.; Rorabacher, D. B. *Inorg. Chem.* **1999**, *38*, 4233−4242.
(70) Liang, J.; Zhang, J.; Zhu, L.; Duarandin, A.; Young, J., V. G.; Geacintov, N.; Canary, J. W. *Inorg. Chem.* **2009**, *48*, 11196−11208.