Inorg. Chem. **2006**, *45*, 9262–9268

Methoxy-Substituted TQEN Family of Fluorescent Zinc Sensors

Yuji Mikata,*,† Motoko Wakamatsu,‡ Ayano Kawamura,‡ Natsuko Yamanaka,‡ Shigenobu Yano,‡ Akira Odani,§ Kazuko Morihiro,|| and Satoshi Tamotsu||

KYOUSEI Science Center, Division of Material Science, and Department of Biological Sciences, Nara Women's University, Nara 630-8506, Japan, and Research Center for Materials Science, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8602, Japan

Received May 11, 2006

Two methoxy-substituted TQEN (*N,N,N′,N′*-tetrakis(2-quinolylmethyl)ethylenediamine) derivatives, T(MQ)EN (*N,N,N′,N′*-tetrakis(6-methoxy-2-quinolylmethyl)ethylenediamine) and T(TMQ)EN (*N,N,N′,N′*-tetrakis(5,6,7-trimethoxy-2-quinolylmethyl)ethylenediamine), have been prepared, and their fluorescence properties with respect to Zn^{2+} coordination were investigated. Introduction of a methoxy substituent at 6-position of the quinoline ring enhances the fluorescence intensity by 10-fold, and the three methoxy substituents in the 5,6,7-positions afford significant enhancement of the long-wavelength component of the fluorescence of zinc complex. The substituents did not alter the binding affinity of these compounds toward zinc ion significantly. T(MQ)EN was proved to be effective in detection of zinc ion in cells by fluorescent microscopy.

Introduction

Zinc is the second most abundant transition metal ion in human body and is known to play many important roles in maintaining function in living organisms.[1−3] Since the mechanism of action of free zinc ion remains poorly understood, development of novel fluorescent probes[4−18] for zinc has attracted the attention of organic and bioinorganic chemists.

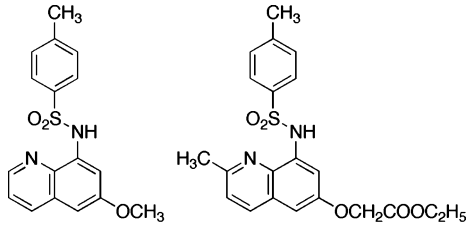
Among such fluorescent probes for zinc, quinoline-based molecules including TSQ[19,20] and Zinquin[21−27] (Chart 1) have been developed extensively.[28−34] Relatively small molecular size, synthetic accessibility, and metal-coordination or hy-

* To whom correspondence should be addressed. E-mail: mikata@cc.nara-wu.ac.jp. Phone/fax: +81-742-20-3095.
† *KYOUSEI* Science Center, Nara Women's University.
‡ Division of Material Science, Nara Women's University.
§ Nagoya University.
|| Department of Biological Sciences, Nara Women's University.

(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) Burdette, S. C.; Lippard, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 3605−3610.
(4) Koike, T.; Watanabe, T.; Aoki, S.; Kimura, E.; Shiro, M. *J. Am. Chem. Soc.* **1996**, *118*, 12696−12703.
(5) Burdette, S. C.; Walkup, G. K.; Spingler, B.; Tsien, R. Y.; Lippard, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 7831−7841.
(6) Burdette, S. C.; Frederickson, C. J.; Bu, W.; Lippard, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 1778−1787.
(7) Nolan, E. M.; Burdette, S. C.; Harvey, J. H.; Hilderbrand, S. A.; Lippard, S. J. *Inorg. Chem.* **2004**, *43*, 2624−2635.
(8) Nolan, E. M.; Lippard, S. J. *Inorg. Chem.* **2004**, *43*, 8310−8317.
(9) Woodroofe, C. C.; Won, A. C.; Lippard, S. J. *Inorg. Chem.* **2005**, *44*, 3112−3120.
(10) Chang, C. J.; Nolan, E. M.; Jaworski, J.; Okamoto, K.-I.; Hayashi, Y.; Sheng, M.; Lippard, S. J. *Inorg. Chem.* **2004**, *43*, 6774−6779.
(11) Hirano, T.; Kikuchi, K.; Urano, Y.; Nagano, T. *J. Am. Chem. Soc.* **2002**, *124*, 6555−6562.
(12) Hanaoka, K.; Kikuchi, K.; Kojima, H.; Urano, Y.; Nagano, T. *J. Am. Chem. Soc.* **2004**, *126*, 12470−12476.
(13) Taki, M.; Wolford, J. L.; O'Halloran, T. V. *J. Am. Chem. Soc.* **2004**, *126*, 712−713.
(14) Dai, Z.; Xu, X.; Canary, J. W. *Chem. Commun.* **2002**, 1414−1415.
(15) Dai, Z.; Proni, G.; Mancheno, D.; Karimi, S.; Berova, N.; Canary, J. W. *J. Am. Chem. Soc.* **2004**, *126*, 11760−11761.
(16) 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.
(17) Goldsmith, C. R.; Lippard, S. J. *Inorg. Chem.* **2006**, *45*, 555−561.
(18) Komatsu, K.; Kikuchi, K.; Kojima, H.; Urano, Y.; Nagano, T. *J. Am. Chem. Soc.* **2005**, *127*, 10197−10204.
(19) Frederickson, C. J.; Kasarskis, E. J.; Ringo, D.; Frederickson, R. E. *J. Neurosci. Methods* **1987**, *20*, 91−103.
(20) Savage, D. D.; Montano, C. Y.; Kasarskis, E. J. *Brain Res.* **1989**, *496*, 257−267.
(21) Zalewski, P. D.; Forbes, I. J.; Betts, W. H. *Biochem. J.* **1993**, *296*, 403−408.
(22) Zalewski, P. D.; Forbes, I. J.; Seamark, R. F.; Borlinghaus, R.; Betts, W. H.; Lincoln, S. F.; Ward, A. D. *Chem. Biol.* **1994**, *1*, 153−161.
(23) Mahadevan, I. B.; Kimber, M. C.; Lincoln, S. F.; Tiekink, E. R. T.; Ward, A. D.; Betts, W. H.; Forbes, I. J.; Zalewski, P. D. *Aust. J. Chem.* **1996**, *49*, 561−568.
(24) Fahrni, C. J.; O'Halloran, T. V. *J. Am. Chem. Soc.* **1999**, *121*, 11448−11458.
(25) Hendrickson, K. M.; Rodopoulos, T.; Pittet, P.-A.; Mahadevan, I.; Lincoln, S. F.; Ward, A. D.; Kurucsev, T.; Duckworth, P. A.; Forbes, I. J.; Zalewski, P. D.; Betts, W. H. *J. Chem. Soc., Dalton Trans.* **1997**, 3879−3882.
(26) Kimber, M. C.; Mahadevan, I. B.; Lincoln, S. F.; Ward, A. D.; Tiekink, E. R. T. *J. Org. Chem.* **2000**, *65*, 8204−8209.
(27) Hendrickson, K. M.; Geue, J. P.; Wyness, O.; Lincoln, S. F.; Ward, A. D. *J. Am. Chem. Soc.* **2003**, *125*, 3889−3895.
(28) Nasir, M. S.; Fahrni, C. J.; Suhy, D. A.; Kolodsick, K. J.; Singer, C. P.; O'Halloran, T. V. *J. Bioinorg. Chem.* **1999**, *4*, 775−783.

10.1021/ic060810x CCC: $33.50
Published on Web 10/14/2006

Chart 1



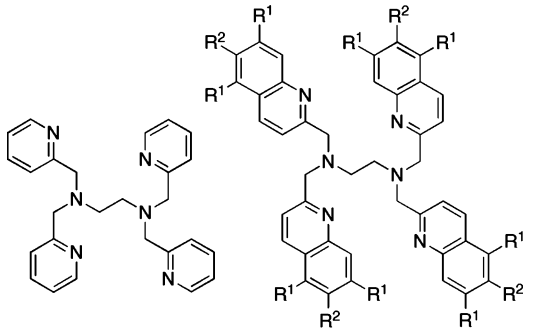
TSQ Zinquin

drogen-bonding ability of ring nitrogen make quinoline derivatives an attractive target for photofunctional molecular devices that are not limited in zinc sensors.[35−37] Steric hindrance caused by peri hydrogen and intermolecular $\pi-\pi$ stacking ability of the quinoline ring also exhibit characteristic coordination architecture in quinoline-based metal complexes.[38−41]

Recently, we synthesized *N*,*N*,*N*′,*N*′-tetrakis(2-quinolylmethyl)ethylenediamine (TQEN), the fluorescent zinc-sensing molecule derived from a well-known heavy metal chelator, *N*,*N*,*N*′,*N*′-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN) (Chart 2).[42] The mechanism of fluorescence of TQEN is based on CHEF (chelation-enhanced fluorescence)[31,43,44] of the (aminomethyl)quinoline ring, including the PET (photoinduced electron-transfer)[43,45] mechanism. TQEN binds to 1 equiv of zinc ion and emits fluorescence in wide range of pH, but short excitation and emission wavelengths as well as dim fluorescence intensity prevented further investigation of the compounds in cells.

In this paper, we report two TQEN derivatives, T(MQ)EN and T(TMQ)EN, in which one or three methoxy groups

Chart 2



TPEN

R^1=R^2=H: **TQEN**
R^1=H, R^2=OMe: **T(MQ)EN**
R^1=R^2=OMe: **T(TMQ)EN**

were introduced to each of quinoline rings of TQEN (Chart 2). Incorporation of methoxy group into TQEN affords an enhancement of fluorescent intensity and long-wavelength shift of the excitation/emission profile.

Experimental Section

General Methods. All reagents and solvents used for ligand synthesis were from commercial sources and used as received. *N*,*N*-Dimethylformamide (DMF, Dojin) was distilled over CaH_2 and passed through alumina column. All aqueous solution were prepared using deionized and redistilled water. ^{1}H NMR (300.07 Hz) and ^{13}C NMR (75.00 Hz) spectra were recorded on a Varian Gemini 2000 spectrometer and referenced to internal TMS or solvent signals. UV−vis and fluorescence spectra were measured on a Jasco V-700 spectrophotometer and Jasco FP-720 spectrofluorometer, respectively.

Caution: Perchlorate salts of metal complexes with organic ligands are potentially explosive. All due precautions should be taken!

***N*,*N*,*N*′,*N*′-Tetrakis(6-methoxy-2-quinolylmethyl)ethylenediamine (T(MQ)EN).** An agitated mixture of 2-(bromomethyl)-6-methoxyquioline (**2**) (0.076 g, 0.302 mmol), ethylenediamine (5 μL, 0.0755 mmol), and potassium carbonate (0.125 g, 0.904 mmol) in acetonitrile (6 mL) was refluxed for 14 h. After removal of the solvent, the residue was partitioned with chloroform/water, and the organic layer was dried and evaporated. The residue was washed with ethanol to give T(MQ)EN as a white powder (18.7 mg, 0.025 mol) in 33% yield. Recrystallization from chloroform−ether gave single crystals suitable for X-ray crystallography (Figure 1). Mp: 168−170 °C.

^{1}H NMR ($CDCl_3$) [δ (ppm)]: 2.84 (s, 4H), 3.89 (s, 8H), 3.91 (s, 12H), 6.94 (d, J = 2.7 Hz, 4H), 7.30 (dd, J = 2.7, 9.2 Hz, 4H), 7.48 (d, J = 8.24 Hz, 4H), 7.78 (d, J = 8.24 Hz, 4H), 7.88 (d, J = 9.2 Hz, 4H). ^{13}C NMR ($CDCl_3$) [δ (ppm)]: 52.78, 55.26, 55.50, 61.55, 105.05, 105.34, 121.35, 121.70, 121.81, 128.20, 130.21, 130.50, 134.87, 135.15, 143.51, 157.49, 157.94. Anal. Calcd for $C_{46}H_{44}N_6O_4$: H, 5.95; C, 74.17; N, 11.28. Found: H, 5.85; C, 73.92; N, 10.94. Crystal data: $C_{46}H_{44}N_6O_4$, monoclinic, space group *C*2/c with a = 12.6761(5) Å, b = 12.7480(5) Å, c = 24.158(1) Å, β = 95.677(2)°, V = 3884.7(3) Å^3, Z = 4, R = 0.052, and R_W = 0.118.

[Zn(T(MQ)EN)](ClO$_4$)$_2$. A solution of T(MQ)EN and an equimolar amount of zinc perchlorate hexahydrate in acetonitrile was stirred for 2 days. The solution was kept in a refrigerator under ether diffusion conditions to give single crystals of [Zn(T(MQ)EN)](ClO_4)$_2$ in 68% yield.

^{1}H NMR (CD_3CN) [δ (ppm)]: 3.14 (s, 4H), 3.86 (s,12H), 4.0 (br, 4H), 4.35 (d, J = 17.4 Hz, 4H), 6.92 (d, J = 8.1 Hz, 4H), 7.1

(29) Pearce, D. A.; Jotterand, N.; Carrico, I. S.; Imperiali, B. *J. Am. Chem. Soc.* **2001**, *123*, 5160−5161.
(30) Shults, M. D.; Pearce, D. A.; Imperiali, B. *J. Am. Chem. Soc.* **2003**, *125*, 10591−10597.
(31) Jotterand, N.; Pearce, D. A.; Imperiali, B. *J. Org. Chem.* **2001**, *66*, 3224−3228.
(32) Xue, G.; Bradshaw, J. S.; Dalley, N. K.; Savage, P. B.; Izatt, R. M.; Prodi, L.; Montalti, M.; Zaccheroni, N. *Tetrahedron* **2002**, *58*, 4809−4815.
(33) Walkup, G. K.; Imperiali, B. *J. Org. Chem.* **1998**, *63*, 6727−6731.
(34) Bronson, R. T.; Bradshaw, J. S.; Savage, P. B.; Fuangswasdi, S.; Lee, S. C.; Krakowiak, K. E.; Izatt, R. M. *J. Org. Chem.* **2001**, *66*, 4752−4758.
(35) Wei, L.; Babich, J.; Eckelman, W. C.; Zubieta, J. *Inorg. Chem.* **2005**, *44*, 2198−2209.
(36) Stephenson, K. A.; Banerjee, S. R.; Besanger, T.; Sogbein, O. O.; Levadala, M. K.; McFarlane, N.; Lemon, J. A.; Boreham, D. R.; Maresca, K. P.; Brennan, J. D.; Babich, J. W.; Zubieta, J.; Valliant, J. F. *J. Am. Chem. Soc.* **2004**, *126*, 8598−8599.
(37) Kubo, H.; Yoshioka, N.; Takeuchi, T. *Org. Lett.* **2005**, *7*, 359−362.
(38) Mikata, Y.; Wakamatsu, M.; So, H.; Abe, Y.; Mikuriya, M.; Fukui, K.; Yano, S. *Inorg. Chem.* **2005**, *44*, 7268−7270.
(39) Kryatov, S. V.; Taktak, S.; Korendovych, I. V.; Rybak-Akimova, E. V.; Kaizer, J.; Torelli, S.; Shan, X.; Mandal, S.; MacMurdo, V. L.; Payeras, A. M.; Que, L., Jr. *Inorg. Chem.* **2005**, *44*, 85−99.
(40) Wei, N.; Murthy, N. N.; Chen, Q.; Zubieta, J.; Karlin, K. D. *Inorg. Chem.* **1994**, *33*, 1953−1965.
(41) Wei, N.; Murthy, N. N.; Karlin, K. D. *Inorg. Chem.* **1994**, *33*, 6093−6100.
(42) Mikata, Y.; Wakamatsu, M.; Yano, S. *Dalton Trans.* **2005**, 545−550.
(43) 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.
(44) Castagnetto, J. M.; Canary, J. W. *Chem. Commun.* **1998**, 203−204.
(45) de Silva, A. P.; Fox, D. B.; Huxley, A. J. M.; Moody, T. S. *Coord. Chem. Rev.* **2000**, *205*, 41−57.

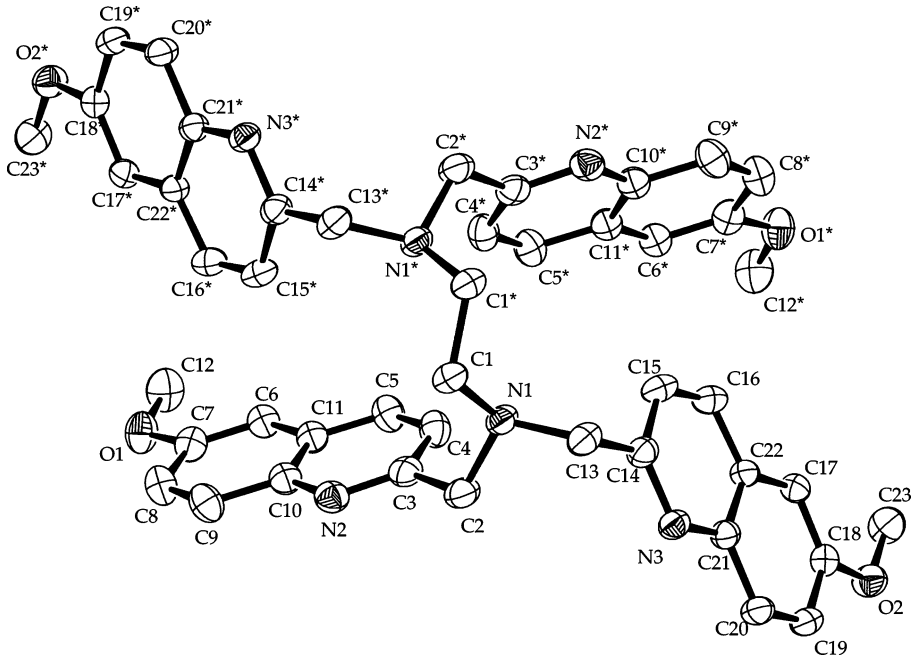


Figure 1. ORTEP plot for T(MQ)EN. Asterisks indicate the atoms generated by the symmetric operation.

(br, 4H), 7.36−7.41 (m, 8H), 8.41 (d, J = 8.1 Hz, 4H). ^{13}C NMR ($CDCl_3$) [δ (ppm)]: 56.65, 56.83, 62.70, 100.95, 108.30, 123.12, 124.67, 127.56, 131.74, 141.22, 157.39, 159.36. Anal. Calcd for $C_{46}H_{44}N_6O_{12}Cl_2Zn$ ([Zn(T(MQ)EN)]$(ClO_4)_2$): H, 4.39; C, 54.75; N, 8.33. Found: H, 4.43; C, 54.58; N, 7.98. Crystal data: $C_{46}H_{44}Cl_2N_6O_{12}Zn$, triclinic, space group $P\bar{1}$ with a = 12.355(3) Å, b = 13.418(3) Å, c = 13.574(3) Å, α = 80.628(6)°, β = 88.203(7)°, γ = 76.681(6)°, V = 2160.4(9) Å^3, Z = 2, R = 0.054, and R_W = 0.167.

5,6,7-Trimethoxyquinaldine (3). To a warm solution of 3,4,5-trimethoxyaniline (5.0 g, 27.3 mmol) in 6 N HCl (35 mL) was added crotonaldehyde (2.0 g, 28.6 mmol) dropwise, and the solution was refluxed for 1 h. After being cooled to room temperature, the reaction mixture was washed with ether, and then $ZnCl_2$ (3.72 g, 27.3 mmol) was added. To the aqueous solution, 5 M sodium hydroxide (15 mL) was added dropwise. After the mixture was stirred for 30 min at room temperature and 15 min at 0 °C, the resulting precipitate was collected and washed with 3 N HCl and 2-PrOH (∼100 mL). The air-dried white precipitate was treated with 40 mL of cold water and 15 mL of concentrated NH_3(aq) and extracted with ether. After the sample was dried over sodium sulfate, the ether was removed to give 1.94 g (8.32 mmol) of **3** as an green oil in 31% yield.

^{1}H NMR ($CDCl_3$) [δ (ppm)]: 2.69 (s, 3H), 3.97 (s, 3H), 4.00 (s, 3H), 4.05 (s, 3H), 7.15 (d, J = 8.5 Hz, 1H), 7.20 (s, 1H), 8.24 (d, J = 8.5 Hz, 1H). ^{13}C NMR ($CDCl_3$) [δ (ppm)]: 56.17, 61.38, 61.69, 61.72, 103.87, 117.49, 119.83, 119.90, 13079, 140.43, 145.96, 147.34, 156.17, 158.64.

5,6,7-Trimethoxy-2-quinolinecarbaldehyde (4). To the agitated mixture of SeO_2 (65 mg, 0.59 mmol) and 1,4-dioxane (5 mL) was added a solution of **3** (148 mg, 0.63 mmol) in the same solvent (1 mL) dropwise at 50 °C. The solution was warmed to 80 °C and stirred for 2 h. After filtration, the filtrate was evaporated to give 132 mg (0.53 mmol) of **4** in 84% yield.

^{1}H NMR ($CDCl_3$) [δ (ppm)]: 4.04 (s, 3H), 4.05 (s, 3H), 4.09 (s, 3H), 7.38 (s, 1H), 7.91 (d, J = 8.5 Hz, 1H), 8.51 (d, J = 8.5 Hz, 1H), 10.18 (s, 1H). ^{13}C NMR ($CDCl_3$) [δ (ppm)]: 56.20, 61.28, 61.61, 104.62, 115.41, 121.78, 131.60, 143.02, 145.81, 146.93, 152.08, 156.92, 193.87.

2-(Hydoxymethyl)-5,6,7-trimethoxyquinoline (5). To the solution of **4** (18.5 mg, 0.075 mmol) in ethanol (2 mL) was added $NaBH_4$ (15.1 mg, 0.375 mmol), and the mixture was stirred for 3 h at room temperature. After acetic acid was added until hydrogen gas evolution ceased, the solvent was evaporated. The residue was partitioned with dichloromethane/water, and the organic layer was washed with saturated $NaHCO_3$(aq) and water, dried, and evaporated to give **5** in quantitative yield.

^{1}H NMR ($CDCl_3$) [δ (ppm)]: 3.98 (s, 3H), 4.03 (s, 3H), 4.07 (s, 3H), 4.88 (s, 2H), 7.16 (d, J = 8.2 Hz, 1H), 7.23 (s, 1H), 8.34 (d, J = 8.2 Hz, 1H). ^{13}C NMR ($CDCl_3$) [δ (ppm)]: 56.05, 61.21, 61.55, 64.07, 103.50, 115.96, 118.45, 131.29, 140.68, 144.63, 147.21, 156.32, 158.54.

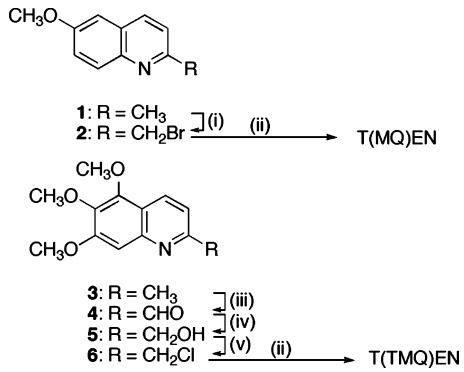
2-(Chloromethyl)-5,6,7-trimethoxyquinoline (6). To the solution of **5** (85.5 mg, 0.343 mmol) in dichloromethane (10 mL) was added thionyl chloride (0.36 mL, 5.2 mmol) dropwise, and the reaction solution was stirred overnight at room temperature. After addition of water, the organic layer was separated and the aqueous solution was extracted with dichloromethane. The combined organic layer was dried and evaporated to give 85 mg (0.32 mmol) of **6** in 93% yield.

^{1}H NMR ($CDCl_3$) [δ (ppm)]: 3.99 (s, 3H), 4.02 (s, 3H), 4.07 (s, 3H), 4.81 (s, 2H), 7.27 (s, 1H), 7.47 (d, J = 8.2 Hz, 1H), 8.41 (d, J = 8.2 Hz, 1H). ^{13}C NMR ($CDCl_3$) [δ (ppm)]: 47.00, 56.13, 61.19, 61.55, 103.70, 118.19, 118.72, 132.04, 141.26, 145.14, 146.97, 156.03, 156.65.

***N,N,N′,N′*-Tetrakis(5,6,7-trimethoxy-2-quinolylmethyl)ethylenediamine (T(TMQ)EN).** To a solution of **6** (260 mg, 0.97 mmol) in acetonitrile (5 mL) was added dropwise an agitatated mixture of ethylenediamine (16 μL, 0.24 mmol) and potassium carbonate (402 mg, 2.9 mmol) in acetonitrile (5 mL). The reaction mixture was refluxed for 4 days. After removal of the solvent, the residue was partitioned with chloroform/water and the aqueous layer was further extracted with chloroform. The combined aqueous layer was dried and evaporated, and the residue was purified by column chromatography (silica gel; eluent, 10:1 chloroform/methanol) to give 49 mg (0.048 mmol) of T(TMQ)EN in 20% yield.

^{1}H NMR ($CDCl_3$) [δ (ppm)]: 2.90 (brs, 4H), 3.89 (brs, 8H), 3.96 (s, 24H), 4.01 (s, 12H), 7.15 (s, 4H), 7.44 (d, J = 8.5 Hz, 4H),

Scheme 1



8.18 (d, J = 8.5 Hz, 4H). ^{13}C NMR ($CDCl_3$) [δ (ppm)]: 52.54, 55.87, 61.01, 61.34, 61.42, 103.66, 117.94, 118.60, 130.36, 140.31, 145.05, 146.74, 155.62, 159.57. Anal. Calcd for $C_{57}H_{72}N_6O_{15}$ (T(TMQ)EN·3CH_3OH): H, 6.71; C, 63.32; N, 7.77. Found: H, 6.13; C, 63.21; N, 7.31.

Cell Experiment. PC-12 rat adrenal pheochromocytoma cells were cultured in RPMI 1640 supplemented with 5% fetal bovine serum (FBS), 10% horse serum (HS), and 1% penicillin−streptomycin (PS). All cells were maintained in a humidified incubator at 37 °C and 5% CO_2. The media was changed to that containing 50 μM T(MQ)EN and 2% DMSO and incubated for 4 h. Cells were rinsed with FBS, soaked in the growth media, and then analyzed with a fluorescent microscope. As a control experiment, cells were incubated without T(MQ)EN and in the presence of T(MQ)EN and Zn−pyrithione (45 μM). In addition, the cells incubated with T(MQ)EN for 4 h were further incubated in the presence of TPEN (660 μM) for 20 min, rinsed with FBS, soaked in the growth media, and analyzed.

Results and Discussion

Synthesis of Ligands. T(MQ)EN and T(TMQ)EN were synthesized from ethylenediamine and 4 equiv of (halomethyl)quinolines (Scheme 1). 2-(Bromomethyl)-6-methoxyquinoline (**2**) was obtained from 6-methoxyquinaldine (**1**) using NBS;[46] however, bromination of 5,6,7-trimethoxyquinaldine (**3**) under similar reaction condition was unsuccessful. Thus, 2-(chloromethyl)-5,6,7-trimethoxyquinoline (**6**) was prepared in three steps as shown in Scheme 1. T(MQ)EN and T(TMQ)EN were obtained as a white powder and characterized by ^{1}H/^{13}C NMR and elemental analysis. T(MQ)EN was further characterized by X-ray crystallography (Figure 1).

Fluorescent Spectra. In aqueous DMF solution (1:1 DMF/water (v/v)), T(MQ)EN exhibits a broad, weak fluorescence (λ_{ex} = 332 nm ($\epsilon = 1.6 \times 10^4$ M^{-1} cm^{-1}), λ_{em} = 389 nm) that is enhanced 13-fold in the presence of 1 equiv of zinc ion (λ_{em} = 408 nm, Figure 2). The T(MQ)EN−Zn complex exhibits 10 times higher emission than unsubstituted TQEN−Zn complex, indicating a significant fluorescence enhancement achieved by the introduction of a methoxy group on each of quinoline rings. A Job plot analysis reveals that T(MQ)EN binds 1 equiv of zinc ion (Figure S1).

Free T(TMQ)EN exhibits moderate fluorescence (λ_{ex} = 332 nm ($\epsilon = 2.7 \times 10^4$ M^{-1} cm^{-1}), λ_{em} = 430 nm) in the same solvent. Upon addition of 1 eqiuv of zinc ion, the fluorescence maximum shifts to 493 nm with a simultaneous 2−3-fold enhancement of fluorescence intensity (Figure 3). The changes in wavelength and intensities allow ratiometric analysis with this compound using I_{493}/I_{430}, which changes from 0.6 to 2.0 upon addition of an equimolar amount of zinc.

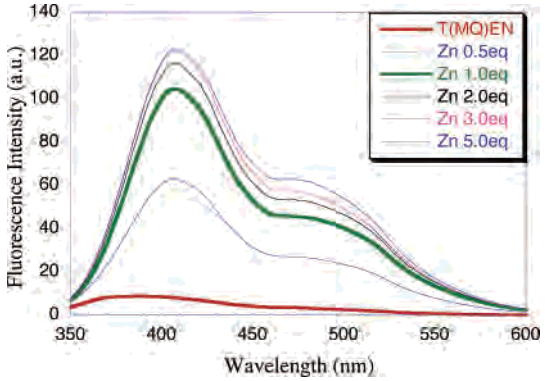


Figure 2. Fluorescence spectra (λ_{ex} = 332 nm) of 34 μM T(MQ)EN in DMF/H_2O (1:1) in the presence of various concentration of Zn^{2+} ranging from 0 to 170 μM.

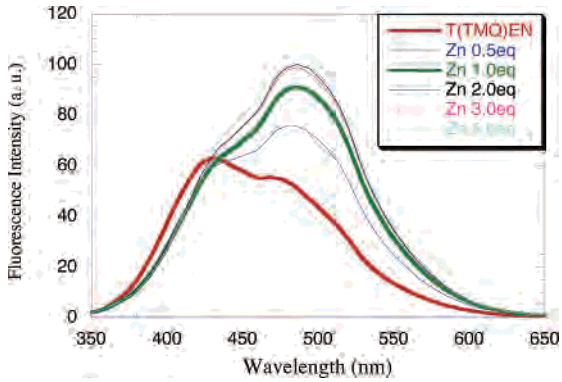


Figure 3. Fluorescence spectra (λ_{ex} = 332 nm) of 34 μM T(TMQ)EN in DMF/H_2O (1:1) in the presence of various concentration of Zn^{2+} ranging from 0 to 170 μM.

The fluorescence spectrum of 6-methoxyquinoline in aqueous solution has two emission bands that vary in intensity with pH.[47−49] At high pH, a single emission band exists with a maximun at 370 nm (neutral form emission), whereas, at low pH, the emission maximum is shifted to 442 nm (cation form emission). Both T(MQ)EN and T(TMQ)EN have two absorption bands similar to neutral form emission (408 and 430 nm) and cation form emission (493 nm). Thus, the fluorescent spectral change of T(TMQ)EN observed in Figure 3 could be attributed to the electronic structure perturbation upon zinc binding. The polarized electronic structure similar to the protonated form stabilized by three methoxy substituents becomes dominant in the zinc complex of T(TMQ)EN. Another explanation for long-wavelength emission of T(TMQ)EN upon zinc binding is excimer formation. Several works in the literature docu-

(46) Jones, P. D.; Glass, T. E. *Tetrahedron* **2004**, *60*, 11057−11065.

(47) Pines, E.; Huppert, D.; Gutman, M.; Nachliel, N.; Fishman, M. *J. Phys. Chem.* **1986**, *90*, 6366−6370.

(48) Pant, D.; Tripathi, H. B.; Pant, D. D. *J. Photochem. Photobiol., A: Chem.* **1991**, *56*, 207−217.

(49) Pant, D.; Tripathi, H. B.; Pant, D. D. *J. Lumin.* **1992**, *51*, 223−230.

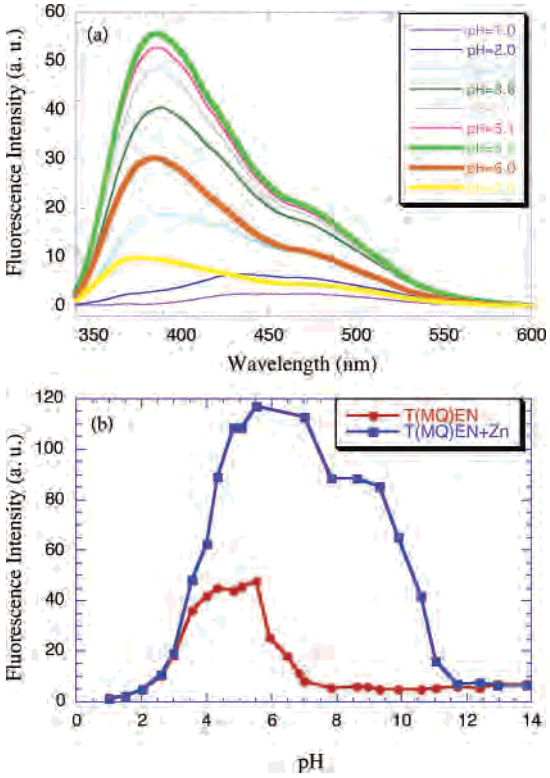


Figure 4. Effect of pH on fluorescence spectra (λ_{ex} = 332 nm) of 34 μM T(MQ)EN in DMF/H_2O (1:1): (a) fluorescence spectra of T(MQ)EN in the absence of zinc ion at pH 1.0−7.0; (b) plot of fluorescence intensity of T(MQ)EN at 408 nm in the absence (red circles) and presence (blue squares) of 1 equiv of Zn^{2+}.

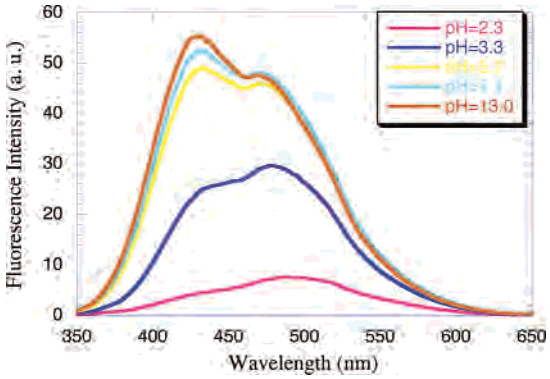


Figure 5. Effect of pH on fluorescence spectra (λ_{ex} = 332 nm) of 34 μM T(TMQ)EN in DMF/H_2O (1:1) in the absence of Zn^{2+}.

mented excimer emission wavelengths of quinoline derivatives around 490 nm.[50,51] The close proximity of quinoline rings to form an excimer in face to face interaction resulting from zinc binding could be responsible for the 493 nm emission of T(TMQ)EN.

Fluorescent intensities of T(MQ)EN and T(TMQ)EN were affected by pH to some extent (Figures 4 and 5). To find out the proton-induced fluorescent species, the pK_a values for TQEN, T(MQ)EN, and T(TMQ)EN were determined by pH titration at 25 °C, I = 0.1 (KCl, 80% (v/v) DMF/H_2O). Although pK_a values for quinoline nitrogen atoms were too low to be determined under the present experimental condition (ligand = 0.1−0.2 mM, [H^+]/[ligand] = 7, 80% (v/v) DMF/H_2O), those for aliphatic nitrogens were successfully determined to be pK_{a6} = 3.76 ± 0.04 and pK_{a5} = 3.63 ± 0.03 for TQEN, pK_{a6} = 3.75 ± 0.03 and pK_{a5} = 2.98 ± 0.07 for T(MQ)EN, and pK_{a6} = 3.81 ± 0.02 and pK_{a5} = 3.46 ± 0.03 for T(TMQ)EN. Considering the fact that pH = 3.7 and 3.0 on our electrode correspond to p{H} = 5.6 and 3.9 under the present experimental condition, respectively, the fluorescent species should be monoprotonated TQEN derivatives. Free and di- (or higher) protonated TQEN derivatives are essentially nonfluorescent except free T(TMQ)EN. For T(MQ)EN, the proton-induced fluorescence enhancement is small compared to those induced by zinc binding and could be negligible above p{H} = 6 (Figure 4b).

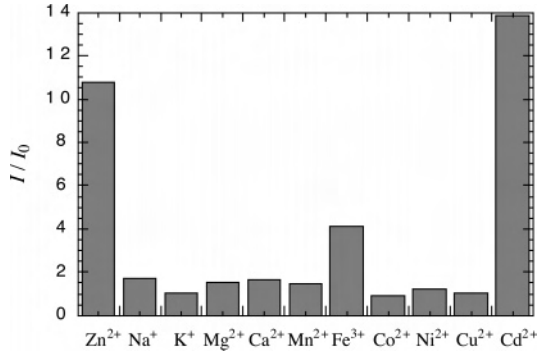


Figure 6. Relative fluorescence intensity at 408 nm of T(MQ)EN (34 μM in DMF/H_2O (1:1)) corresponding to 1 equiv of metal ions at 25 °C (λ_{ex} = 332 nm). I_0 is the emission intensity at 408 nm of T(MQ)EN (34 μM) in the absence of metal ions.

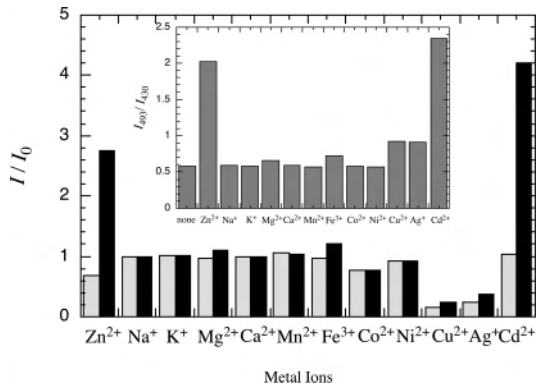


Figure 7. Relative fluorescence intensity at 430 nm (striped bar) and 493 nm (solid bar) of T(TMQ)EN (34 μM in DMF/H_2O (1:1)) responding to 1 equiv of metal ions at 25 °C (λ_{ex} = 332 nm). I_0 is the emission intensity of T(TMQ)EN (34 μM) in the absence of metal ions. Inset: Ratiometric fluorescent analysis (I_{493}/I_{430}) of T(TMQ)EN responding to 1 equiv of metal ions.

Metal Ion Selectivity in Fluorescence Spectral Change. Figures 6 and 7 show the fluorescent response of T(MQ)EN and T(TMQ)EN in the presence of other metal ions. For T(TMQ)EN, metal selectivity is also analyzed by the I_{493}/I_{490} ratiometric measurements (Figure 7, inset). These measurements reveal that the sensors are sensitive to cadmium ion as well as zinc. These results are consistent with the previous studies with TQEN and other quinoline- or DPA(dipicolylamine)-based fluorescent zinc sensors which also exhibits Zn^{2+}- and Cd^{2+}-dependent fluorescence

(50) Chen, T.-R. *J. Mol. Struct.* **2005**, *737*, 35−41.
(51) Ghosh, K.; Adhikari, S. *Tetrahedron Lett.* **2006**, *47*, 3577−3581.

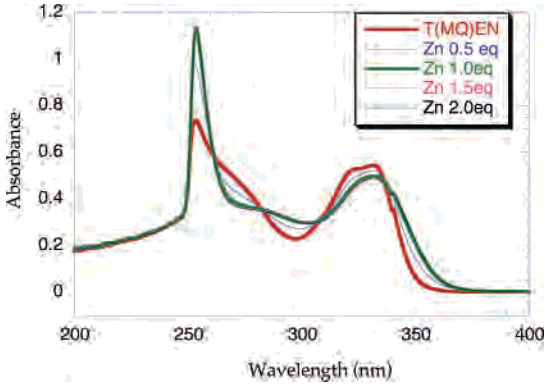


Figure 8. UV−vis spectra of 34 μM T(MQ)EN in DMF/H_2O (1:1) in the presence of various concentration of Zn^{2+} ranging from 0 to 68 μM.

enhancement.[6−8,12,16,17,30,52] It is interesting to note that the increasing number of methoxy substituents enhances cadmium complex emission relative to zinc (I_{Cd}/I_{Zn} = 0.6 for TQEN, 1.3 for T(MQ)EN, and 1.5 for T(TMQ)EN (λ_{em} = 490 nm)). The filled d^{10} shell of Cd^{2+}, as well as Zn^{2+}, induces efficient fluorescence enhancement of TQEN derivatives by PET inhibition; however, this does not cause a problem in cellular experiment because a very small amount of Cd^{2+} exists in cells.

The presence of equimolar amount of Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, and Ni^{2+} does not interfere with fluorescence from the zinc complex; however, the absence of emission from T(MQ)EN− and T(TMQ)EN−Zn complexes in the presence of an equimolar amount of Zn^{2+} and Cu^{2+} or Co^{2+} indicates the affinity of Zn^{2+} is weaker than that for Cu^{2+} and Co^{2+}. The binding affinity of these molecules with cadmium is also higher than that for zinc because the emission intensity for cadmium complex was not affected by addition of zinc. The order of the binding affinity (Fe < Cu > Zn) is in good agreement with the Irving−Williams series of complex stability for all compounds in the TQEN family.[53]

Absorption Spectra. Absorption changes (Figure 8) of T(MQ)EN with titration of increasing amounts of zinc ion exhibit isosbestic points at 261, 282, 308, and 337 nm. Similar absorbance changes were observed for the metal ions that have a higher affinity for the ligand than zinc (Cu^{2+}, Co^{2+}, and Cd^{2+}). Other metal ions do not affect the absorbance spectra of T(MQ)EN. Zinc ion induced very small absorbance change for T(TMQ)EN (Figure S2).

Binding Affinity and Quantum Yield. Binding affinity was estimated in 50% (v/v) aqueous DMF from analysis of fluorescence titration data by a nonlinear curve fit procedure (see Supporting Information for details). Obtained K_d values are $(7.0 \pm 3.2) \times 10^{-6}$, $(1.2 \pm 0.3) \times 10^{-6}$, and $(2.9 \pm 1.5) \times 10^{-6}$ M for TQEN, T(MQ)EN, and T(TMQ)EN, respectively. The 6-methoxy substituent slightly enhances complexation to some extent, but the effect is small.

Fluorescence quantum yields were determined to be 0.02 for T(MQ)EN−Zn complex and 0.03 for T(TMQ)EN−Zn complex. These values are rather low but sufficient for fluorescent microscopic analysis.[36]

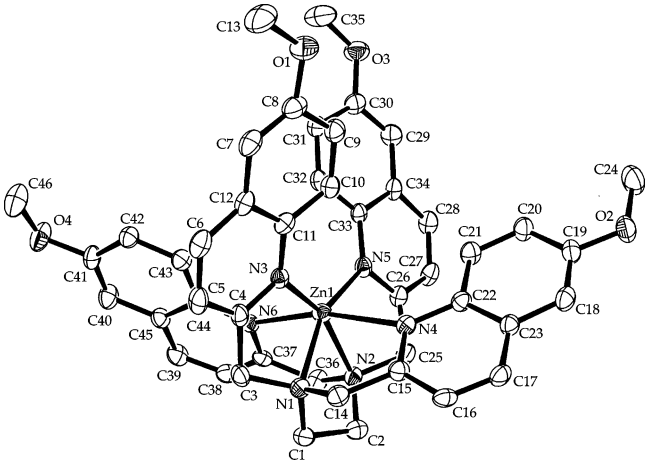


Figure 9. ORTEP plot for $[Zn(T(MQ)EN)](ClO_4)_2$. Counteranions were omitted for clarity. Selected interatomic distances (Å): Zn1−N1, 2.167(4); Zn1−N2, 2.192(3); Zn1−N3, 2.157(3); Zn1−N4, 2.430(3); Zn1−N5, 2.119(3); Zn1−N6, 2.277(3).

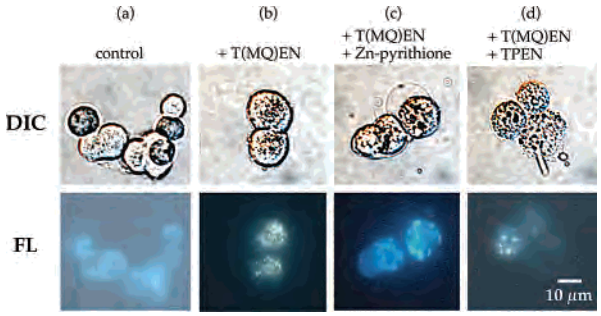


Figure 10. Differential interference contrast (DIC, top) and fluorescent (FL, bottom) micrographs of cultured PC-12 rat adrenal cells: (a) control; (b) incubated with T(MQ)EN (50 μM); (c) incubated with T(MQ)EN + Zn−pyrithione (45 μM); (d) incubated with T(MQ)EN and then incubated with TPEN (660 μM).

Crystal Structure. The structure of the T(MQ)EN−Zn complex was analyzed by crystallographic analysis of single crystals (Figure 9). T(MQ)EN binds zinc ion by six nitrogen atoms in a manner analogous to the TQEN−Zn and TPEN−Zn complexes.[42] The structural analysis reveals that the coordination environment around zinc is similar to the TQEN−Zn and TPEN−Zn complexes with respect to bond lengths. Compared to TQEN−Zn complex, T(MQ)EN−Zn complex has longer N1,N2,N4−Zn distances, similar N3,N5−Zn distances, and a significantly shortened N6−Zn bond length. The twist angle between two closely located quinoline rings containing N3 and N5 is greater for T(MQ)EN−Zn complex (~30°) than TQEN−Zn complex (~22°). This structure could support the excimer mechanism for T(TMQ)-EN emission.

Cellular Experiment. A cell experiment was performed using PC-12 rat adrenal pheochromocytoma (Figure 10). The cells become fluorescent after incubation with 50 μM T(MQ)EN. This fluorescence is enhanced by co-incubation with Zn−pyrithione but quenched by subsequent incubation with TPEN. The cell permeability of T(MQ)EN and reversibility of fluorescence upon zinc binding is thus ensured.

(52) Walkup, G. K.; Burdette, S. C.; Lippard, S. J.; Tsien, R. Y. *J. Am. Chem. Soc.* **2000**, *122*, 5644−5645.
(53) Irving, H.; Williams, R. J. P. *J. Chem. Soc.* **1953**, 3192−3210.

Conclusions

Methoxy-subtituted, hexadentate nitrogen ligands based on TPEN-like structures, T(MQ)EN and T(TMQ)EN, have been prepared. The quinoline moiety of these TQEN family molecules acts not only as a recognition site but also as a fluorophore with CHEF mechanism. Introduction of one methoxy group into each of the quinoline rings of TQEN affords an enhancement of fluorescence intensity, whereas three methoxy substituents on the quinoline moiety produce a ratiometric fluorescent sensor. The benefits of methoxy substitution in TQEN derivatives will lead not only to a new generation of fluorescent sensors for metal ions but also to a rational design for quinoline-based photofunctional molecules.

Acknowledgment. This work was supported by a Nara Women's University Intramural Grant for Project Research and a Grant-in Aid for Scientific Research from the MEXT of Japan.

Supporting Information Available: Experimental procedure, Table S1 for crystallographic data, Figures S1 and S2, and CIF files. This material is available free of charge via the Internet at http://pubs.acs.org.

IC060810X