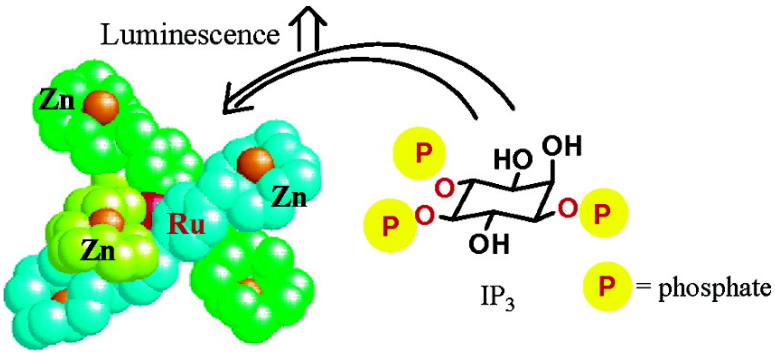
Article

A Luminescence Sensor of Inositol 1,4,5-Triphosphate and Its Model Compound by Ruthenium-Templated Assembly of a Bis(Zn-Cyclen) Complex Having a 2,2‘-Bipyridyl Linker (Cyclen = 1,4,7,10-Tetraazacyclododecane)

Shin Aoki, Mohd Zulkefeli, Motoo Shiro, Masanori Kohsako, Kei Takeda, and Eiichi Kimura

J. Am. Chem. Soc., **2005**, 127 (25), 9129-9139• DOI: 10.1021/ja050876b • Publication Date (Web): 04 June 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 5 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
ARTICLES
Published on Web 06/04/2005

A Luminescence Sensor of Inositol 1,4,5-Triphosphate and Its Model Compound by Ruthenium-Templated Assembly of a Bis(Zn^{2+}-Cyclen) Complex Having a 2,2′-Bipyridyl Linker (Cyclen = 1,4,7,10-Tetraazacyclododecane)

Shin Aoki,*[,†,‡] Mohd Zulkefeli,[§] Motoo Shiro,[⊥] Masanori Kohsako,[§] Kei Takeda,[§] and Eiichi Kimura[||]

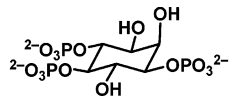
Contribution from the Faculty of Pharmaceutical Sciences and Center for Drug Delivery Research, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba, 278-8510 Japan, Division of Medicinal Chemistry, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima, 734-8551 Japan, Rigaku Corporation X-ray Research Laboratory, 3-9-12 Matsubaracho, Akishima, Tokyo, 196-8666 Japan, and Faculty of Integrated Arts and Sciences, Hiroshima University,1-7-1 Kagamiyama, Higashi-Hiroshima, 739-8521 Japan

Received February 10, 2005; E-mail: shinaoki@rs.noda.tus.ac.jp

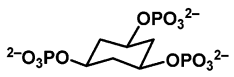
Abstract: A new supramolecular complex ($Ru(Zn_2L^4)_3$) was designed and synthesized as a luminescence sensor for inositol 1,4,5-triphosphate (IP_3), which is one of the important second messengers in intracellular signal transduction, and its achiral model compound, *cis,cis*-1,3,5-cyclohexanetriol triphosphate (CTP_3), by a ruthenium(II)-templated assembly of three molecules of a bis(Zn^{2+}-cyclen) complex having a 2,2-bipyridyl linker (Zn_2L^4). Single-crystal X-ray diffraction analysis of a racemic mixture of $Ru(Zn_2L^4)_3$ showed that three of the six Zn^{2+}-cyclen units are orientated to face the opposite side of the molecule with three apical ligands (Zn^{2+}-bound HO^-) of each of the three Zn^{2+} located on the same face. ^1H NMR and UV titrations of $Ru(Zn_2L^4)_3$ with CTP_3 indicated that $Ru(Zn_2L^4)_3$ forms a 1:2 complex with CTP_3, $(Ru(Zn_2L^4)_3)-((CTP_3)^{6-})_2$, in aqueous solution at neutral pH. In the absence of guest molecules, $Ru(Zn_2L^4)_3$ (10 μM) has an emission maximum at 610 nm at pH 7.4 (10 mM HEPES with $I = 0.1$ ($NaNO_3$)) and 25 °C (excitation at 300 nm). An addition of 2 equiv of CTP_3 induced a 4.2-fold enhancement in the emission of $Ru(Zn_2L^4)_3$ at 584 nm. In this article, we describe that $Ru(Zn_2L^4)_3$ is the first chemical sensor that directly responds to CTP_3 and IP_3 and discriminates these triphosphates from monophosphates and diphosphates. The photodecomposition of $Ru(Zn_2L^4)_3$, which is inhibited upon complexation with CTP_3, and the stereoselective complexation of chiral IP_3 by $Ru(Zn_2L^4)_3$ are also described.

Introduction

Inositol 1,4,5-triphosphate (IP_3) is one of the important second messengers in intracellular signal transduction.[1] The hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) located in the plasma membrane by a specific phospholipase C (PLC) releases IP_3, which induces an increase of Ca^{2+} concentrations in living cells. To date, a large number of fluorescent probes for Ca^{2+} have been developed and used to investigate intracellular events accompanied by the increase in intracellular free Ca^{2+} concentrations.[2,3] Thus far, only a few biological and chemical sensing systems for IP_3 and related phosphates have been developed because IP_3 does not have a chromophore,[4−8] and specific chemical motifs for IP_3 recognition have not been explored.



Inositol 1,4,5-triphosphate (IP_3)



cis,cis-1,3,5-Cyclohexanetriol triphosphate (CTP_3)

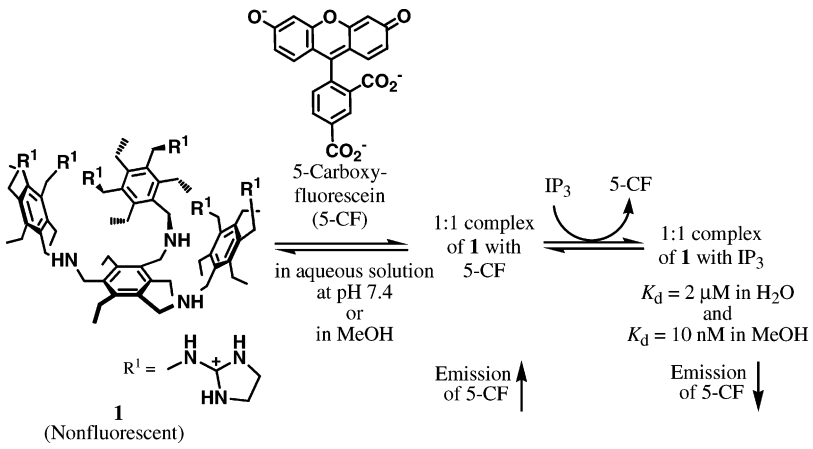
As for biological IP_3 sensors, Hirose and co-workers have developed the green fluorescent protein (GFP)-tagged pleckstrin

† Faculty of Pharmaceutical Sciences, Tokyo University of Science.
‡ Center for Drug Delivery Research, Tokyo University of Science.
§ Graduate School of Biomedical Sciences, Hiroshima University.
⊥ Rigaku Corporation.
|| Faculty of Integrated Arts and Sciences, Hiroshima University.

(1) (a) Reitz, A. B. *Inositol Phosphates and Derivatives*; American Chemical Society: Washington, DC, 1991. (b) Berridge, M. J. *Nature* **1993**, *361*, 315−325. (c) Potter, B. V. L.; Lampe, D. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1933−1972. (d) Taylor, C. W. *Biochim. Biophys. Acta* **1998**, *1436*, 19−33. (e) Shears, S. B. *Biochim. Biophys. Acta* **1998**, *1436*, 49−67. (f) Fishman, H. A.; Greenwald, D. R.; Zare, R. N. *Annu. Rev. Biophys. Biomol. Struct.* **1998**, *27*, 165−198. (g) Hinterding, K.; Alonso-Díaz, D.; Waldmann, H. *Angew. Chem., Int. Ed.* **1998**, *37*, 688−749. (h) Taylor, C. W. *Biochim. Biophys. Acta* **1998**, *1436*, 19−33. (i) Irvine, R. *Curr. Biol.* **2001**, *11*, R172−R174. (j) Taylor, C. W.; Thorn, P. *Curr. Biol.* **2001**, *11*, R352−R355.

(2) Haugland, R. R. *Handbook of Fluorescent Probes and Research Products*; Molecular Probes: Eugene, OR, 2002.
(3) (a) Grynkiewicz, G.; Poenie, M.; Tsien, R. Y. *J. Biol. Chem.* **1985**, *260*, 3440−3450. (b) Minta, A.; Kao, J. P. Y.; Tsien, R. Y. *J. Biol. Chem.* **1989**, *264*, 8171−8178. (c) Miyawaki, A.; Griesbeck, O.; Heim, R.; Tsien, R. Y. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 2135−2140.
(4) Hirose, K.; Kadowaki, S.; Tanabe, M.; Takeshima, H.; Iino, M. *Science* **1999**, *284*, 1527−1530.

Scheme 1



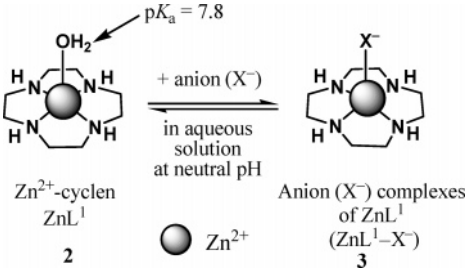
homology (PH) domain of PLC-δ_1 (GFP−PH), which binds to PIP_2 within the plasma membrane and to IP_3 in the cytoplasma.[4] Intracellular translocation of IP_3, which is produced by PLC-catalyzed hydrolysis of PIP_2, from the plasma membrane into cytoplasmic regions was monitored by GFP−PH, revealing that the spatiotemporal dynamics in the concentration of IP_3 is synchronous with Ca^{2+} oscillations. Allbritton et al. have utilized cultured cells as IP_3 detectors, in which the effluent from a sampling/electrophoresis capillary containing IP_3 was directed onto permeabilized *Xenopus* oocyte cells, from which Ca^{2+} is released. Changes in the resultant Ca^{2+} concentration was monitored by Ca^{2+}-selective fluorophores.[5] In 2002, Bayley et al. reported stochastic sensing of IP_3 utilizing a staphylococcal α-hemolysin homoheptamer modified with arginine (Arg) residues in the lumen of the heptameric pore. IP_3 specifically blocks channel conductivity of the heptameric pore of engineered α-hemolysin, permitting the detection of IP_3 at nanomolar concentrations.[6]

In contrast, progress in chemical IP_3 sensors has been much slower than that in biological sensors while many phosphate receptors and sensors have been reported.[9,10] A displacement-based assay system developed by Anslyn and co-workers using a combination of a nonfluorescent receptor and 5-carboxyfluorescein (5-CF) is the only chemical IP_3 sensing (Scheme 1) reported.[11,12] A C_3-symmetric receptor having six guanidinium cations **1** binds strongly to 5-CF as well as to IP_3. Fluorescence emission of 5-CF is enhanced upon formation of the 1:1 **1**−5-CF complex (excitation at 450 nm and emission at ca. 530 nm). An addition of IP_3 to a solution of **1**−5-CF complex induced displacement of 5-CF, yielding the **1**−IP_3 complex, resulting in a decrease in the fluorescent emission of 5-CF, which allows sensing of IP_3. To the best of our knowledge, no chemical sensors that directly respond to IP_3 have been reported.

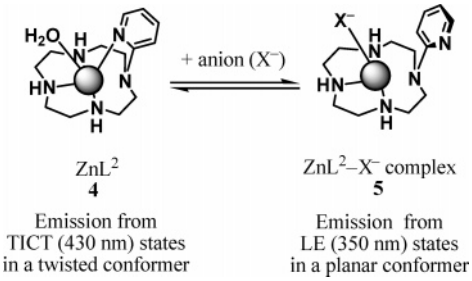
It has been proven that Zn^{2+} complexes of macrocyclic tetraamine derivatives such as Zn^{2+}-cyclen **2** (ZnL^1) are good models for Zn^{2+} enzymes (cyclen = 1,4,7,10-tetraazacyclododecane) and form 1:1 complexes (such as **3**) with anions (X^-) including phosphate monoesters, imidates (e.g., thymine), and thiolates in aqueous solution at neutral pH (Scheme 2).[13,14] Therefore, Zn^{2+}-cyclen derivatives are promising recognition

(5) (a) Luzzi, V.; Sims, C. E.; Soughayer, J. S.; Allbritton, N. L. *J. Biol. Chem.* **1998**, *273*, 28657−38662. (b) Luzzi, V.; Murtazina, D.; Allbritton, N. L. *Anal. Biochem.* **2000**, *277*, 221−227.
(6) Cheley, S.; Gu, L.-Q.; Bayley, H. *Chem. Biol.* **2002**, *9*, 829−838.
(7) Yamakoshi, M.; Takahashi, M.; Kouzuma, T.; Imamura, S.; Tsuboi, I.; Kawazu, S.; Yamagata, F.; Tominaga, M.; Noritake, M. *Clin. Chim. Acta* **2003**, *328*, 163−171.
(8) Anslyn, E. V.; Shear, J. B. H. *Chem. Biol.* **2002**, *9*, 779−780.
(9) (a) Czarnik, A. W. *Fluorescent Chemosensors for Ion and Molecule Recognition*; American Chemical Society: Washington, DC, 1992. (b) Lehn, J.-M. *Supramolecular Chemistry*: *Concepts and Perspectives*; VCH: Weinheim, Germany, 1995. (c) *Supramolecular Chemistry of Anions*; Bianchi, A., Bowman-James, K., Garcia-Espana, E., Eds.; Wiley-VCH: New York, 1996. (d) Sessler, J. L.; Andrievsky, A.; Genge, J. W. In *Advances in Supramolecular Chemistry*; Gokel, G. W., Ed.; JAI Press Inc.: Greenwich, CT, 1997; Vol. 4, pp 97−142. (e) Valeur, B. *Molecular Fluorescence*; Wiley-VCH: Weinheim, Germany, 2002. (f) Seel, C.; Galán, A.; de Mendoza, J. *Top. Curr. Chem.* **1995**, *175*, 101−132. (g) Scheerder, J.; Engbersen, J. F. J.; Reinhoudt, D. N. *Recl. Trav. Chim. Pays-Bas* **1996**, *115*, 307−320. (h) 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. (i) Linton, B.; Hamilton, A. D. *Chem. Rev.* **1997**, *97*, 1669−1680. (j) Rybak-Akimova, E. V. *Rev. Inorg. Chem.* **2001**, *21*, 207−298. (k) Martínez-Mánez, R.; Sancenón, F. *Chem. Rev.* **2003**, *103*, 4419−4476.
(10) (a) Hosseini, M. W.; Blacker, A. J.; Lehn, J.-M. *J. Am. Chem. Soc.* **1990**, *112*, 3896−3904. (b) Vance, D. H.; Czarnik, A. W. *J. Am. Chem. Soc.* **1994**, *116*, 9397−9398. (c) Baudoin, O.; Gonnet, F.; Teulade-Fichou, M.-P.; Vigneron, J.-P.; Tabet, J.-C.; Lehn, J.-M. *Chem.−Eur. J.* **1999**, *5*, 2762−2771. (d) Berger, M.; Schmidtchen, F. P. *J. Am. Chem. Soc.* **1999**, *121*, 9986−9993. (e) Parker, D.; Senanayake, P. K.; Williams, J. A. G. *J. Chem. Soc., Chem. Commun.* **1997**, 1777−1778. (f) Parker, D.; Senanayake, P. K.; Williams, J. A. G. *J. Chem. Soc., Perkin Trans. 2* **1998**, 2129−2138. (g) Nishizawa, S.; Kato, Y.; Teramae, N. *J. Am. Chem. Soc.* **1999**, *121*, 9463−9464. (h) Dickins, R. S.; Aime, S.; Batsanov, A. S.; Beeby A.; Botta, M.; Bruce, J. I.; Howard, J. A. K.; Love, C. S.; Parker, D.; Peacock, R. D.; Puschmann, H. *J. Am. Chem. Soc.* **2002**, *124*, 12697−12705. (i) Mizukami, S.; Nagano, T.; Urano, Y.; Odani, A.; Kikuchi, K. *J. Am. Chem. Soc.* **2002**, *124*, 3920−3925. (j) Ojida, A.; Mito-oka, Y.; Inoue, M.; Hamachi, I. *J. Am. Chem. Soc.* **2002**, *124*, 6256−6258. (k) Ojida, A.; Inoue, M.; Mito-oka, Y.; Hamachi, I. *J. Am. Chem. Soc.* **2003**, *125*, 10184−10185. (l) Ojida, A.; Mito-oka Y.; Sada, K.; Hamachi, I. *J. Am. Chem. Soc.* **2004**, *126*, 2454−2463. (m) Lakshmi, C.; Hanshaw, R. G.; Smith, B. D. *Tetrahedron* **2004**, *60*, 11307−11315. (n) Amendola, V.; Basteianello, E.; Fabbrizzi, L.; Mangano, C.; Pallavicini, P.; Perotti, A.; Lanfredi, A. M.; Ugozzoli, F. *Angew. Chem., Int. Ed.* **2000**, *39*, 2917−2920. (o) Aldakov, D.; Anzenbacher, P. *J. Am. Chem. Soc.* **2004**, *126*, 4752−4753.
(11) Niikura, K.; Mertzger, A.; Anslyn, E. V. *J. Am. Chem. Soc.* **1998**, *120*, 8533−8534.
(12) (a) Snowden, T.; Anslyn, E. V. *Curr. Opin. Chem. Biol.* **1999**, *3*, 740−746. (b) Lavigne, J. J.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **2001**, *40*, 3119−3130. (c) Wiskur, S. L.; Ait-Haddou, H.; Anslyn, E. V.; Lavigne, J. J. *Acc. Chem. Res.* **2001**, *34*, 963−972.
(13) For reviews, see: (a) Kimura, E.; Koike, T.; Shionoya, M. In *Structure and Bonding: Metal Site in Proteins and Models*; Sadler, P. J., Ed.; Springer: Berlin, 1997; Vol. 89, pp 1−28. (b) Kimura, E.; Koike, T. In *Comprehensive Supramolecular Chemistry*; Reinhoudt, D. N., Ed.; Pergamon: Tokyo, 1996; Vol. 10, pp 429−444. (c) Kimura, E.; Koike, T. *J. Chem. Soc., Chem. Commun.* **1998**, 1495−1500. (d) Kimura, E.; Koike, T. In *Bioinorganic Catalysis*; Reedijk, J., Bouwman, E., Eds.; Marcel Dekkar: New York, 1999; pp 33−54. (e) Kimura, E.; Kikuta, E. *J. Biol. Inorg. Chem.* **2000**, *5*, 139−155. (f) Kimura, E.; Aoki, S. *BioMetals* **2001**, *14*, 191−204. (g) Aoki, S.; Kimura, E. In *Comprehensive Coordination Chemistry II*; Que, L. Jr.; Tolman, W. B. Eds.; Elsevier: Amsterdam, 2004; Vol. 8, pp 601−640. (h) Aoki, S.; Kimura, E. *Chem. Rev.* **2004**, *104*, 769−787.
(14) (a) Aoki, S.; Kimura, E. *Rev. Mol. Biotechnol.* **2002**, *90*, 129−155. (b) Aoki, S.; Shiro, M.; Koike, T.; Kimura, E. *J. Am. Chem. Soc.* **2000**, *122*, 576−584. (c) Aoki, S.; Iwaida, K.; Hanamoto, N.; Shiro, M.; Kimura, E. *J. Am. Chem. Soc.* **2002**, *124*, 5256−5257. (d) Aoki, S.; Jikiba, A.; Takeda, K.; Kimura, E. *J. Phys. Org. Chem.* **2004**, *17*, 489−497.

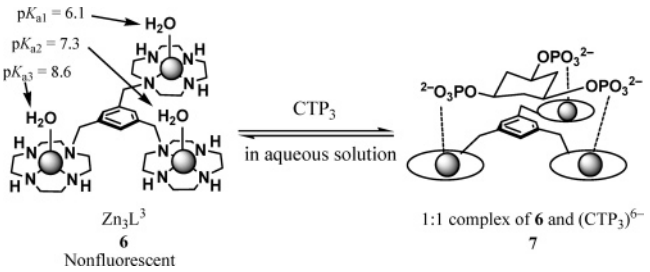
Scheme 2



Scheme 3



Scheme 4

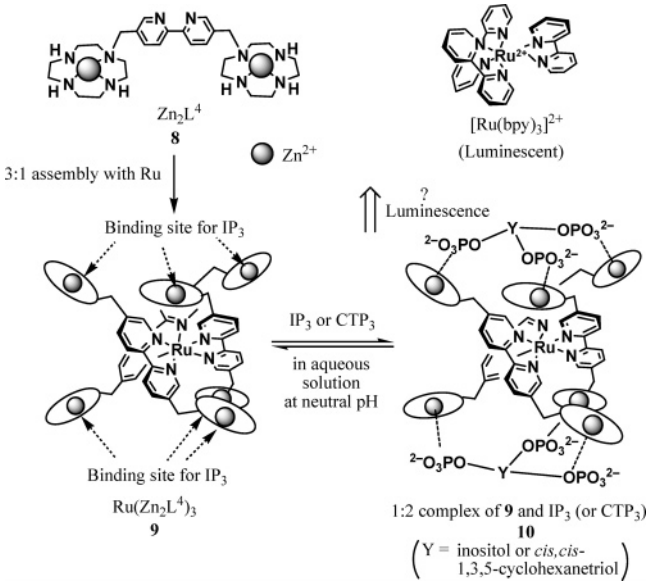


sites of fluorescence or luminescence anion sensors. Indeed, a Zn^{2+}-*N*-(2-pyridyl)cyclen complex **4** (ZnL^2) is a fluorescence sensor for anions based on the concepts of metal-chelation control of twisted intramolecular charge transfer (TICT) (Scheme 3).[15] Emission of **4** at 430 nm from a twisted conformer fixed by N(pyridine)-Zn^{2+} chelation shifts to 350 nm (emission from locally excited (LE) states) upon complexation with anions, which bind to Zn^{2+} and induce a conformational change from a twisted conformer **4** to a planar conformer **5**.

Prior to the design and synthesis of novel IP_3 sensors, we postulated that a C_3-symmetric tris(Zn^{2+}-cyclen) complex **6** (Zn_3L^3),[16] which has been utilized as a building block for three-dimensional supramolecular complexes,[17] could bind to IP_3 through three O(phosphate)-Zn^{2+} coordination bonds in aqueous solution at neutral pH (Scheme 4). In the first part of this article, we describe that **6** forms a stable 1:1 complex **7** with *cis,cis*-1,3,5-cyclohexanetriol triphosphate (CTP_3),[18] which has a similar Ca^{2+}-releasing activity as does IP_3 and is readily available on a large scale, as examined by potentiometric pH and ^1H NMR titrations.

Next, we designed a supramolecular complex **9** ($Ru(Zn_2L^4)_3$) possessing a luminescent tris(2,2′-bipyridyl)ruthenium (Ru-

Scheme 5



(bpy)$_3$, bpy = 2,2′-bipyridine) center[19] by Ru-templated assembly of three molecules of a bis(Zn^{2+}-cyclen) having a 2,2′-bipyridyl linker **8** (Zn_2L^4) (Scheme 5). We hypothesized that three of six Zn^{2+}-cyclen moieties of **9** on opposite sides of the molecule would cooperatively bind to three phosphate groups of IP_3 to yield a 1:2 complex **10**, resulting in a luminescence response to IP_3. In this work, we have synthesized **9** as a racemic mixture (Δ and Λ forms) and examined the interaction of racemic **9** mainly with CTP_3, an achiral model for IP_3 to avoid the complexity of analysis due to the formation of diastereomeric complexes with chiral IP_3. We describe discrimination of CTP_3 and IP_3 from monophosphates and diphosphates by **9**. The photodecomposition of **9** by UV and its inhibition upon complexation with CTP_3 and the stereoselective recognition of IP_3 by **9** will also be described.

Results and Discussion

Recognition of CTP_3 by a Nonluminescent Tris(Zn^{2+}-Cyclen) 6 (Zn_3L^3) Examined by Potentiometric pH and ^1H NMR Titrations. Prior to the synthesis of a new supramolecular sensor **9**, the interaction of a C_3-symmetric tris(Zn^{2+}-cyclen) **6** (Zn_3L^3) with CTP_3[20] was examined by potentiometric pH and ^1H NMR titrations. Previously, we have reported the crystal structure of **6**, in which the $Zn^{2+}-Zn^{2+}$ distances are about 10 Å.[16] Molecular model studies of CTP_3 suggested that the distances between phosphate groups of CTP_3 would be 9−10 Å.

Three deprotonation constants, $pK_{ai}(Zn_3L^3)$, of Zn^{2+}-bound water of **6** (defined by eq 1, where a_{H+} is the activity of H^+) were previously reported to be 6.08 ± 0.03, 7.25 ± 0.03, and 8.63 ± 0.03 at 25 °C with $I = 0.1$ ($NaNO_3$).[16] Analysis of typical potentiometric pH titration curves for 0.5 mM CTP_3Na_6 + 3.0 mM HNO_3 (Figure S1 in the Supporting Information) by using the software program "BEST"[21] gave six $pK_{ai}(CTP_3)$ values of

(15) Aoki, S.; Kagata, D.; Shiro, M.; Takeda, K.; Kimura, E. *J. Am. Chem. Soc.* **2004**, *126*, 13377−13390.
(16) Kimura, E.; Aoki, S.; Koike, T.; Shiro, M. *J. Am. Chem. Soc.* **1997**, *119*, 3068−3076.
(17) (a) Aoki, S.; Shiro, M.; Koike, T.; Kimura, E. *J. Am. Chem. Soc.* **2000**, *122*, 576−584. (b) Aoki, S.; Shiro, M.; Kimura, E. *Chem.−Eur. J.* **2002**, *8*, 929−939. (c) Aoki, S.; Zulkefeli, M.; Shiro, M.; Kimura, E. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4894−4899.
(18) Schultz, C.; Gebauer, G.; Metschies, T.; Rensing, L.; Jastorff, B. *Biochem. Biophys. Res. Commun.* **1990**, *166*, 1319−1327.
(19) (a) Kavarnos, G. J.; Turro, N. J. *Chem. Rev.* **1986**, *86*, 401−449. (b) Sauvage, J.-P.; Colin, J.-P.; Chambron, J.-C.; Guillerez, S.; Coudret, C. *Chem. Rev.* **1994**, *94*, 993−1019.
(20) Synthesis of CTP_3 and *cis*-1,3-cyclohexanediol diphosphate (CDP_2) is described in the Supporting Information.
(21) Martell, A. E.; Motekaitis, R. J. *Determination and Use of Stability Constants*, 2nd ed.; VCH: New York, 1992.

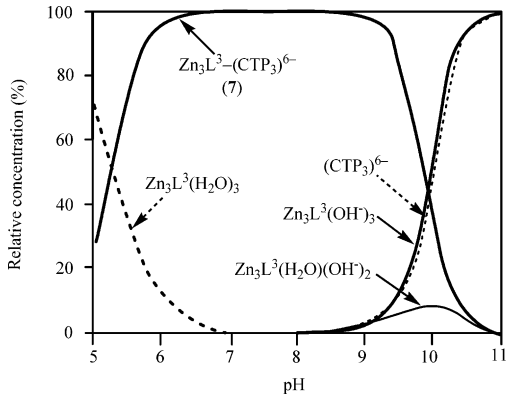


Figure 1. Speciation diagram calculated for a mixture of 0.5 mM **6** (Zn_3L^3) and 0.5 mM CTP_3Na_6 as a function of pH at 25 °C with $I = 0.1$ ($NaNO_3$). For clarity, the species less than 5% were omitted.

CTP_3 (defined by eq 2) of <3, <3, <3, 6.67 ± 0.05, 7.33 ± 0.05, and 8.59 ± 0.05. Analysis of a titration curve for a mixture of 0.5 mM **6** + 0.5 mM CTP_3Na_6 with $I = 0.1$ ($NaNO_3$) at 25 °C (Figure S1 in the Supporting Information) gave a complexation constant defined by eq 3, log K_s(**7**), of 10.2 ± 0.1. From this log K_s(**7**) value, an apparent complexation constant, log K_{app}(**7**), and a dissociation constant, K_d(**7**), defined by eqs 4−7, at pH 7.4 were calculated to be 8.0 ± 0.1 and 10 nM, respectively. Figure 1 shows a distribution diagram for a mixture of 0.5 mM Zn_3L^3 and 0.5 mM CTP_3, in which **7** is formed almost quantitatively in the pH range of ∼6.6−9.0.

$$Zn_3L^3(H_2O)_{4-i}(HO^-)_{i-1} \rightleftarrows Zn_3L^3(H_2O)_{3-i}(HO^-)_i + H^+ \quad (i = 1\sim3)$$

$$K_{ai}(Zn_3L^3) = [Zn_3L^3(H_2O)_{3-i}(HO^-)_i]a_{H^+}/[Zn_3L^3(H_2O)_{4-i}(HO^-)_{i-1}] \quad (1)$$

$$(H_{(7-i)}CTP_3)^{(i-1)-} \rightleftarrows (H_{6-i}CTP_3)^{i-} + H^+ \quad (i = 1\sim6)$$

$$K_{ai}(CTP_3) = [(H_{6-i}CTP_3)^{i-}]a_{H^+}/[(H_{(7-i)}CTP_3)^{(i-1)-}] \quad (2)$$

$$Zn_3L^3(H_2O)_6 + (CTP_3)^{6-} \rightleftarrows Zn_3L^3{-}(CTP_3)^{6-} \text{ complex } (\mathbf{6})$$

$$K_s(\mathbf{7}) = [\mathbf{7}]/[Zn_3L^3(H_2O)_6][(CTP_3)^{6-}]\ (M^{-1}) \quad (3)$$

$$K_{app}(\mathbf{7}) = [\mathbf{7}]/[Zn_3L^3]_{free}[CTP_3]_{free}\ (M^{-1}) \quad (4)$$

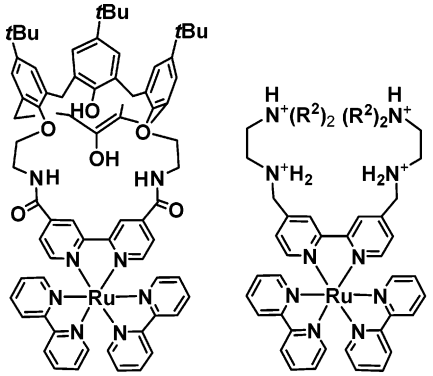
$[Zn_3L^3]_{free}$ = total concns of uncomplexed $Zn_3L^3(H_2O)_{3-i}(HO^-)_i$ $(i = 1\sim3)$ (5)

$[CTP_3]_{free}$ = total concns of uncomplexed $(H_{(7-i)}CTP_3)^{(i-1)-}$ $(i = 1\sim6)$ (6)

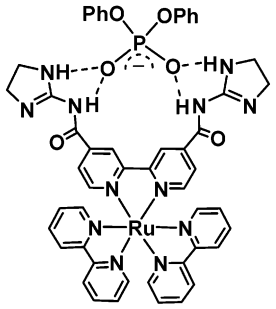
$$K_d(\mathbf{7}) = 1/K_{app}(\mathbf{7})\ (M) \quad (7)$$

The aromatic signals on the ^{1}H NMR spectra of **6** (2 mM) in the presence of 0.5 equiv (1 mM) of CTP_3 in D_2O at pD 7.0 and 35 °C showed two broad ^{1}H signals for several complexation species of **6** and CTP_3 (Figure S2 in the Supporting Information). The ^{1}H NMR spectra of **6** (2 mM) with 1.0 equiv (2 mM) and 2.0 equiv (4 mM) of CTP_3 exhibited one broad ^{1}H signal, indicating that single complexation species were formed. Dissociation of **7** in an alkaline solution indicated in Figure 1 was confirmed by ^{1}H NMR spectra at pD 11 (Figure S2 in the Supporting Information).

Design and Synthesis of 9 ($Ru(Zn_2L^4)_3$). Since **6** (Zn_3L^3) has low molecular extinction coefficients (e.g., $\epsilon_{265} = 3.0 \times 10^2$ ($M^{-1}\cdot cm^{-1}$))[16] and is nonluminescent, we decided to replace a phenyl group of **6** with a luminescent unit. A tris(2,2′-bipyridyl)ruthenium ($Ru(bpy)_3$) moiety is known to be luminescent due to metal-to-ligand charge transfer (MLCT)[19] and has been used as a luminescent center for phosphate sensors[9k,22,23] such as **11**,[24] **12**,[25] and **13**.[26] Thus, we have designed a new supramolecular receptor **9** ($Ru(Zn_2L^4)$) (Scheme 4). A dimeric zinc(II) complex **8** (Zn_2L^4) was chosen as a building block to reduce the numbers of stereoisomers of **9**.



11 **12** (R^2 = H or Me)

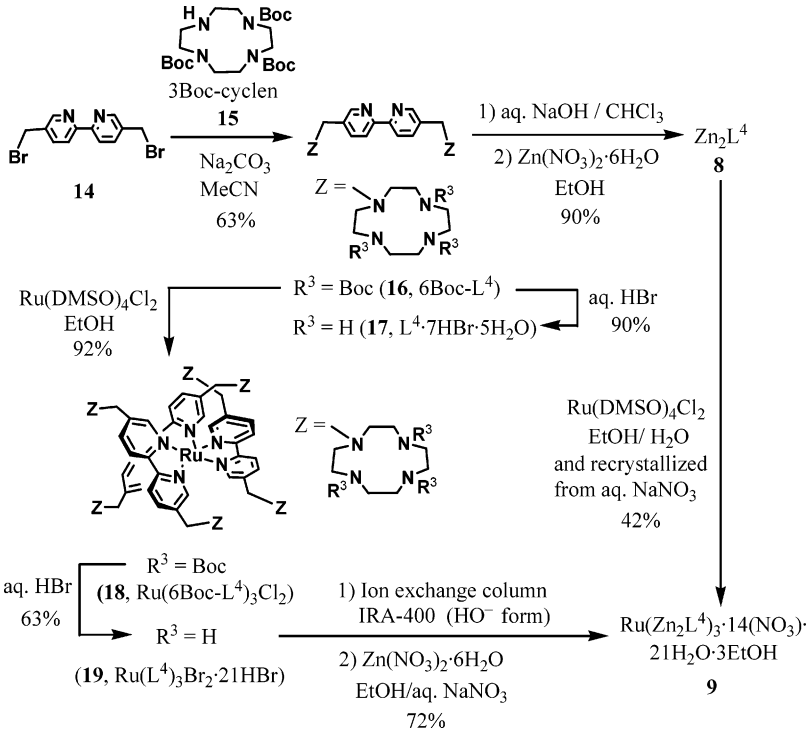


13-diphenyl phosphate complex

The synthesis of **9** and a Zn^{2+}-free **19** ($Ru(L^4)_3$) is summarized in Scheme 6. A reaction of 5,5′-bis(bromomethyl)-2,2′-bipyridine **14**[27] with 3Boc-cyclen **15**[16] gave **16** (6Boc-L^4), whose Boc groups were removed with aqueous HBr to yield **17** as a HBr salt ($L^4\cdot 7HBr\cdot 5H_2O$). Metal free-L^4 was reacted with 2

(22) (a) Balzani, V.; Juris, A.; Venturi, M.; Campagna, S.; Serroni, S. *Chem. Rev.* **1996**, *96*, 759−833. (b) Schubert, U. S.; Eschbaumer, C. *Angew. Chem., Int. Ed.* **2002**, *41*, 2893−2926.
(23) (a) Beer, P. D. *J. Chem. Soc., Chem. Commun.* **1996**, 689−696. (b) Beer, P.; Schmitt, P. *Curr. Biol. Chem. Biol.* **1997**, *1*, 475−482. (c) Beer, P. D. *Acc. Chem. Res.* **1998**, *31*, 71−80. (d) Beer, P. D.; Cadman, J. *J. Chem. Soc., Chem. Commun.* **1999**, 347−349. (e) Beer, P. D.; Cadman, J. *Coord. Chem. Rev.* **2000**, *205*, 131−155. (f) Keefe, M. H.; Benkstein, K. D.; Hupp, J. T. *Coord. Chem. Rev.* **2000**, *205*, 201−208. (g) Beer, P. D.; Gale, P. A. *Angew. Chem., Int. Ed.* **2001**, *40*, 486−516. (h) Deetz, M. J.; Smith, B. D. *Tetrahedron Lett.* **1998**, *39*, 6841−6844.
(24) Szemes, F.; Hesek, D.; Chen, Z.; Dent, S. W.; Drew, M. G. B.; Goulden, A. J.; Graydon, A. R.; Grieve, A.; Mortimer, R. J.; Wear, T.; Weightman, J. S.; Beer, P. D. *Inorg. Chem.* **1996**, *35*, 5868−5879.
(25) Beer, P. D.; Gale, P. A. *New J. Chem.* **1999**, *23*, 347−349.
(26) Watanabe, S.; Onogawa, O.; Komatsu, K.; Yoshida, K. *J. Am. Chem. Soc.* **1998**, *120*, 229−584.
(27) Schubert, U. S.; Eschbaumer, C.; Hochwimmer, G. *Synthesis* **1999**, *5*, 779−782.

Scheme 6



equiv of $Zn(NO_3)_2 \cdot 6H_2O$ to give a dimeric zinc(II) complex, **8** (Zn_2L^4). A reaction of **8** with $Ru(DMSO)_4Cl_2$[28] in $EtOH/H_2O$ followed by recrystallization from $EtOH/H_2O$ gave **9** ($Ru(Zn_2L^4)_3 \cdot 14(NO_3) \cdot 21H_2O \cdot 3EtOH$) as an orange powder.[29]

Alternatively, **16** was reacted with $Ru(DMSO)_4Cl_2$ in EtOH to yield a 3:1 complex **18** ($Ru(6Boc\text{-}L^4)_3Cl_2$), whose Boc groups were removed by aqueous HBr to yield **19** as a HBr salt ($Ru(L^4)_3Br_2 \cdot 21HBr$). After being passed through ion exchange column IRA-400 (HO^- form), acid-free **19** was reacted with $Zn(NO_3)_2 \cdot 6H_2O$ in $EtOH/H_2O$ to yield **9**.

X-ray Crystal Structure of 9 ($Ru(Zn_2L^4)_3$). The orange prism of **9** obtained as $Ru(Zn_2L^4)_3 \cdot 3(HO^-) \cdot 11(NO_3) \cdot 20H_2O$ by recrystallization from *an aqueous solution at pH 7.5* was subjected to single-crystal X-ray diffraction analysis (Table 1). Parts a and b of Figure 2 are space-filling drawings of **9** viewed from the pseudo C_3 and the pseudo C_2 axes, respectively. The three Zn_2L^4 units comprising **9** are presented in yellow, light blue, and light green; Ru^{2+} is red, Zn^{2+} is orange, and the Zn^{2+}-bound oxygen (HO^-) is blue. Interestingly, three of the six Zn^{2+}-cyclen units are orientated to face the opposite side of the molecule with three apical ligands (Zn^{2+}-bound HO^-) of each of the three Zn^{2+} located on the same face.[30] The $Zn^{2+}-Zn^{2+}$ distances of **9** are ca. 11.5, 11.7, and 11.8 Å, which are close to the assumed distances between the two O(phosphate)'s of CTP_3. Each crystal of **9** contains either of two enantiomers, the Δ or Λ form, implying that the crystals of **9** are conglomerate.

Table 1. Selected Crystal Data for **9**

formula	$C_{84}H_{187}N_{41}O_{56}RuZn_6$
M_r	3160.99
crystal system	hexagonal
space group	$P6_122$ (No. 178)
color of crystal	orange
a (Å)	20.000(4)
b (Å)	20.000(4)
c (Å)	71.74(2)
α (deg)	90
β (deg)	90
γ (deg)	120
V (Å^3)	24852(10)
Z	6
D_{calcd} (g·cm^{-3})	1.267
λ (Cu Kα) (Å)	1.5419
μ (Cu Kα) (cm^{-1})	23.93
R ($I > 2\sigma(I)$)	0.1330
R_w	0.3660
temp of data collection (K)	93.1
no. of reflns used for least squares	8607
no. of variables	705

^{1}H NMR Titrations of 9 ($Ru(Zn_2L^4)_3$) with CTP_3. We have performed ^{1}H NMR titrations of **9** ($Ru(Zn_2L^4)_3$ with CTP_3 in D_2O at pD 7.4 and 25 °C. In the absence of CTP_3, three signals for aromatic protons of **9** (2 mM) appear at 7.84, 8.22, and 8.77 ppm (Figure 3a). In the presence of 1 equiv (2 mM) and 2 equiv (4 mM) of CTP_3, these three signals shifted to 7.58, 8.24, and 8.74, and 7.55, 8.26, and 8.73, respectively (Figure 3b,c). The addition of 2 equiv of CTP_3 ([CTP_3] = 8 mM in total) to that shown in Figure 3c caused a very small change (Figure 3d). The results of Job plot experiments of **9** with CTP_3 (Figure S4 in the Supporting Information) strongly indicated the formation of the 1:2 complex **10**, $(Ru(Zn_2L^4)_3)-(CTP_3)_2$, as we predicted in Scheme 5.[31] At pD 11.0, similar spectra were obtained for **9** in the absence (Figure 3e) and presence (Figure 3f) of CTP_3,

(28) Evans, I. P.; Spencer, A.; Wilkinson, G. *J. Chem. Soc., Dalton Trans.* **1973**, 204−209.

(29) Negligible complexation of cyclen with $RuCl_3$ or $Ru(DMSO)_4Cl_2$ was observed in H_2O and $EtOH/H_2O$.

(30) Crystal packing of **9** is shown in Figure S3 in the Supporting Information, in which Zn^{2+}-bound H_2O is deprotonated and coordinate to two Zn^{2+} in an intermolecular manner.

(31) It should be mentioned that ^{1}H NMR spectra of a mixture of **9** and CTP_3 in D_2O at pD 7.0 and 25 °C exhibited averaged signals of uncomplexed and complexed species of **9** (Figure 3b−d), indicating that complexes of **9** with CTP_3 are thermodynamically stable but are kinetically labile on the NMR time scale (400 and 500 MHz).

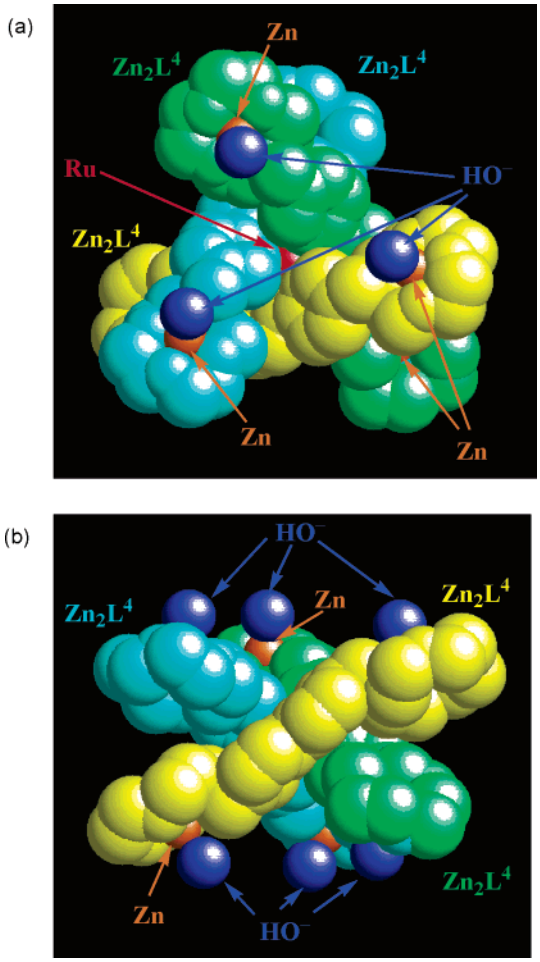


Figure 2. Space-filling drawings of **9** ($Ru(Zn_2L^4)_3$) viewed along the pseudo C_3-symmetry axis (a) and the pseudo C_2-symmetry axis (b). The three Zn_2L^4 units comprising **9** are presented in yellow, light blue, and light green, Ru^{2+} is red, Zn^{2+} is orange, and the Zn^{2+}-bound oxygen (HO^-) is blue. All NO_3^- and H_2O were omitted.

indicating negligible interaction of **9** with CTP_3 in an alkaline solution (see below).

UV Spectrophotometric and Luminescence (Quick Scanning) Titrations of 9 with CTP_3. UV titrations of **9** (50 μM) with CTP_3 were performed at pH 7.4 (10 mM HEPES with I = 0.1 ($NaNO_3$)) and 25 °C. Uncomplexed **9** has absorption maxima at 296 nm ($\epsilon_{296} = 7.8 \times 10^4$) and 452 nm ($\epsilon_{452} = 1.1 \times 10^4$). The latter absorbance (dashed curve in Figure 4) is a characteristic MLCT band for $Ru(bpy)_3$.[32] Upon addition of CTP_3, the absorption maxima at 452 nm shifted to 470 nm. The inset shows an increasing curve for ϵ_{470}, suggesting 1:2 complexation of **9** with CTP_3.[33]

The results of luminescence titrations of 10 μM **9** with CTP_3 at pH 7.4 (10 mM HEPES with I = 0.1 ($NaNO_3$)) and 25 °C (excitation at 300 nm) are shown in Figure 5. An emission spectrum of 10 μM **9** has emission maxima (dashed curve in Figure 5a) at 610 nm, which is close to that (590 nm) of $Ru(bpy)_3$.[19] Interestingly, the addition of CTP_3 caused a considerable increase in emission with blue shifts from 610 to 584 nm (4.2-fold enhancement at 584 nm), as shown in Figure 5a and

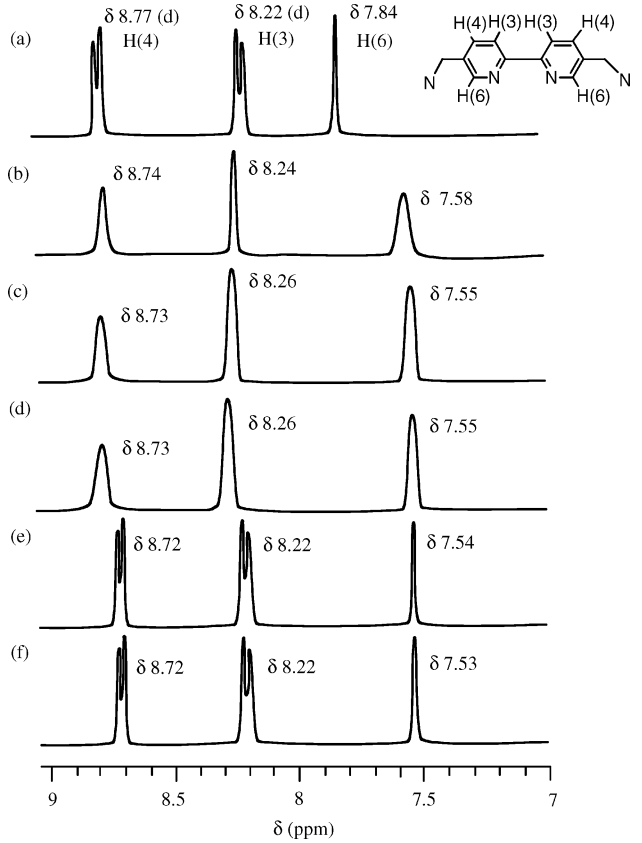


Figure 3. ^1H NMR spectra (aromatic region) of (a) 2 mM **9** at pD 7.4, (b) 2 mM **9** + 2 mM CTP_3 at pD 7.4, (c) 2 mM **9** + 4 mM CTP_3 at pD 7.4, (d) 2 mM **9** + 8 mM CTP_3 at pD 7.4, (e) 2 mM **9** at pD 11.0, and (f) 2 mM **9** + 4 mM CTP_3 at pD 11.0 in D_2O at 25 °C.

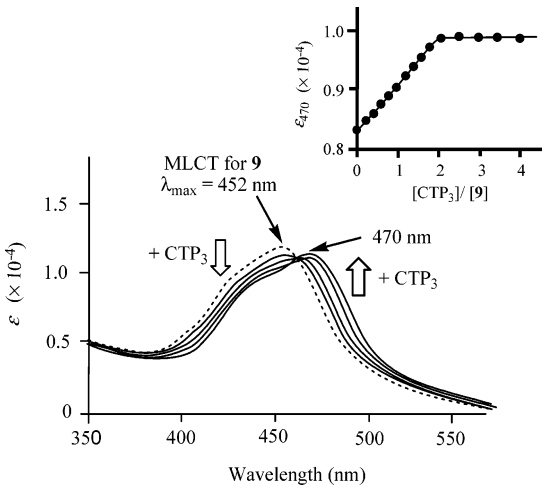


Figure 4. UV absorption spectral change of **9** ($Ru(Zn_2L^4)_3$) (50 μM) upon addition of CTP_3 at pH 7.4 (10 mM HEPES with I = 0.1 ($NaNO_3$)) and 25 °C. A dashed curve is a UV spectrum of uncomplexed **9** having an absorption maxima at 470 nm, which corresponds to MLCT. The inset shows an increase in ϵ at 470 nm upon addition of CTP_3.

plotted in Figure 5b (●).[34–36] We presume that the enhancement of emission of **9** is due to the restriction of the conformation of **9** upon complexation with CTP_3, as previously proposed.[23–26]

(32) For comparison, UV spectra of **9** and $Ru(bpy)_3$ (10 μM) in 10 mM HEPES (pH 7.4 with I = 0.1 ($NaNO_3$) at 25 °C are shown in Figure S5 in the Supporting Information.
(33) Change in UV spectra of **9** upon addition of phenyl phosphate (PP) is shown in Figure S6 in the Supporting Information.
(34) Quantum yields (Φ) for luminescence of **9** (10 μM) in the absence and the presence of 2 equiv of CTP_3 are 1.3×10^{-2} and 3.4×10^{-2}, respectively, in 10 mM HEPES (pH 7.4) with I = 0.1 ($NaNO_3$) at 25 °C.
(35) The emission spectrum of **9** obtained by excitation at 452 nm at pH 7.4 had the same emission maxima as that obtained by excitation at 300 nm.
(36) Change in the excitation spectra of **9** upon addition of CTP_3 and PP are shown in Figure S7 in the Supporting Information.

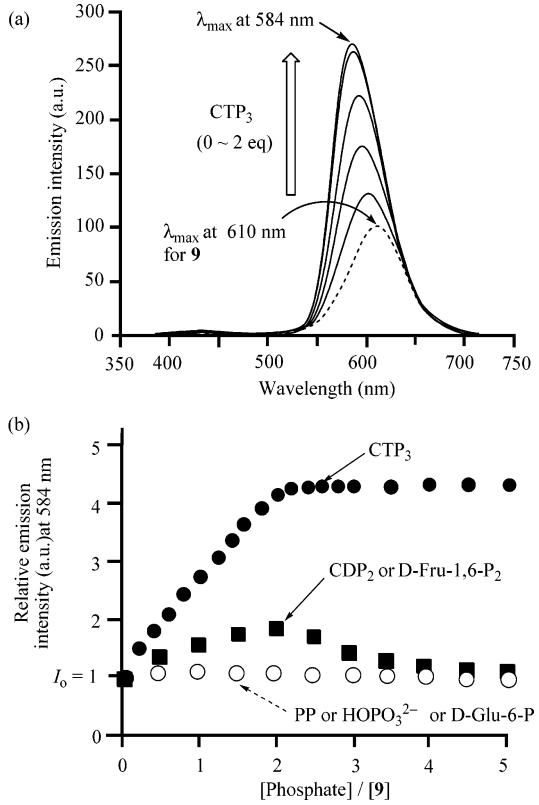


Figure 5. (a) Change in the luminescence emission of 10 μM **9** ($Ru(Zn_2L^4)_3$) upon addition of CTP_3 at pH 7.4 (10 mM HEPES with I = 0.1 ($NaNO_3$)) and 25 °C (excitation at 300 nm). Arbitrary unit is a.u. (b) Luminescence response of **9** (10 μM) at 584 nm to CTP_3 (●), monophosphates such as PP, $HOPO_3^{2-}$, and D-Glu-6-P (○), and diphosphates including CDP_3 and D-Fru-1,6-P_2 (■) at pH 7.4 (10 mM HEPES with I = 0.1 ($NaNO_3$)) and 25 °C (excitation at 300 nm). I_0 is an emission intensity of **9** at 584 nm in the absence of CTP_3.

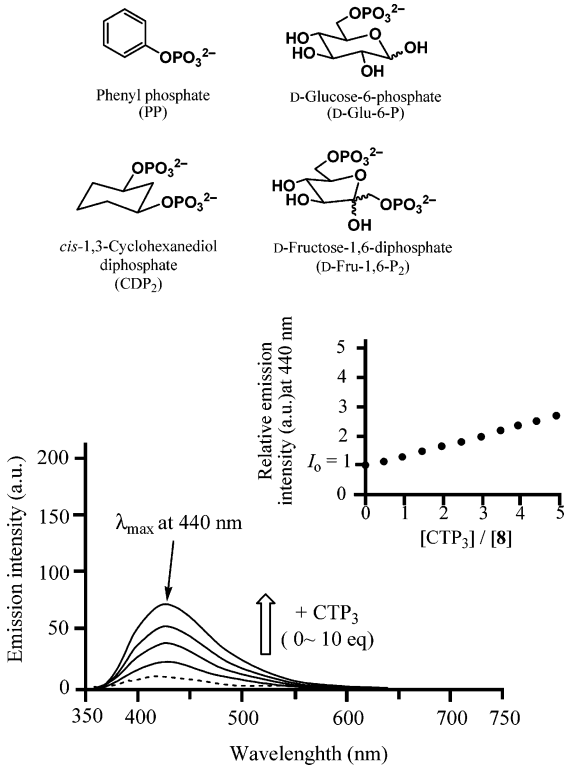


Figure 6. Change in the luminescence emission of 10 μM **8** (Zn_2L^4) upon addition of CTP_3 at pH 7.4 (10 mM HEPES with I = 0.1 ($NaNO_3$)) and 25 °C (excitation at 300 nm). The inset shows the increase in emission intensity of **8** at 440 nm, where I_0 is emission intensity of **8** at 440 nm in the absence of CTP_3.

As we describe later, **9** undergoes photodecomposition by UV exposure. Reproducible luminescence spectra of **9** were obtained by a quick scanning of the emission wavelength (~500−1000 nm/min).

For comparison, the emission spectral change of 10 μM **8** (Zn_2L^4) upon the addition of CTP_3 at pH 7.4 (10 mM HEPES with I = 0.1 ($NaNO_3$)) and 25 °C is shown in Figure 6. The dashed curve in Figure 6 is the emission spectrum of uncomplexed **8**. As the concentration of CTP_3 increased, emission at 440 nm gradually increased, as plotted in the inset of Figure 6. Negligible change was observed in the emission of $Ru(bpy)_3$ and Zn^{2+}-free **19** ($Ru(L^4)_3$) upon addition of CTP_3 under the same conditions,[37] implying negligible interactions of $Ru(bpy)_3$ and **19** with CTP_3 (Figure S8 in the Supporting Information).

Moreover, an addition of monophosphates such as inorganic phosphate (HPO_4^{2-}), phenyl phosphate (PP), and D-glucose-6-phosphate (D-Glu-6-P) induced negligible change in the emission spectra of **9**, as shown in Figure 5b (○), indicating very weak interaction of **9** with the monophosphates.[38] Addition of diphosphates such as *cis*-1,3-cyclohexanediol diphosphate (CDP_2)[20,39] and D-fructose-1,6-diphosphate (D-Fru-1,6-P_2) induced a complicated change (■ in Figure 5b). Therefore, we concluded that a supramolecular sensor **9** discriminates CTP_3 from monophosphates and diphosphates.[40−42] The presence of 100 μM inorganic phosphate did not interfere with the luminescence sensing of CTP_3 by **9**.

Complexation Behaviors of 9 ($Ru(Zn_2L^4)_3$) with CTP_3 Studied by Potentiometric pH Titrations. Curve a in Figure 7 shows a typical potentiometric pH titration curve of 0.25 mM **9** $(Ru(Zn_2L^4)_3{\cdot}14(NO_3){\cdot}21H_2O{\cdot}3EtOH)$ at 25 °C with I = 0.1 ($NaNO_3$). Deprotonation constants (defined by eq 7), pK_{ai}($Ru(Zn_2L^4)_3$), of the Zn^{2+}-bound water of **9** (defined by eq 8) were calculated to be 6.71 ± 0.05, 7.06 ± 0.05, 7.71 ± 0.05, 8.63 ± 0.05, 9.04 ± 0.05, and 9.46 ± 0.05[43] by the software program "BEST".[21]

(37) Quantum yields (Φ) for luminescence of **8** (Zn_2L^4) and **19** ($Ru(L^4)_3$) were 1.6×10^{-3} and 1.4×10^{-2}, respectively, in 10 mM HEPES (pH 7.4) with I = 0.1 ($NaNO_3$) at 25 °C.

(38) A stoichiometry for complexation of **9** with PP in aqueous solution was examined by Job plot experiments in ^1H NMR in D_2O at 35 °C (Figure S4b in the Supporting Information). Additionaly, an orange-yellow powder isolated from a mixture of **9** and PP (6 equiv) in an aqueous solution at pH 7.4 was suggested to be the 1:2 complex of **9** and PP by ^1H NMR spectra and elemental analysis. Although we could not obtain fine crystals of **9**−PP complex, we presume that three Zn^{2+}-cyclen units of **9** coordinate to three O(phosphate) of PP as does **6** (ref 16).

(39) Shuey, S. W.; Deerfield, D. W., II.; Amburgey, J. C.; Cabaniss, S. E.; Huh, N.-W.; Charifson, P. S.; Pedersen, L. G.; Hiskey, R. G. *Bioorg. Chem.* **1993**, *21*, 95−108.

(40) Luminescence titrations of **9** with citric acid at pH 7.4 (10 mM HEPES with I = 0.1 ($NaNO_3$)) and 25 °C gave almost the same results as those with diphosphates.

(41) Luminescence titration of **9** with inositol hexaphosphate (phytic acid, IP_6) and adenosine 5′-triphsophate (ATP) gave somehow complicated results, as shown in Figure S9 in the Supporting Information.

(42) In preliminary experiments, lifetimes of luminescence of ruthenium complexes, **9**, **19**, and $Ru(bpy)_3$ ([Ru complex] = 10 μM) measured at 660 nm (excitation at 300 nm) in 10 mM HEPES (pH 7.4) with I = 0.1 ($NaNO_3$) at 25 °C were 0.68 ± 0.03, 0.76 ± 0.03, and 0.65 ± 0.03 μs, respectively. In the literature, the lifetime of $Ru(bpy)_3$ was reported to be 0.58 μs (ref 19a). Lifetimes of **9** in the presence of 20 μM (2 equiv) of CTP_3 and 100 μM (10 equiv) of PP were 0.60 ± 0.03 and 0.68 ± 0.03 μs, respectively.

(43) The separated pK_a values of **9** are explained by intramolecular hydrogen bondings between the Zn^{2+}-bound waters, as we discussed previously (ref 16).

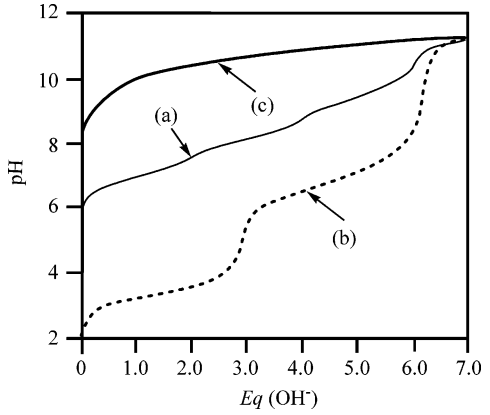


Figure 7. Typical potentiometric pH titration curves for (a) 0.25 mM **9** ($Ru(Zn_2L^4)_3 \cdot 14NO_3 \cdot 21H_2O \cdot 3EtOH$), (b) 0.25 mM CTP_3Na_6 + 1.5 mM HNO_3, and (c) 0.25 mM **9** ($Ru(Zn_2L^4)_3 \cdot 14NO_3 \cdot 21H_2O \cdot 3EtOH$) + 0.5 mM CTP_3Na_6 at 25 °C with $I = 0.1$ ($NaNO_3$). $Eq(OH^-)$ is the number of equivalents of base (NaOH) added.

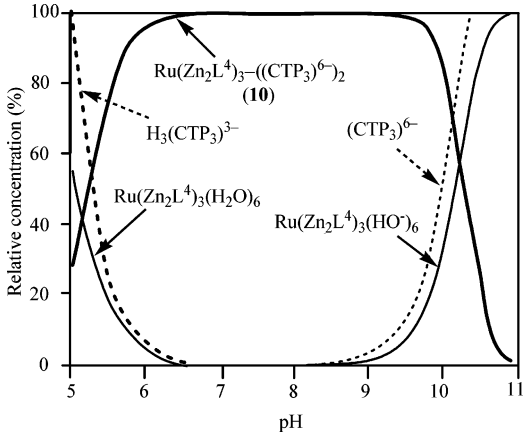
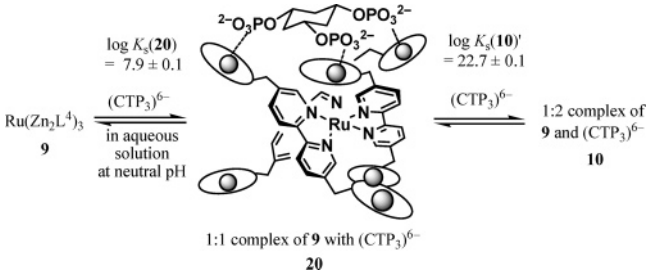


Figure 8. Speciation diagrams for a mixture of 10 μM **9** ($Ru(Zn_2L^4)_3$) and 20 μM CTP_3 as a function of pH at 25 °C with $I = 0.1$ ($NaNO_3$) (the concentrations for luminescent titrations). For clarity, the species less than 5% were omitted.

Scheme 7



For analysis of curve c in Figure 7 for a mixture of 0.25 mM **9** + 0.5 mM CTP_3Na_6 with $I = 0.1$ ($NaNO_3$) at 25 °C, equilibria between **9** and CTP_3 were hypothesized as shown in Scheme 7 and eqs 9−11, including the 1:1 complex of **9** and CTP_3 (**20**). From UV and luminescent titrations of **9** with CTP_3 at pH 7.4 described earlier (linear changes in ϵ_{470} and luminescence at 584 nm of **9** upon addition of up to 2 equiv of CTP_3), we assumed that the log K_s(**10**)′ is larger than log K_s(**20**). Therefore, two log K_s values of 7.9 ± 0.2 and 22.7 ± 0.2 were assigned to be log K_s(**20**) (for **9** + CTP_3 ⇄ **20**) and log K_s(**10**)′ (for **20** + CTP_3 ⇄ **10**), respectively. From these values, the log K_s(**10**) defined by eq 11 and an apparent complexation constant defined by eqs 6, 12, and 13, log K_{app}(**10**), at pH 7.4, were calculated to be 30.6 ± 0.2 and 19.0 ± 0.2, respectively.

$$Ru(Zn_2L^4)_3(H_2O)_{(7-i)}(HO^-)_{(i-1)} \rightleftarrows Ru(Zn_2L^4)_3(H_2O)_{(6-i)}(HO^-)_i + H^+ \quad (i = 1 \sim 6)$$

$$K_{ai}(Ru(Zn_2L^4)_3) = [Ru(Zn_2L^4)_3(H_2O)_{(6-i)}(HO^-)_i]q_{H^+}/[Ru(Zn_2L^4)_3(H_2O_{(7-i)}(HO^-)_{(i-1)}] \quad (8)$$

$$Ru(Zn_2L^4)_3(H_2O)_6\ (\mathbf{9}) + (CTP_3)^{6-} \rightleftarrows 1{:}1\ Ru(Zn_2L^4)_3{-}(CTP_3)^{6-}\ \text{complex}\ (\mathbf{20})$$

$$K_s(\mathbf{20}) = [\mathbf{20}]/[\mathbf{9}][(CTP_3)^{6-}]\ (M^{-1}) \quad (9)$$

$$\mathbf{20} + (CTP_3)^{6-} \rightleftarrows 1{:}2\ Ru(Zn_2L^4)_3{-}((CTP_3)^{6-})_2\ \text{complex}\ (\mathbf{10})$$

$$K_s(\mathbf{10})' = [\mathbf{10}]/[\mathbf{20}][(CTP_3)^{6-}]\ (M^{-1}) \quad (10)$$

$$K_s(\mathbf{10}) = [\mathbf{10}]/[\mathbf{9}][(CTP_3)^{6-}]^2\ (M^{-2}) \quad (11)$$

$$K_{app}(\mathbf{10}) = [\mathbf{10}]/[\mathbf{9}]_{free}[(CTP_3)_{free}]^2\ (M^{-2}) \quad (12)$$

$[\mathbf{9}]_{free}$ = total concns of uncomplexed

$$Ru(Zn_2L^4)_3(H_2O)_m(HO^-)_n\ (m = 6 - n,\ n = 0 \sim 6) \quad (13)$$

In ^{1}H NMR titrations of **9** with CTP_3, addition of the 1 equiv of CTP_3 caused a considerable upfield shift from δ 7.84 (Figure 3a) to δ 7.55 (Figure 3c) for H(6) of **9**. Further addition of CTP_3 caused negligible chemical shifts (δ 7.55 in Figure 3c,d). These results suggested that formation of the 1:1 complex **20** facilitates formation of the 1:2 complex **10**. A speciation diagram for a mixture of 10 μM **9** and 20 μM CTP_3 is shown in Figure 8 (the concentration for the luminescence titrations described above), in which more than 95% of the 1:2 complex **10** is formed in the range of pH ~5.8−9.6. Dissociation of **10** in acidic and alkaline solutions at 25 °C was confirmed by ^{1}H NMR spectra of a mixture of 0.25 mM **9** and 0.5 mM CTP_3 at pD 3.0 (data not shown) and 11.0 (Figure 3e,f) and the pH emission profile of **9** in the absence and presence of CTP_3 shown in Figure S10 in the Supporting Information.

Photodecomposition of 9 by UV Light and Its Inhibition by CTP_3. During the luminescence titrations, we became aware that the emission spectra of **9** were somehow not reproducible. After careful experimentation, we noticed that the change in the emission spectra is dependent on the duration of the UV exposure (Figure 9a). Indeed, emission of 10 μM **9** at 610 nm shifted to 435 nm after UV exposure for 3 h and its emission spectra showed negligible change while being kept in the dark, as summarized in Figure 9b.[44] The emission of Zn^{2+}-free **19** ($Ru(L^4)_3$) at 608 nm was also reduced after UV exposure. In contrast, UV irradiation of $Ru(bpy)_3$ for 5 hr in 10 mM HEPES (at pH 7.4 with $I = 0.1$ ($NaNO_3$)) and in H_2O caused a little change (<5% decomposition) in the emission spectra.

Photodecomposition of $Ru(bpy)_3$ has been described in some literature.[45] Porter et al. described that the decomposition of

(44) UV irradiation experiments of an aqueous solution of **9** with or without phosphates were performed with Hitachi F-3000 fluorescence spectrophotometers in 1-cm quartz cuvettes. The averaged light intensity at 300 nm was 1.1 J·min^{-1}·cm^{-2} (2.5×10^{-6} einstein·min^{-1}·cm^{-2}), as measured by chemical actinometry utilizing photoreduction of Fe^{3+} to Fe^{2+}, whose quantum yield had been reported to be 1.24 at 313 nm (Murov, S. L.; Carmichael, I.; Hug, G. L. *Handbook of Photochemistry*, 2nd ed.; Marcel Dekker: New York, 1993). Φ for the photodecomposition of **9** was 1.8×10^{-6}.

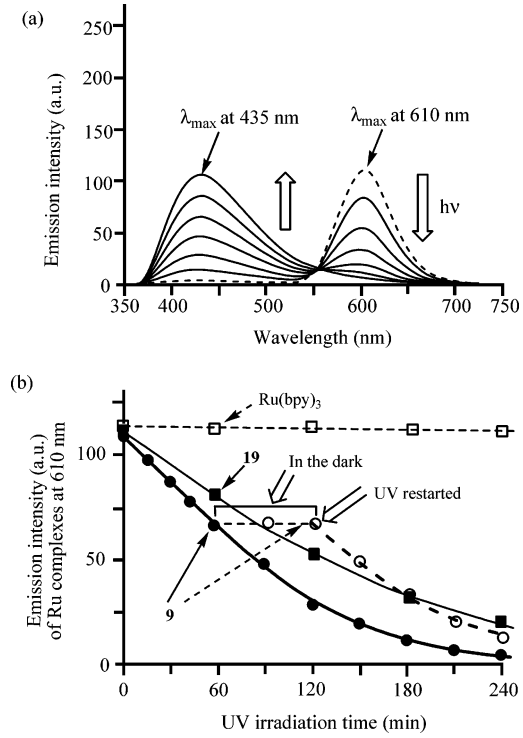


Figure 9. (a) Change in the luminescent spectra of **9** (10 μM) after photoreaction (irradiation at 300 nm) at pH 7.4 (10 mM HEPES with I = 0.1 ($NaNO_3$)) and 25 °C (spectra were obtained by quick scanning of emission wavelength). A dashed line is an emission spectrum of **9** before UV exposure. (b) Photodecomposition of ruthenium complexes, **9** ($Ru(Zn_2L^4)_3$, bold curve), **19** ($Ru(L^4)_3$, plain curve), and $Ru(bpy)_3$ (dashed curve). A bold dashed curve with open circles indicates that emission spectra of **9** shows negligible change in the dark (from 60 to 120 min).

$Ru(bpy)_3$ is accelerated by anions such as Cl^-, Br^-, and NCS^- in DMSO.[45d] Recently, Dutta's group reported that the photodecomposition of $Ru(bpy)_3$ in aqueous solution is much slower than that in nonaqueous solution and is accelerated in acetate buffer (0.025−2 M) or 2 M phosphate (2 M) buffer at pH 5.0.[45f,46] The effects of guest molecules (CTP_3, CDP_2, and inorganic phosphate) on the photodecomposition of **9** (10 μM) were examined at pH 7.4 (10 mM HEPES with I = 0.1 ($NaNO_3$)) at 25 °C. Figure 10a shows the emission spectra of 10 μM **9** before UV irradiation (dashed curve) and after UV irradiation at 330 nm for 3 h in the absence (plain curve) and presence of 10 μM CTP_3 (bold dashed curve), and of 20 μM CTP_3 (bold curve). In the absence of CTP_3, luminescence emission from **9** shifts to 435 nm after photoreaction for 3 h (plain curve). Very interestingly, the addition of 2 equiv of CTP_3 stabilized **9** and the emission at 584 nm remained unchanged,

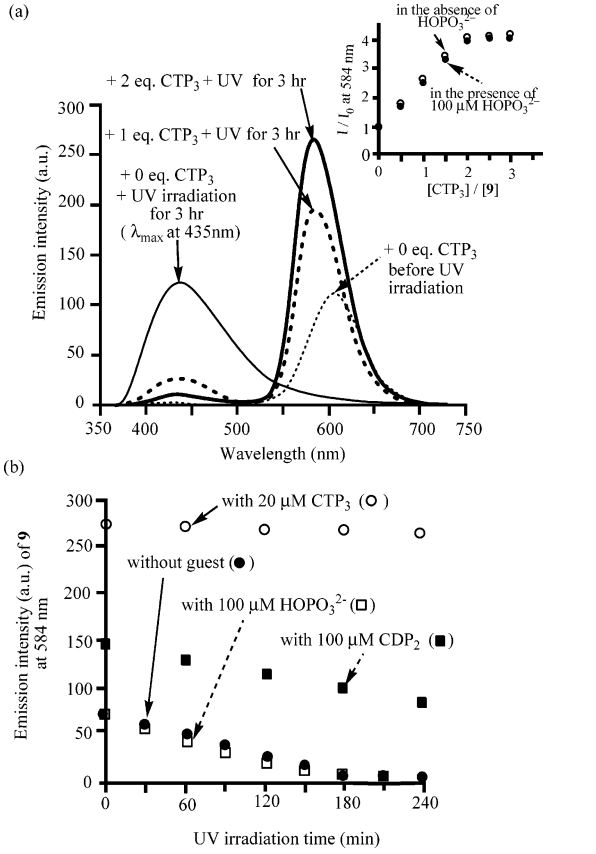
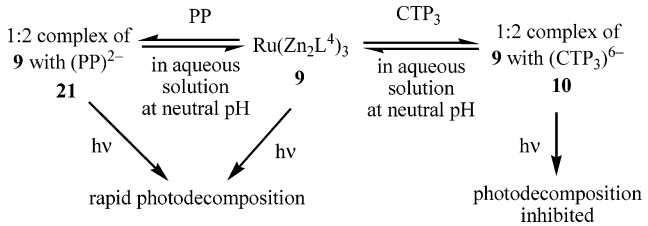


Figure 10. (a) Change in the luminescent spectra of **9** (10 μM) after photoreaction for 3 h (irradiation at 300 nm) in the absence (plain curve) and presence of 10 μM CTP_3 (bold dashed curve) and 20 μM CTP_3 (bold curve) at pH 7.4 (10 mM HEPES with I = 0.1 ($NaNO_3$)) and 25 °C. A dashed line is an emission spectrum of **9** (10 μM) before UV irradiation (spectra were obtained by quick scanning of emission wavelength). The inset shows change in relative emission intensity of **9** (10 μM) at 584 nm after UV irradiation for 3 h at the increasing concentration of CTP_3 in the absence (○) and the presence (●) of 100 μM inorganic phosphate at pH 7.4 (10 mM HEPES with I = 0.1 ($NaNO_3$)) and 25 °C. (b) Changes in the emission intensity of **9** at 584 nm after UV irradiation (at 300 nm) in the absence (●) and presence of inorganic phosphate (□), CDP_2 (■), and CTP_3 (○).

Scheme 8



as plotted in Figure 10b (○), which may allow determination of CTP_3 concentrations even after UV exposure. The presence of inorganic phosphate (□) or CDP_2 (■) did not affect stability of **9**−$(CTP_3)_2$ complex **10** (Figure 10a, inset). We therefore concluded that only CTP_3 effectively inhibits the photodecomposition of **9**, as summarized in Scheme 8.

Stereoselective Interaction of 9 with Chiral IP_3. The stereoselective interaction of **9** (*racemic*) with IP_3, which is a chiral molecule, was studied by luminescence titrations at pH 7.4 (10 mM HEPES with I = 0.1 ($NaNO_3$)) and 25 °C. As shown in Figure 11, the addition of 3 equiv of IP_3 induced ca. a 2-fold increase in the emission of **9** at 584 nm. Further addition of achiral CTP_3 to this reaction mixture resulted in an increase

(45) (a) Van Houten, J.; Watts, R. J. *J. Am. Chem. Soc.* **1976**, *98*, 4853−4858. (b) Van Houten, J.; Watts, R. J. *Inorg. Chem.* **1978**, *17*, 3381−3385. (c) Gleria, M.; Minto, F.; Beggiato, G.; Bortolus, P. *J. Chem. Soc., Chem. Commun.* **1978**, 285. (d) Hoggard, P. E.; Porter, G. B. *J. Am. Chem. Soc.* **1978**, *100*, 1457−1463. (e) Durham, B.; Caspar, J. V.; Nagle, J. K.; Meyer, T. J. *J. Am. Chem. Soc.* **1982**, *104*, 4803−4810. (f) Vaidyalingam, A.; Dutta, P. K. *Anal. Chem.* **2000**, *72*, 5219−5224.

(46) Athough the mechanism involved in the photodecomposition of **9** and **19** are unknown, we assume that alkyl groups possessing cyclen moieties at 5,5′-position of 2,2′-bpy might accelerate photodecomposition of $Ru(bpy)_3$ centers. Although attempts to characterize the products of photodecomposition of **9** were carried out, ^1H NMR spectra of the photoreaction mixture from **9** showed a mixture of several products, which were not identical with **8** (Zn_2L^4). In the UV spectra of a mixture of 100 μM **9** with or without 100 μM D-Glu-6-P after UV irradiation at 300 nm for 8 h in 10 mM HEPES (pH 7.4) with I = 0.1 ($NaNO_3$) at 25 °C, MLCT absorption at ca. 460 nm has disappeared (data not shown), strongly indicating that $Ru(bpy)_3$ moiety is dissociated.

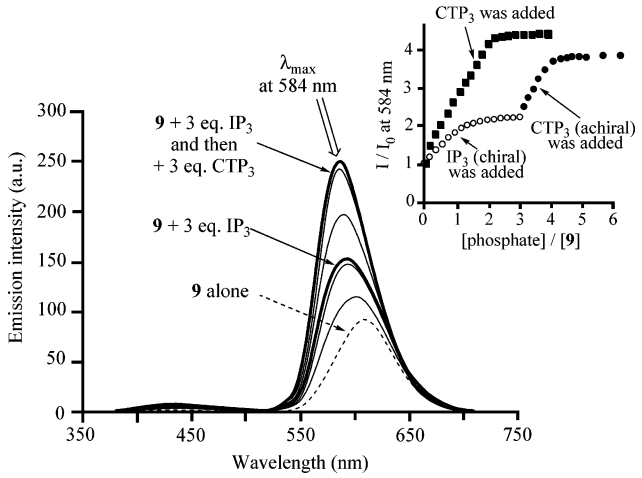
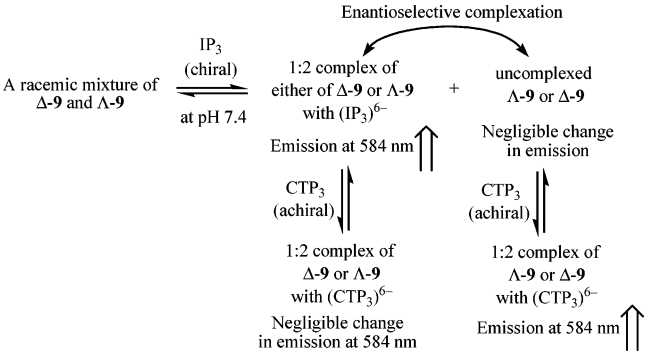


Figure 11. Luminescence response of **9** (10 μM) at 584 nm (excitation at 300 nm) against successive addition of IP_3 and CTP_3 at pH 7.4 (10 mM HEPES with $I = 0.1$ ($NaNO_3$)) and 25 °C. IP_3 was added up to 3 equiv against **9**, and then CTP_3 was added up to 3 equiv (see also the inset). Closed squares in the inset show increasing emission of **9** (10 μM) upon addition of CTP_3 (the same curve as that in Figure 6).

Scheme 9



in the emission by a factor of 4. These results imply that chiral IP_3 binds to either **Δ-9** or **Λ-9** (5 μM) and that another enantiomer of **9** (5 μM) remains uncomplexed (Scheme 9). The CTP_3 added to this solution binds to the remaining enantiomer of **9** to enhance its emission at 584 nm, indicating that either enantiomer of **9** binds to chiral IP_3 with a high degree of enantioselectivity.

Figure 12 clearly shows that a supramolecular sensor **9** (10 μM) selectively responds to CTP_3 and IP_3. The intensity of luminescence from a mixture of 10 μM **9** and 20 μM IP_3 is almost half that from a mixture of 10 μM **9** and 20 μM CTP_3, which shows good coincidence with Figure 11.

Conclusion

In this article, we first described that a tris(Zn^{2+}-cyclen) **6** $(Zn_3L^3)_3$ forms a stable 1:1 complex **7** with CTP_3, whose K_d is 10 nM at pH 7.4, through three Zn^{2+}−O(phosphate) coordination bonds. Since **6** has low molar extinction coefficients (ϵ) and is nonfluorescent, we have designed and synthesized a new supramolecular sensor **9** ($Ru(Zn_2L^4)_3$) having a luminescent Ru(bpy)$_3$ center and six Zn^{2+}-cyclen units as phosphate binding sites for IP_3 and CTP_3. Potentiometric pH, ^{1}H NMR, and UV titrations confirmed that **9** forms a very stable 1:2 complex **10** with CTP_3 in aqueous solution at neutral pH. Interestingly, luminescence emission of **9** was enhanced by a factor of 4.2 upon complexation with CTP_3. We concluded that **9** is the first

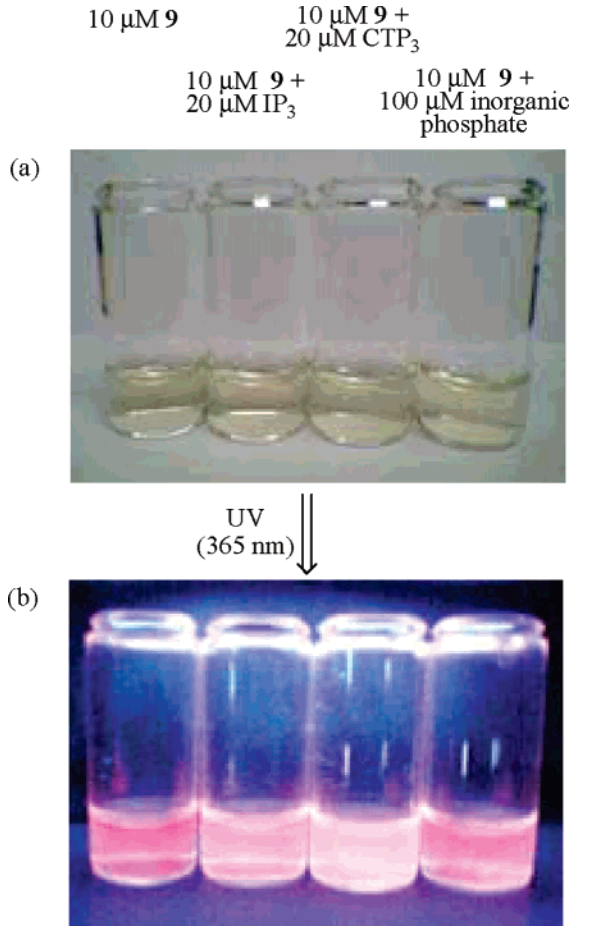


Figure 12. (a) Photograph showing solution of 10 μM **9**, 10 μM **9** + 20 μM IP_3, 10 μM **9** + 20 μM CTP_3, and 10 μM **9** + 100 μM $HOPO_3^{2-}$ (from left to right) at pH 7.4 (10 mM HEPES with $I = 0.1$ ($NaNO_3$)) and 25 °C. (b) Luminescence from 10 μM **9**, 10 μM **9** + 20 μM IP_3, 10 μM **9** + 20 μM CTP_3, and 10 μM **9** + 100 μM $HOPO_3^{2-}$ (from left to right) excited by UV light at 365 nm.

luminescence supramolecular sensor that directly responds to IP_3 and CTP_3 and that discriminates these triphosphates from monophosphates and diphosphates in aqueous solution at neutral pH. Potent recognition of CTP_3 (and IP_3) by **6** and **9** might be useful in the design of effective inhibitors of IP_3. Unexpectedly, we found that **9** decomposes by exposure to UV light with a considerable emission shift from 610 to 440 nm (excitation at 300 nm) and that photodecomposition of **9** is effectively inhibited upon complexation with CTP_3. These facts may suggest some aspects of the mechanism involved in the photodecomposition of Ru(bpy)$_3$.

In natural biological systems, the Zn^{2+} ions are used as a structural factor (as seen in zinc finger proteins and enzymes such as alcohol dehydrogenase), a catalytic factor (in zinc(II) enzymes including carbonic anhydrase and alcohol dehydrogenase), and a cocatalytic factor (in aminopeptidase from *Aeromonas proteolytica*).[47] In our previous supramolecular complexes such as a cuboctahedral capsule and trigonal prisms,[17] Zn^{2+} ions are five-coordinated and work as structural factors. In contrast, Zn^{2+} ions in an IP_3 sensor **9** function as recognition

(47) (a) Vallee, B. L.; Falchuk, K. H. *Physiol. Rev.* **1993**, *73*, 79−118. (b) Vallee, B. L.; Auld, D. S. *Acc. Chem. Res.* **1993**, *26*, 543−551. (c) Auld, D. S. In *Handbook of Metalloproteins*; Bertini, I., Sigel, A., Sigel, H., Eds.; Marcel Dekker: New York, 2001; pp 881−959. (d) Auld, D. S. *BioMetals* **2001**, *14*, 271−313.

factors for phosphates. This knowledge will be useful in designing novel supramolecular complexes and their applications in bioorganic chemistry, bioinorganic chemistry, and other related scientific fields.

Acknowledgment. We thank Professor Yoshifumi Tanimoto and Dr. Yoshihisa Fujiwara of the Graduate School of Science, Hiroshima University, for their measurements of the lifetimes of the Ru complexes. This work was supported by the grants-in-aid from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan (Nod. 13557195 and 15590008) and the High-Tech Research Center Project for Private Universities (matching fund subsidy from MEXT). M.Z. is grateful for the grants from the Ministry of Education, Science and Culture of Japan. S.A. is appreciative of the grants from the Mitsubishi Chemical Corporation Fund (Tokyo), the Toray Science Foundation (Chiba), the Terumo Life Science Foundation (Kanagawa), and the Mochida Memorial Foundation for Medical and Pharmaceutical Research (Tokyo). We also thank the Research Center for Molecular Medicine (RCMM) at Hiroshima University for use of the NMR instruments (for the JEOL Alpha (400 MHz)).

Supporting Information Available: Experimental section (general information and synthetic procedures of Ru complexes and phosphates), Figures S1−S9, and tables and CIF file for **9** (Ru(Zn_2L^4)$_3$). This material is available free of charge via the Internet at http://pubs.acs.org.

JA050876B