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Isoquinoline-Based TQEN Family as TPEN-Derived Fluorescent Zinc Sensors

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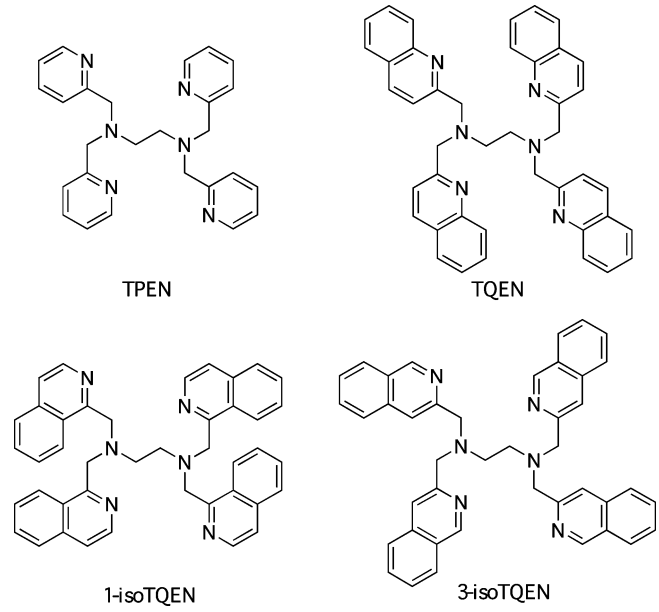
The hexadentate nitrogen ligands 1-isoTQEN (*N,N,N′,N′*-tetrakis(1-isoquinolylmethyl)ethylenediamine) and 3-isoTQEN (*N,N,N′,N′*-tetrakis(3-isoquinolylmethyl)ethylenediamine) have been prepared. The structures of these ligands are based on that of TPEN (*N,N,N′,N′*-tetrakis(2-pyridylmethyl)ethylenediamine). The introduction of a benzene ring into TPEN affords fluorescence ability upon zinc-ion binding. Compared to the quinoline isomer TQEN, isoquinoline derivatives 1-isoTQEN and 3-isoTQEN exhibit a lower-energy shift in the excitation and emission wavelengths and an enhanced fluorescence intensity, probably because of the energy-transfer mechanism between adjacent isoquinoline rings. Importantly, an increase in the Zn^{2+}/Cd^{2+} discriminating ability and a reduction in the background fluorescence induced by pH were also achieved for isoquinoline derivatives. The zinc-ion-induced fluorescence of these isoTQENs was not quenched by an addition of TPEN, which demonstrates the significantly high zinc-ion binding ability of these isoTQEN ligands.

Introduction

The design and synthesis of fluorescent sensors that selectively respond to zinc ions is of significant interest. Cellular zinc ions exist as protein-bound, relatively free forms. Their protein-binding qualities are considered to play many important roles in signaling processes. Because the concentration of a zinc ion in a living cell lies over a very broad range (on the order of nanomolar to millimolar),[1] the investigation of zinc sensors with various affinities for the zinc ion is very important. Fluorescent zinc sensors prepared recently include fluorescein,[2–7] coumarin,[8–10] dansylamide,[11,12] BODIPY,[13] quinoline,[12,14–19] and other[20–28] fluorophores. Except for several examples in which mostly quinoline moieties are utilized as both the metal binding site

Chart 1



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and the fluorophere,[29–38] the reported zinc probes have a discrete metal-binding site with various zinc-binding affinities[2,3,5–7] attached to a fluorescent molecule.

A widely used ligand for both transition and lanthanoid metals is TPEN (*N,N,N′,N′*-tetrakis(2-pyridylmethyl)ethylenediamine (Chart 1).[39] X-ray crystal structures have been reported for complexes of this ligand with Zn^{2+},[15,39] Fe^{2+},[40] Ru^{2+},[41] Co^{3+},[42] Re^{4+}═O,[43] Tl^{3+},[44] La^{3+},[45] and so forth. In these metal complexes, high binding affinity results from an ideal octahedral binding geometry generated by the six nitrogens of TPEN. In contrast to EDTA, which also has six coordinating atoms, the neutral nature of TPEN allows it to penetrate cell membranes, and thus TPEN is often used as a membrane-permeable, intracellular transition-metal sequestering agent.[46,47] We have recently developed the quinoline-based ligand TQEN (*N,N,N′,N′*-tetrakis(2-quinolylmethyl)ethylenediamine (Chart 1), which is derived from TPEN as a fluorescent zinc-sensing molecule.[14,15] The introduction of an extra benzene ring into the TPEN skeleton generates weak to moderate fluorescence upon zinc binding. No other metal ions except Cd^{2+} exhibited fluorescence upon chelation by TQEN. The binding affinity of TQEN with a zinc ion is significantly weaker than that of TPEN because of the steric repulsion of the quinoline rings.

Herein, we introduce the isoquinoline-based TQEN family (Chart 1), 1-isoTQEN (*N,N,N′,N′*-tetrakis(1-isoquinolylmethyl)ethylenediamine) and 3-isoTQEN (*N,N,N′,N′*-tetrakis(3-isoquinolylmethyl)ethylenediamine), as a new platform for fluorescent zinc sensors. Although several metal complexes with 1,1′- or 3,3′-biisoquinoline and their cyclometalated analogs have been extensively investigated as luminescent devices,[48,49] very few examples have been found in the literature in which the aminomethylisoquinoline moiety is used as a ligand for metal ions.[50] It was expected that a change in the position of the benzene ring of TQEN would afford a higher metal-binding affinity because steric repulsion between the metal ion and the perihydrogen and between intramolecular quinoline rings could be avoided.

Experimental Section

General. All reagents and solvents used for the ligand synthesis were from commercial sources and were used as received. All aqueous solutions were prepared with deionized and redistilled water. ^{1}H NMR (300 MHz) and ^{13}C NMR (75.5 MHz) spectra were recorded on a Varian GEMINI 2000 spectrometer and referenced to internal TMS or solvent signals. Ultraviolet−visible and fluorescence spectra were measured on a Jasco V-700 spectrophotometer and a Jasco FP-720 spectrofluorometer, respectively. ***Caution!*** *Perchlorate salts of metal complexes with organic ligands are potentially explosive. All due precautions should be taken.*

Materials. Via (i) the oxidation to isoquinolinealdehyde by SeO_2,[54] (ii) the reduction to isoquinolinemethanol[54] by $NaBH_4$,[14] and (iii) the chlorination of the OH group by $SOCl_2$, 1-chlorom-

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Table 1. Crystallographic Data for 1-isoTQEN, 3-isoTQEN, [Zn(1-isoTQEN)]$(ClO_4)_2$·4DMF, and [Zn(3-isoTQEN)]$(ClO_4)_2$·CH_3CN

	1-isoTQEN	3-isoTQEN	[Zn(1-isoTQEN)]$(ClO_4)_2$·4DMF	[Zn(3-isoTQEN)]$(ClO_4)_2$·CH_3CN
formula	$C_{42}H_{36}N_6$	$C_{42}H_{36}N_6$	$C_{54}H_{64}Cl_2N_{10}O_{12}Zn$	$C_{44}H_{39}Cl_2N_7O_8Zn$
fw	624.79	624.79	1181.42	930.12
space group	$P\bar{1}$	$P2_1/c$	$Fdd2$	$P2_1/c$
a, Å	8.497(5)	16.686(3)	30.5337(9)	10.3252(4)
b, Å	9.061(6)	5.8007(9)	38.0595(13)	18.9257(8)
c, Å	11.129(7)	17.173(3)	9.6447(2)	21.3899(8)
α, deg	100.338(8)	90	90	90
β, deg	92.288(7)	108.688(2)	90	92.035(2)
γ, deg	107.504(8)	90	90	90
V, Å^3	799.9(9)	1574.5(5)	11208.1(6)	4177.2(3)
Z	1	2	8	4
D_{calcd}, g·cm^{-3}	1.297	1.318	1.400	1.479
μ, cm^{-1}	0.779	0.791	6.04	7.797
$2\theta_{max}$, deg	55.0	55.0	55.0	55.0
temp, K	173	173	173	153
no. reflns collected	3895	3610	28885	29290
no. reflns used	1357	2672	6781	7819
no. of params	218	290	361	716
final R1 ($I > 2\theta(I)$)[a]	0.0662	0.0440	0.0351	0.0408
wR2 (all data)[b]	0.1545	0.1260	0.1017	0.1041
GOF	0.891	1.051	1.129	1.095

[a] $R1 = (\sum||F_o| - |F_c||)/(\sum|F_o|)$. [b] $wR2 = \{[\sum w(F_o^2 - F_c^2)^2]/[\sum w(F_o^2)^2]\}^{1/2}$.

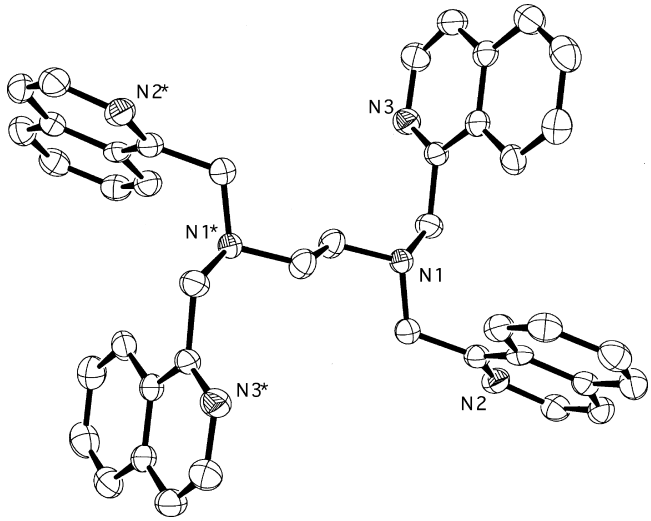

Figure 1. ORTEP plot (50% probability) for 1-isoTQEN. Hydrogen atoms were omitted for clarity. The symmetry operation needed to generate the atoms is marked with an asterisk.

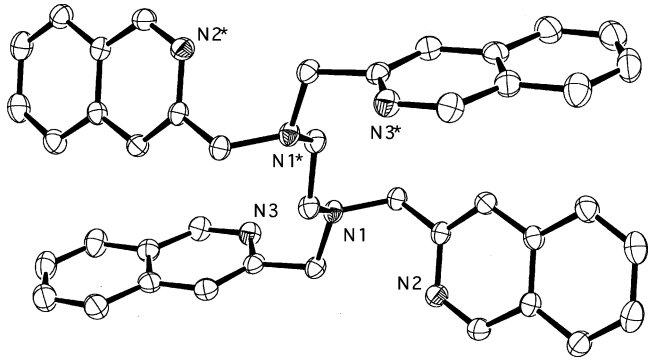

Figure 2. ORTEP plot (50% probability) for 3-isoTQEN. Hydrogen atoms were omitted for clarity. The symmetry operation needed to generate the atoms is marked with an asterisk.

ethylisoquinoline[51] and 3-chloromethylisoquinoline[52,53] were prepared from corresponding methylisoquinolines.[14,53]

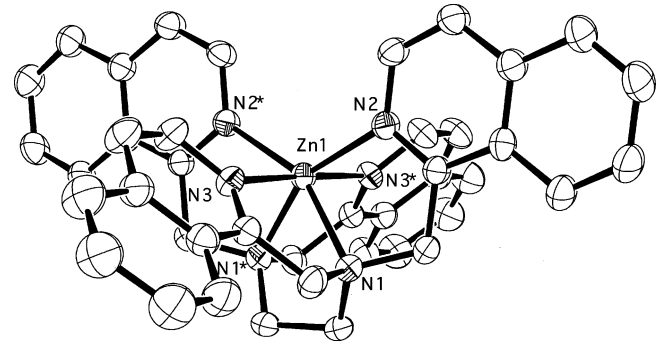

Figure 3. ORTEP plot (50% probability) for [Zn(1-isoTQEN)]$(ClO_4)_2$·4DMF. Counteranions, solvents, and hydrogen atoms were omitted for clarity. The symmetry operation needed to generate the atoms is marked with an asterisk.

***N,N,N′,N′*-Tetrakis(1-isoquinolylmethyl)ethylenediamine (1-isoTQEN).** An agitated mixture of 1-chloromethylisoquinoline (357 mg, 2.0 mmol), ethylenediamine (30 mg, 0.50 mmol), and cesium carbonate (1.8 g, 5.5 mmol) in acetonitrile (30 mL) was refluxed for 5 days. After the solvent was removed, the residue was extracted with chloroform/water, and the organic layer was dried and evaporated. The residue was washed with acetone to produce 1-isoTQEN as a white powder (145 mg, 0.23 mol) in 46% yield. ^{1}H NMR ($CDCl_3$, δ): 2.83 (s, 4H), 4.11 (s, 8H), 7.11 (dd, J = 7.8, 7.8 Hz, 4H), 7.47 (d, J = 5.7 Hz, 4H), 7.53 (dd, J = 7.8, 7.8 Hz, 4H), 7.70 (d, J = 8.1 Hz, 4H), 7.84 (d, J = 8.1 Hz, 4H), 8.33 (d, J = 5.7 Hz, 4H). ^{13}C NMR ($CDCl_3$, δ): 52.0, 60.3, 120.5, 126.5, 126.6, 126.7, 127.4, 129.7, 136.1, 141.3, 158.4. Anal. Calcd for $C_{43.2}H_{38.8}N_{6.4}O_{0.4}$ (1-isoTQEN·0.4DMF): H, 5.98; C, 79.34; N, 13.71. Found: H, 5.82; C, 79.33; N, 13.44. Recrystallization from DMF−ether produced single crystals suitable for X-ray crystallography (Figure 1). Crystal data are summarized in Table 1.

***N,N,N′,N′*-Tetrakis(3-isoquinolylmethyl)ethylenediamine (3-isoTQEN).** An agitated mixture of 3-chloromethylisoquinoline (200 mg, 1.1 mmol), ethylenediamine (17 mg, 0.28 mmol), and cesium

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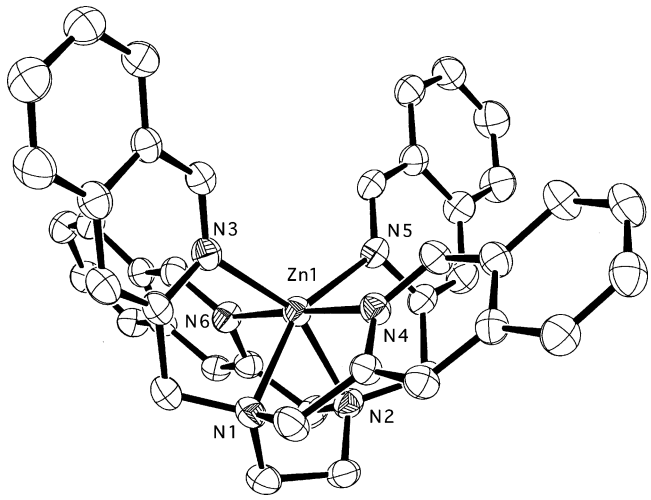

Figure 4. ORTEP plot (50% probability) for [Zn(3-isoTQEN)]-$(ClO_4)_2 \cdot CH_3CN$. Counteranions, solvents, and hydrogen atoms were omitted for clarity.

Chart 2

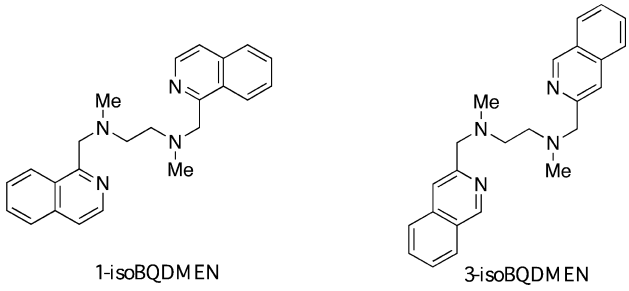

Table 2. Selected Bond Distances (Angstroms) and Angles (deg) for [Zn(1-isoTQEN)]$(ClO_4)_2 \cdot$4DMF and [Zn(3-isoTQEN)]$(ClO_4)_2 \cdot CH_3CN$[a]

	[Zn(1-isoTQEN)] $(ClO_4)_2 \cdot$4DMF	[Zn(3-isoTQEN)] $(ClO_4)_2 \cdot CH_3CN$
Zn−N(1)	2.1945(16)	2.2167(18)
Zn−N(2)		2.2123(17)
Zn−N(3)	2.1269(17)	2.1675(16)
Zn−N(4)	2.1742(15)	2.1591(17)
Zn−N(5)		2.1484(16)
Zn−N(6)		2.1608(17)
N(1)−Zn−N(2)	81.89(9)	80.46(6)
N(1)−Zn−N(3)	78.24(6)	79.04(6)
N(1)−Zn−N(4)	79.05(7)	78.93(6)
N(1)−Zn−N(5)	155.11(6)	155.97(6)
N(1)−Zn−N(6)	99.23(7)	100.24(6)
N(2)−Zn−N(3)	155.11(6)	156.02(6)
N(2)−Zn−N(4)	99.23(7)	98.84(6)
N(2)−Zn−N(5)	78.24(6)	78.95(6)
N(2)−Zn−N(6)	79.05(7)	79.34(6)
N(3)−Zn−N(4)	91.57(7)	89.32(6)
N(3)−Zn−N(5)	124.52(9)	123.50(6)
N(3)−Zn−N(6)	89.47(7)	92.17(6)
N(4)−Zn−N(5)	89.47(7)	92.11(6)
N(4)−Zn−N(6)	177.76(12)	178.12(6)
N(5)−Zn−N(6)	91.57(7)	88.01(6)

[a] Atoms N(2), N(3), N(4), N(5), and N(6) correspond to N(1)*, N(2), N(3), N(2)*, and N(3)*, respectively, in the crystal data for [Zn(1-isoTQEN)]$(ClO_4)_2 \cdot$4DMF.

carbonate (1.0 g, 3.1 mmol) in acetonitrile (10 mL) was refluxed for 2.5 days. After the solvent was removed, the residue was extracted with chloroform/water, and the organic layer was dried and evaporated. The residue was washed with ethanol to produce 3-isoTQEN as a white powder (110 mg, 0.17 mol) in 62% yield. ^{1}H NMR ($CDCl_3$, δ): 3.03 (s, 4H), 4.06 (s, 8H), 7.47−7.58 (m, 12H), 7.79 (s, 4H), 7.87 (d, J = 8.1 Hz, 4H), 9.11 (s, 4H). ^{13}C NMR ($CDCl_3$, δ): 52.5, 60.7, 118.7, 126.49, 126.54, 127.4, 127.6, 130.1, 136.3, 151.9, 153.1. Anal. Calcd for $C_{42}H_{36}N_6$ (3-isoTQEN): H, 5.81; C, 80.74; N, 13.45. Found: H, 5.81; C, 80.65; N, 13.66. Recrystallization from acetonitrile−ether produced single crystals suitable for X-ray crystallography (Figure 2). Crystal data are summarized in Table 1.

[Zn(1-isoTQEN)]$(ClO_4)_2 \cdot$4DMF. Single crystals of [Zn(1-isoTQEN)]$(ClO_4)_2 \cdot$4DMF suitable for X-ray crystallography were prepared from 1-isoTQEN and $Zn(ClO_4)_2 \cdot 6H_2O$ in DMF−ether (Figure 3). Crystal data are summarized in Table 1. Anal. Calcd for $C_{48}H_{52}Cl_2N_8O_{11}Zn$ ([Zn(1-isoTQEN)]$(ClO_4)_2 \cdot$2DMF$\cdot H_2O$): H, 4.98; C, 54.75; N, 10.64. Found: H, 4.89; C, 54.84; N, 10.57.

[Zn(3-isoTQEN)]$(ClO_4)_2 \cdot CH_3CN$. Single crystals of [Zn(3-isoTQEN)]$(ClO_4)_2 \cdot CH_3CN$ suitable for X-ray crystallography were prepared from 3-isoTQEN and $Zn(ClO_4)_2 \cdot 6H_2O$ in acetonitrile−ether (Figure 4). Crystal data are summarized in Table 1. Anal. Calcd for $C_{48}H_{52}Cl_2N_8O_{11}Zn$ ([Zn(3-isoTQEN)]$(ClO_4)_2 \cdot$2DMF$\cdot H_2O$): H, 4.98; C, 54.75; N, 10.64. Found: H, 4.92; C, 54.72; N, 10.51.

***N,N'*-Bis(1-isoquinolylmethyl)-*N,N'*-dimethylethylenediamine (1-isoBQDMEN).** An agitated mixture of 1-chloromethylisoquinoline (55 mg, 0.31 mmol), *N,N'*-dimethylethylenediamine (15.2 mg, 0.17 mmol), and cesium carbonate (245 mg, 0.75 mmol) in acetonitrile (15 mL) was refluxed for 2 days. After the solvent was removed, the residue was extracted with chloroform/water, and the organic layer was dried and evaporated. The residue was subjected to column chromatography (alumina; eluent, ethyl acetate) to produce 1-isoBQDMEN as a yellow oil (27.5 mg, 0.074 mol) with a 48% yield. ^{1}H NMR ($CDCl_3$, δ): 2.25 (s, 6H), 2.78 (s, 4H), 4.11 (s, 4H), 7.40 (ddd, J = 8.1, 6.6, 1.2 Hz, 2H), 7.55 (d, J = 5.7 Hz, 2H), 7.60 (ddd, J = 8.1, 6.6, 1.2 Hz, 2H), 7.77 (d, J = 7.8 Hz, 2H), 8.42 (d, J = 5.7 Hz, 2H), 8.47 (d, J = 8.4 Hz, 2H). ^{13}C NMR ($CDCl_3$, δ): 42.7, 55.7, 62.9, 120.5, 126.3, 126.85, 126.92, 127.6, 129.9, 136.3, 141.4, 158.3.

***N,N'*-Bis(3-isoquinolylmethyl)-*N,N'*-dimethylethylenediamine (3-isoBQDMEN).** An agitated mixture of 3-chloromethylisoquinoline (150 mg, 0.84 mmol), *N,N'*-dimethylethylenediamine (38 mg, 0.42 mmol), and cesium carbonate (820 mg, 2.5 mmol) in acetonitrile (20 mL) was refluxed for 2 days. After the solvent was removed, the residue was extracted with chloroform/water, and the organic layer was dried and evaporated. The residue was subjected to column chromatography (alumina; eluent: ethyl acetate/methanol = 4/1) to produce 1-isoBQDMEN as a yellow oil (90 mg, 0.24 mol) in 57% yield. ^{1}H NMR ($CDCl_3$, δ): 2.36 (s, 6H), 2.78 (s, 4H), 3.87 (s, 4H), 7.55 (m, 2H), 7.64 (m, 2H), 7.72 (s, 2H), 7.73 (m, 2H), 7.94 (d, J = 8.1 Hz, 2H), 9.20 (s, 2H). ^{13}C NMR ($CDCl_3$, δ): 42.9, 55.4, 63.9, 119.2, 126.5, 126.8, 127.5, 127.6, 130.3, 136.3, 152.1.

Results and Discussion

Ligand Synthesis. Hexadentate nitrogen ligands 1- and 3-isoTQEN were prepared from the corresponding chloromethylisoquinolines and from 0.25 equiv of ethylenediamine refluxing in acetonitrile in the presence of cesium carbonate. These compounds were characterized by ^{1}H and ^{13}C NMR, elemental analysis, and X-ray crystallography (Figures 1 and 2). Bisisoquinoline derivatives, 1- and 3-isoBQDMEN (Chart 2), were prepared via a similar method.

Crystal Structure of Zinc Complexes. X-ray crystallographic analysis of the zinc complexes with 1- and 3-isoTQEN reveals their coordination geometries (Figures

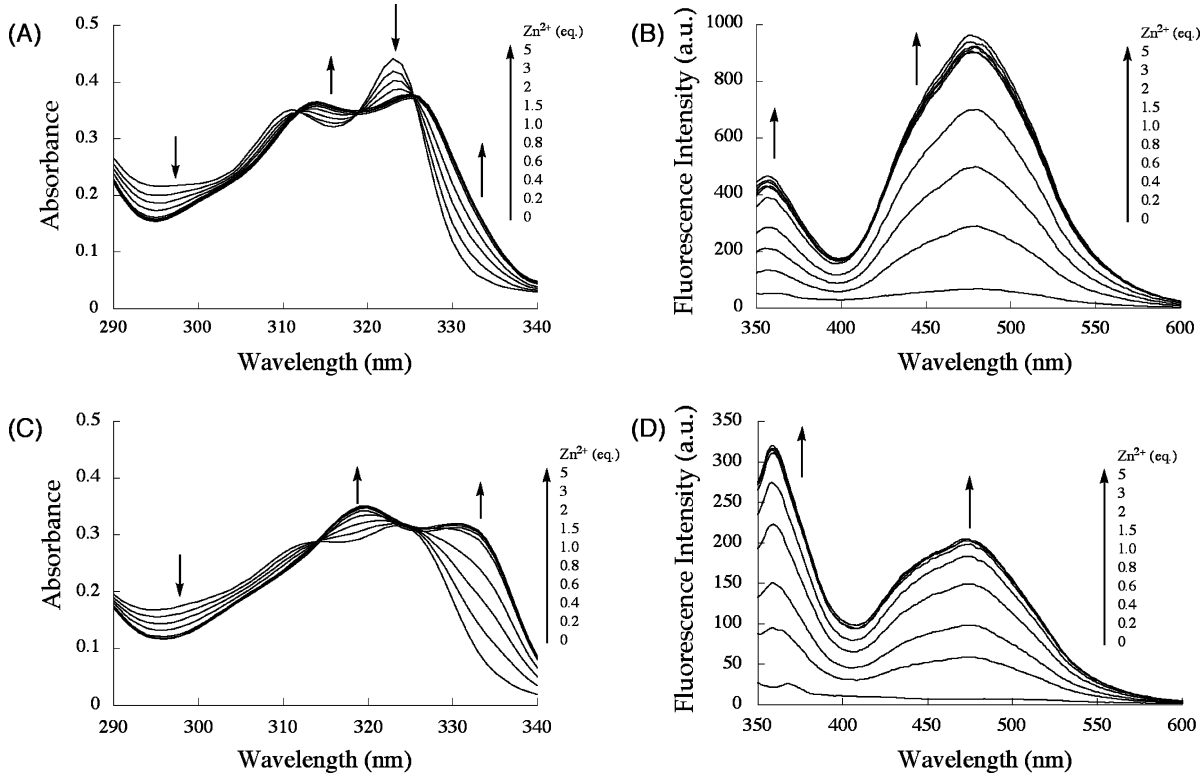

Figure 5. (a) UV−vis absorbance spectra of 1-isoTQEN, (b) fluorescence spectra of 1-isoTQEN (λ_{ex} = 328 nm), (c) UV−vis absorbance spectra of 3-isoTQEN, and (d) fluorescence spectra of 3-isoTQEN (λ_{ex} = 332 nm) in the presence of an increasing number of zinc ions in a 34 μM DMF/H_2O (1/1) solution.

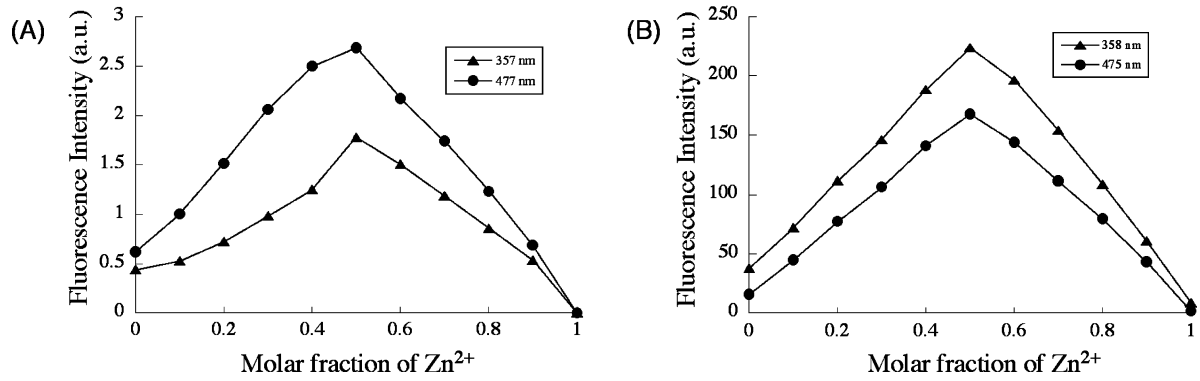

Figure 6. Job plot analysis for fluorescence intensity of zinc complexes with (a) 1-isoTQEN monitored at 357 and 477 nm and (b) 3-isoTQEN monitored at 358 and 475 nm in DMF/H_2O (1/1). The sum of the concentrations of isoTQEN and Zn^{2+} is 34 μM.

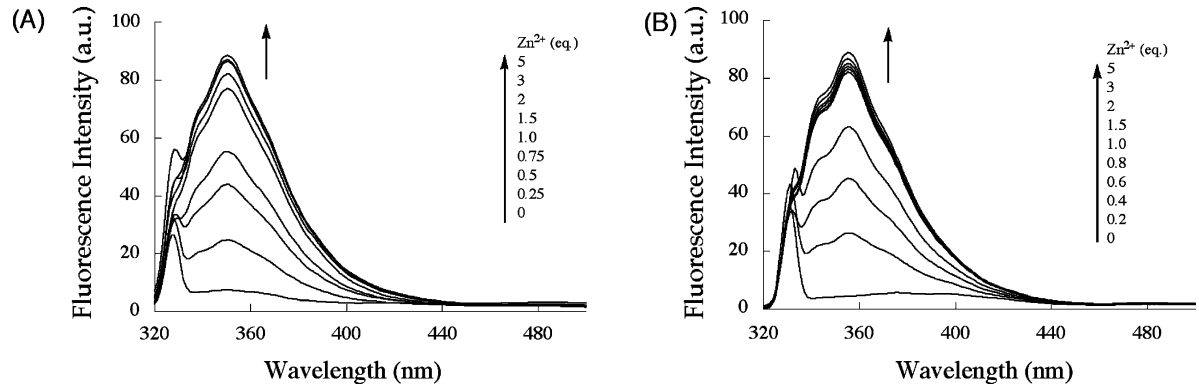

Figure 7. (a) Fluorescent spectra of 1-isoBQDMEN (λ_{ex} = 329 nm) and (b) 3-isoBQDMEN (λ_{ex} = 329 nm) in the presence of an increasing number of zinc ions in 34 μM DMF/H_2O (1/1).

3 and 4). Single crystals suitable for X-ray crystallography were obtained from a DMF−ether solution for the 1-isoTQEN−Zn complex and from an acetonitrile−ether solution for the 3-isoTQEN−Zn complex. The details of the X-ray measurement and the crystal data are summarized in Table 1. For the 1-isoTQEN−Zn complex, which crystallizes in the *Fdd*2

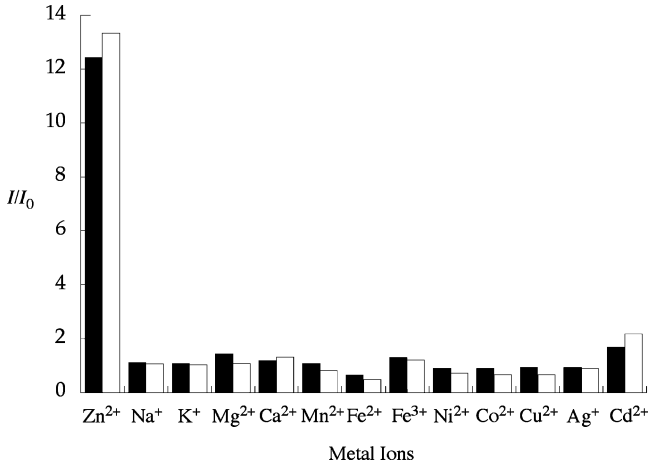

Figure 8. Relative fluorescence intensity of 1-isoTQEN at 477 nm (filled bars) and 3-isoTQEN at 475 nm (open bars) in the presence of 1 equiv of metal ions. I_0 is the emission intensity in the absence of metal ions. Spectra were recorded in a 34 μM DMF/H_2O (1/1) solution.

space group, the crystallographic twofold axis is found in the center of the complex cation, relating the right half of the molecule to the left half. The selected bond distances and angles are listed in Table 2.

Compared to the structure of the TQEN−Zn complex, the isoTQEN−Zn complexes exhibit diminished steric repulsion between adjacent isoquinoline rings. The large deviation from 180° observed in N4−Zn−N6 of the TQEN−Zn complex (166°)[15] was minimized for 1-isoTQEN−Zn (N3−Zn−N3*, 178°) and 3-isoTQEN (178°). The twist angle between two (iso)quinoline rings containing N3 and N5 (N2 and N2* for 1-isoTQEN) was also significantly reduced in 1-isoTQEN−Zn (4°) compared to those of the 3-isoTQEN−Zn (21°) and TQEN−Zn (22°) complexes. These values for 1-isoTQEN are comparable to those for TPEN−Zn complexes (171−175° for N4−Zn−N6 and 8° for the interplane angle).[15,39]

Zinc Ion-Induced UV−Visible and Fluorescence Spectral Changes. Because both 1-isoTQEN and 3-isoTQEN are insoluble in water, the following experiments were performed in a 50% aqueous DMF solution. The addition of zinc ion to an aqueous DMF solution (DMF/H_2O = 1/1 (v/v)) of 1- or 3-isoTQEN induces UV−vis and fluorescence spectral changes (Figure 5). The UV−vis spectra both exhibit distinct isosbestic points at 312, 319, and 325 nm for 1-isoTQEN and at 314 nm for 3-isoTQEN. Weak emissions of 1- and 3-isoTQEN in the absence of a zinc ion increased as zinc ion was added at 357 and 477 nm for 1-isoTQEN and at 358 and 475 nm for 3-isoTQEN. Both changes stopped at the point of the addition of 1 equiv of zinc ion. The 1/1 ligand-to-zinc ion stoichiometry was further confirmed by Job plot analysis (Figure 6). In the presence of 1 equiv of zinc ion, the fluorescence intensity increased 12-fold at 477 nm for 1-isoTQEN and 13-fold at 475 nm for 3-isoTQEN. The fluorescence quantum yields of zinc complexes of 1- and 3-isoTQEN are relatively low, but the zinc-induced spectral changes are clearly detectable and provide a detection limit of $\sim 10^{-7}$ M.

Both the excitation and emission wavelengths of the isoquinoline derivatives shift to lower energy compared to those of the parent compound, TQEN (λ_{ex} = 317 nm, λ_{em} = 383 nm). In particular, the emission spectra of the isoTQENs consist of two distinct components at ~360 and ~480 nm. The short-wavelength emission is due to the combination of photoinduced electron-transfer (PET) and coordination-enhanced fluorescence (CEF), as found for the TQEN-Zn complex.[15] The significant increase in the intensity of the long-wavelength emission is attributed to an isoquinoline−isoquinoline interaction upon zinc complexation because the bisisoquinoline analogs, 1- and 3-isoBQDMEN (Chart 2), completely lack this component in their fluorescence spectra even in the presence of zinc ion (Figure 7). It is reasonable to assume that the close proximity of isoquinoline rings in zinc-bound isoTQENs found in the crystal structure (Figures 3 and 4) promotes the energy transfer between aromatic rings to induce the long-wavelength emission.

Fluorescence Spectral Change Induced by Other Metal Ions and pH. The isoTQENs both exhibit excellent zinc ion discriminating ability, as shown in Figure 8. It is well known that the cadmium ion often exhibits a significant response in these types of fluorescent ligands; however, 1- and 3-isoTQEN exhibit very weak fluorescence in the presence of cadmium ion. The observed Cd^{2+} fluorescence is <15% of that in the presence of an equivalent number of zinc ions. The zinc-induced fluorescence enhancement of isoTQENs was prevented by equimolar amounts of Mn^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Ag^+, and Cd^{2+}, demonstrating the preferred binding of these metal ions with isoTQENs rather than zinc ion (data not shown). This does not cause a serious problem

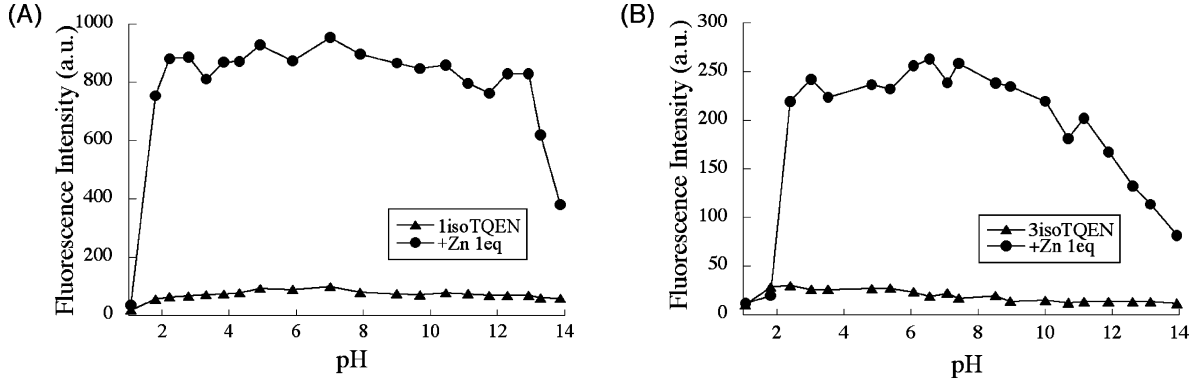

Figure 9. Effect of pH on fluorescence intensity of (a) 1-isoTQEN at 477 nm and (b) 3-isoTQEN at 475 nm in the absence (▲) and presence (●) of 1 equiv of zinc ion. Spectra were recorded in a 34 μM DMF/H_2O (1/1) solution.

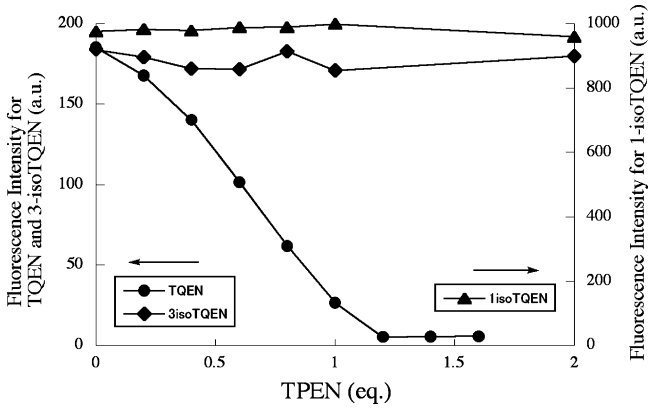

Figure 10. Effect of TPEN on the fluorescence intensity of zinc complexes of 1-isoTQEN at 477 nm (▲), 3-isoTQEN at 475 nm (♦), and TQEN (●). Spectra were recorded in 34 μM ligands and Zn^{2+} in a DMF/H_2O (1/1) solution with increasing amounts of TPEN.

in the cellular experiment because these metals rarely exist in a free form in cells.

These fluorescent ligands strictly differentiate the zinc ion from protons (Figure 9). Changes in pH do not induce any modifications in the fluorescence spectra of 1- and 3-isoTQEN. In the presence of zinc ion, 1- and 3-isoTQEN responded to zinc ion in the pH 2−13 and 2−10 regions, respectively, demonstrating the wide pH windows of these zinc sensors.

Competition Experiment. Because the binding constants of these ligands with zinc ion are too high to be determined by conventional titration methods, the competition experiments were performed using TPEN (Figure 10). To our surprise, the fluorescence intensities of zinc complexes with 1- and 3-isoTQEN were not affected by the addition of TPEN. To the best of our knowledge, this is the first example of a fluorescent zinc sensor from which TPEN does not completely sequester zinc ion. When 1- and 3-isoTQEN were added to a solution of the TPEN−Zn complex, only weak fluorescence corresponding to free isoTQENs was observed (data not shown). These results suggest that the dissociation rate constant k_{off} is very low for 1- and 3-isoTQEN.

Conclusions

The introduction of an isoquinoline ring in place of the quinoline ring of TQEN results in (i) the enhancement of the fluorescence intensity, (ii) red shifts in the excitation and emission wavelengths, (iii) an increased Zn^{2+}/Cd^{2+} discriminating ability, (iv) the reduction of background fluorescence induced by pH, and (v) the elimination of steric hindrance upon zinc binding to afford a ligand with a significantly strong metal binding affinity. These findings provide very important information for the future design of TPEN-based fluorescent sensors. The poor water solubility and the insufficient fluorescence intensity of 1- and 3-isoTQEN for cellular experiment will be optimized by the introduction of appropriate substituents into isoquinoline rings or the ethylenediamine framework.

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