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Fluorescent Sensing of IP_3 with a Trifurcate Zn(II)-Containing Chemosensing Ensemble System

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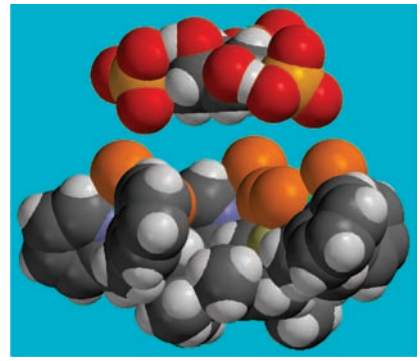
ABSTRACT



A trifurcate receptor containing Zn(II)-dipicolylamine ligands is developed for the fluorescent sensing of IP_3, *myo*-inositol 1,4,5-tris(phosphate), through an indicator displacement approach. The chemosensing ensemble containing the Zn(II) complex and eosin Y as indicator shows the maximum fluorescence restoration for IP_3 among various other anions including phosphate derivatives in water buffered at pH = 7.

myo-Inositol 1,4,5-tris(phosphate) (IP_3) is an important signaling molecule involved in intracellular signal transduction.[1] The biological importance of IP_3 and its analogues makes them attractive targets for the development of chemosensors. Significant progress has been made in the development of chemosensors for simple mono- and diphosphate ions and their nucleic acid derivatives.[2] However, chemosensors for tris(phosphate) such as IP_3 or higher homologues have been less explored, and only a few chemical sensing systems for IP_3 have been reported so far.[3,4] To develop an abiological sensing system for tris(phosphate) analytes such as IP_3, we need a recognition motif that recognizes the three asymmetrically substituted phosphate groups on the *myo*-inositol backbone, *cis*-1,2,3,5-*trans*-4,6-cyclohexanehexol. Anslyn and co-workers devised a cleft-like receptor that provides six peripheral guanidinium groups for chemical sensing of IP_3 through a competition assay.[3a]

(1) (a) Berridge, M. J. *Nature* **1993**, *361*, 315. (b) Clapham, D. E. *Cell* **1995**, *80*, 259. (c) Hinterding, K.; Alonso-Diaz, D.; Waldmann, H. *Angew. Chem., Int. Ed.* **1998**, *37*, 688. (d) Irvine, R. *Curr. Biol.* **2001**, *11*, R172. (e) Taylor, C. W.; Thorn, P. *Curr. Biol.* **2001**, *11*, R352.

(2) (a) Jose, D. A.; Mishra, S.; Ghosh, A.; Shrivastav, A.; Mishra, S. K.; Das, A. *Org. Lett.* **2007**, *9*, 1979. (b) Nishizawa, S.; Kato, Y.; Teramae, N. *J. Am. Chem. Soc.* **1999**, *121*, 9463. (c) Aldakov, D.; Anzenbacher, P, Jr. *J. Am. Chem. Soc.* **2004**, *126*, 4752. (d) Zyryanov, G. V.; Palacios, M. A.; Anzenbacher, P. *Angew. Chem., Int. Ed.* **2007**, *46*, 7849. (e) Lee, D. H.; Lee, K. H.; Hong, J.-I. *Org. Lett.* **2001**, *3*, 5. (f) Mizukami, S.; Nagano, T.; Urano, Y.; Odani, A.; Kikuchi, K. *J. Am. Chem. Soc.* **2002**, *124*, 3920. (g) Yamaguchi, S.; Yoshimura, I.; Kohira, T.; Tamaru, S.; Hamachi, I. *J. Am. Chem. Soc.* **2005**, *127*, 11835. (h) Han, M. S.; Kim, D. H. *Angew. Chem., Int. Ed.* **2002**, *41*, 3809. (i) Lee, D. H.; Im, J. H.; Son, S. U.; Chung, Y. K.; Hong, J.-I. *J. Am. Chem. Soc.* **2003**, *125*, 7752. (j) Lee, H. N.; Swamy, K. M. K.; Kim, S. K.; Kwon, J. -Y.; Kim, Y.; Kim, S. -J.; Yoon, Y. J.; Yoon, J. *Org. Lett.* **2007**, *9*, 243.

(3) (a) Nikura, K.; Anslyn, E. V. *J. Am. Chem. Soc.* **1998**, *120*, 8533. (b) Aoki, S.; Zulkefeli, M.; Shiro, M.; Kohsako, M.; Takeda, K.; Kimura, E. *J. Am. Chem. Soc.* **2005**, *127*, 9129.

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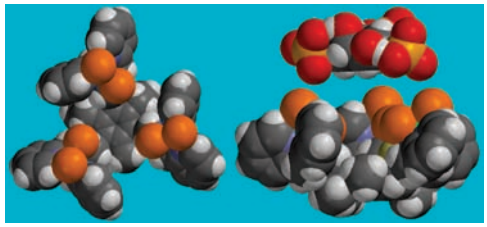
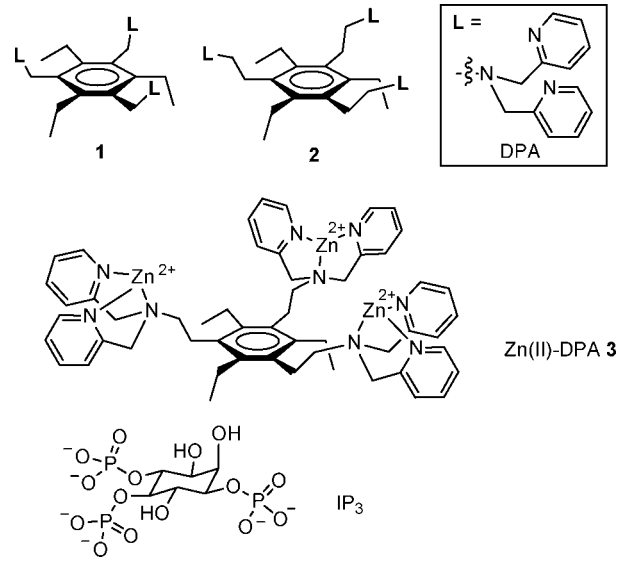


Figure 1. Modeled structures of **3** ($ZnCl_2$-complex) (left) and IP_3 stacked on **3** (right).

Kimura and co-workers devised a luminescent ruthenium(II)-templated assembly that provides two tripodal Zn(II)-cyclen units for fluorescent sensing of IP_3 and its analogues.[3b] This ruthenium complex recognizes two molecules of a C_3-symmetric *cis*,*cis*-1,3,5-cyclohexanetriol triphosphate (CTP_3) in a stepwise manner. We were interested in a simpler tripodal recognition system that has three phosphate-binding units in a matched geometry with the three phosphate groups of IP_3 or its sereoisomers. Here, we report the new tripodal platform for chemical sensing of IP_3 through the competition assay.

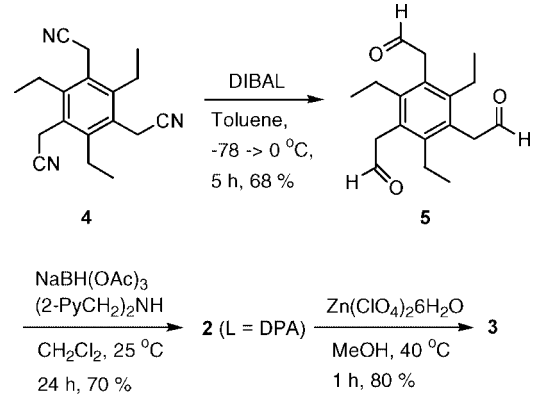
Initially, we focused on a benzene-based tripodal system such as **1**,[5] which readily provides three binding units (L) in the same hemisphere through conformational preorganization owing to the steric hindrance between the adjacent substituents. As the phosphate binding unit, we chose metal complexes of dipicolylamine (DPA), which have been successfully used for the recognition of phosphate derivatives.[2g–j] Thus, we synthesized a tripodal Cu(II)-complex of **1** (L = DPA) and its application to a competition assay for phytate, a hexakis(phosphate) anion.[6] However, the complex could not provide geometry wide enough to accommodate phytate or its analogues such as IP_3 in a 1:1 chelation mode. We need an extended tripodal platform to accommodate IP_3 or its stereoisomers. Thus, we have devised a new platform **2** on the base of a molecular modeling study.



Although the tripodal system **2** (L = DPA) has the extended structure, its metal complex shows a well-preorganized conformation, particularly suitable for the recognition of IP_3 or CTP_3. A modeled structure for a Zn(II) complex of **2** (L = DPA) shows that the three Zn(II)-DPA groups can have the "*all-syn*" conformation with little distortion. Furthermore, this extended structure seems to adequately accommodate IP_3, as shown in the stacked structure (Figure 1).

The ligand **2** (L = DPA) can be synthesized readily by reductive amination of tris(formylmethyl)benzene **5** with dipicolylamine using sodium acetoxyborohydride in 70% yield. Aldehyde **5** was prepared from the tris(cyanomethyl)benzene **4**[5g] by treatment with DIBAL in 68% yield. Treatment of tris(dipicolylamine) **2** with zinc perchlorate hexahydrate in methanol afforded the corresponding Zn(II) complex **3** in 80% isolated yield (Scheme 1).

Scheme 1. Synthesis of Zn(II) Complex **3**



The NMR spectrum of ligand **2** (L = DPA) taken at room temperature showed a C_3 symmetric structure in solution,

(4) For biosensors of IP_3, see: (a) Hirose, K.; Kadowaki, S.; Tanabe, M.; Takeshima, H.; Iino, M. *Science* **1999**, *284*, 1527. (b) Luzzi, V.; Murtazina, D.; Allbritton, N. L. *Anal. Biochem.* **2000**, *277*, 221. (c) Cheley, S.; Gu, L.-Q.; Bayley, H. *Chem. Biol.* **2002**, *9*, 829. (d) Yamakoshi, M.; Takahashi, M.; Kouzuma, T.; Imanmura, S.; Tsuboi, I.; Kawazu, S.; Yamagata, F.; Tominaga, M.; Noritake, M. *Clin. Chim. Acta* **2003**, *328*, 163. (e) Morii, T.; Sugimoto, K.; Makino, K.; Otsuka, M.; Imoto, K.; Mori, Y. *J. Am. Chem. Soc.* **2002**, *124*, 1138. (f) Sugimoto, K.; Nishida, M.; Otsuka, M.; Makino, K.; Ohkubo, K.; Mori, Y.; Morii, T. *Chem. Biol.* **2004**, *11*, 475.

(5) For selected examples of benzene-based tripodal receptors used in molecular recognition, see: (a) Metzger, A.; Lynch, V. M.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **1997**, *36*, 862. (b) Kimura, E.; Aoki, S.; Koike, T.; Shiro, M. *J. Am. Chem. Soc.* **1997**, *119*, 3068. (c) Chin, J.; Walsdorff, C.; Stranix, B.; Oh, J.; Chung, H. J.; Park, S.-M.; Kim, K. *Angew. Chem., Int. Ed.* **1999**, *38*, 2756. (d) Lavigne, J. L.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **1999**, *38*, 3666. (e) Abouderbala, L. O.; Belcher, W. J.; Boutelle, M. G.; Cragg, P. J.; Steed, J. W.; Turner, D. R.; Wallace, K. J. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5001. (f) Tobe, S. L.; Anslyn, E. V. *Org. Lett.* **2003**, *5*, 2029. For some of our own contributions, see: (g) Kim, S.-G.; Ahn, K. H. *Chem.*−*Eur. J.* **2000**, *6*, 3399. (h) Kim, S.-G.; Kim, K.-H.; Jung, J.; Shin, S. K.; Ahn, K. H. *J. Am. Chem. Soc.* **2002**, *124*, 591. (i) Kim, S. J.; Kim, K. H.; Kim, Y. K.; Shin, S. K.; Ahn, K. H. *J. Am. Chem. Soc.* **2003**, *125*, 13819. (j) Kim, J.; Kim, S.-G.; Seong, H. R.; Ahn, K. H. *J. Org. Chem.* **2005**, *70*, 7227. (k) Kim, K.; Raman, B.; Ahn, K. H. *J. Org. Chem.* **2006**, *71*, 38.

(6) Oh, D. J.; Han, M. S.; Ahn, K. H. *Supramol. Chem.* **2007**, *19*, 315–320.

whereas that of $Zn(ClO_4)_2$ complex **3** showed broad peaks in the temperature range of 25−73 °C, indicating that the metal complex is sterically bulky and thus conformational change is slow.

Because Zn(II) complex **3** does not contain a good chromo- or fluoro-genic moiety, a direct sensing of IP_3 is not feasible. Therefore, we have investigated the competition assay using a chemosensing ensemble system that consists of a receptor for an analyte and a dye competitive with the analyte. Such a chemical ensemble system has been demonstrated to be useful in sensing various analytes for its operational simplicity and versatility in the receptor design.[7] The chemosensing ensembles based on metal coordination complexes seem to be promising for the development of assay systems for phosphate groups, because such coordination complexes can provide strong binding affinity toward the anions even in aqueous media.[8] Thus far, only a few trifurcated sensing systems containing metal complexes as the binding units have been reported.[6,9]

First, we evaluated several dyes such as eosin Y, 5-carboxy-fluorescein, and coumarine 343 to find an optimal chemosensing ensemble for Zn(II)-DPA **3**. Fluorescence titrations of eosin Y (3.0 μM) with **3** (0−6.0 μM) in water buffered at pH 7.0 (HEPES, 10 mM) resulted in continuous quenching up to two equivalent points, which suggested a 1:2 binding mode (Figure S1). The other dyes showed small or little quenching. A Job plot for the complex formation between eosin Y and Zn(II) complex **3** supported the 1:2 (**3**/eosin Y) binding stoichiometry (Figure S2). By carrying out UV/vis titrations between eosin Y and Zn(II) complex **3** (Figure S3), we could determine association constants for the dye binding processes: $K_1 = 1.2 \times 10^6\ M^{-1}$, $K_2 = 8.2 \times 10^4\ M^{-1}$.

On the basis of the titration experiments, an ensemble system containing Zn(II) complex **3** and eosin Y was chosen for the chemical sensing study. Thus, a chemical ensemble system composed of 3.0 μM of eosin Y and 2.0 μM of **3** in water at pH 7.0 (HEPES, 10 mM) was titrated with increasing amount of IP_3 (0−6.0 μM) and the resulting changes in the fluorescence spectra were recorded with an excitation wavelength at 517 nm. As shown in Figure 2, the fluorescence of eosin Y restores as it is replaced by IP_3 added, showing a saturation behavior after about 2.2 equivalents of IP_3 were added.

The fluorescence titration was carried out at different pH, from 6.0 to 8.5 (Figure 3). The data indicate that the present chemosensing ensemble system works better for the physiological pH range (pH 7.3−7.4). The difference in fluorescence intensity before and after addition of IP_3 becomes smaller both at the lower and higher pH ranges.

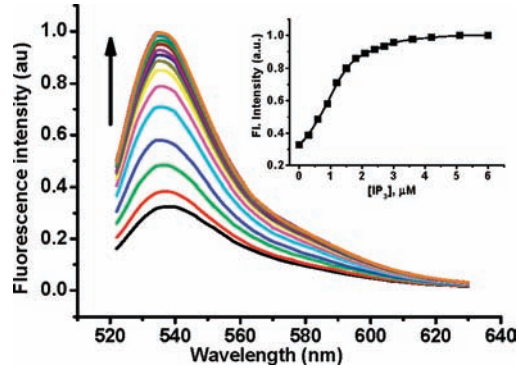


Figure 2. Fluorescence titration of the chemical ensemble (a solution containing 3.0 μM eosin Y and 2.0 μM Zn(II) complex **3**) with IP_3 (0−6.0 μM) in water buffered at pH 7.0 (HEPES 10 mM), with the excitation wavelength at 517 nm. (Inset) Plot of the fluorescence intensity (peak height at 536 nm) against concentration of IP_3.

An attempt to determine the association constant between Zn(II)-DPA **3** and IP_3 (K_5) directly by isothermal titration calorimetry (ITC) was not successful. To carry out a competition calorimetry we needed a competitive analyte that binds Zn(II) complex **3** in a 1:1 stoichiometry and with lower binding affinity. We have found that benzene-1,3,5-tricarboxylate (BTC), trimesic acid sodium salt, can be used as the competitive analyte. The Zn(II) complex **3** was found to bind BTC with an association constant of $2.0 \times 10^4\ M^{-1}$ and with near 1:1 binding stoichiometry ($n = 0.90$), as determined by ITC titrations carried out in 20% DMSO-water buffered at pH 7.0 (HEPES, 10 mM) at 303 K (Figure S4). Although the n value deviated a little from unity, indicating that other minor binding modes are involved, we used BTC as the competition ligand to determine the binding

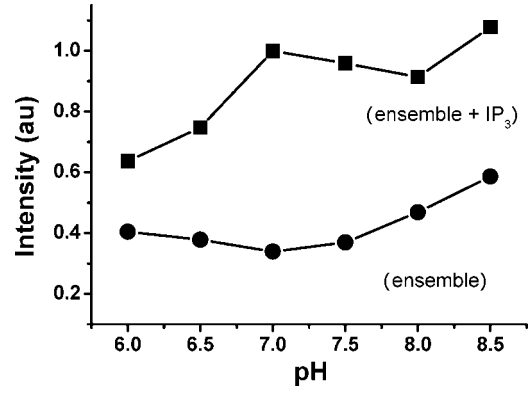


Figure 3. Fluorescence changes depending on pH; ●, the ensemble only (2.0 μM eosin Y and 2.0 μM**3**); ■, the ensemble with IP_3 (2.0 μM) in water buffered at various pH's: pH 6.0 (MES 10 mM), pH 6.5 (MES 10 mM), pH 7.0 (HEPES 10 mM), pH 7.5 (HEPES 10 mM), pH 8.0 (HEPES 10 mM), and pH 8.5 (CHES 10 mM).

(7) (a) Metzger, A.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **1998**, *37*, 649. (b) Niikura, K.; Metzger, A.; Ansyln, E. V. *J. Am. Chem. Soc.* **1998**, *120*, 8533. (c) Lavigne, J. J.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **2001**, *40*, 3119. (d) Wiskur, S. L.; Ait-Haddou, H.; Lavigne, J. J.; Anslyn, E. V. *Acc. Chem. Res.* **2001**, *34*, 963.

(8) (a) Han, M. S.; Kim, D. H. *Angew. Chem., Int. Ed.* **2002**, *41*, 3809. (b) Han, M. S.; Kim, D. H. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1079. (c) Hanshaw, R. G.; O'Neil, E. J.; Foley, M.; Carpenter, R. T.; Smith, B. D. *J. Mater. Chem.* **2005**, *15*, 2707.

(9) (a) Kimura, E.; Aoki, S.; Koike, T.; Shiro, M. *J. Am. Chem. Soc.* **1997**, *119*, 3068–3076. (b) Morey, J.; Orell, M.; Barceló, M. À; Deyà, P. M.; Costa, A.; Ballester, P. *Tetrahedron Lett.* **2004**, *45*, 1261. (c) Fabbrizzi, L.; Foti, F.; Taglietti, A. *Org. Lett.* **2005**, *7*, 2603.

affinity of Zn(II) complex **3** toward IP_3. Thus, from competitive ITC titrations for a 1:1 mixture of **3** and BTC with IP_3, we were able to obtain an approximate association constant between **3** and IP_3 in an aqueous buffer solution (pH 7.0, HEPES 10 mM) containing 20% DMSO at 303 K: $K_5 = 4.6\ (\pm 1.4) \times 10^8\ M^{-1}$. The complexation involved a large enthalpy change, $\Delta H^\circ = 8.8\ (\pm 0.3)$ kcal·mol^{-1} (Figure S5). With the K_1, K_2, K_5 values, we could also determine equilibrium constants for K_3 and K_4 according to the literature precedent:[10]

$$I + H \rightleftharpoons HI\ (K_1 = 1.2\ 10^6\ M^{-1})$$

$$HI + I \rightleftharpoons HI_2\ (K_2 = 8.2 \times 10^4\ M^{-1})$$

$$HI_2 + A \rightleftharpoons HI + HA\ (K_3 = 5.6 \times 10^3\ M^{-1})$$

$$HI + A \rightleftharpoons I + HA\ (K_4 = 3.9 \times 10^2\ M^{-1})$$

$$H + A \rightleftharpoons HA\ (K_5 = 4.6 \times 10^8\ M^{-1})$$

where H, I, and A represent Zn(II) complex **3**, eosin Y, and IP_3, respectively. In this calculation, we have assumed that Zn(II) complex **3** binds IP_3 in a 1:1 stoichiometry. An evidence for the 1:1 complexation between **3** and IP_3 was obtained by electrospray ionization mass spectrometry, which gave peaks responsible for 1:1 complexes (Figure S6). The competitive ITC titration data shows an inflection point at $[IP_3]/[\mathbf{3}] = 1.24$, a small deviation from the equimolar ratio. This deviation indicates that other higher-order complexes exist even though they are minor components. However, to a good approximation, we assumed that Zn(II) complex **3** interacts with IP_3 in a 1:1 binding mode.

On the basis of the fluorescence titration results, we evaluated chemical sensing ability of the ensemble system toward various other anions such as pyrophosphate (PPi), ATP, ADP, AMP, chloride, bromide, nitrate, sulfate, azide, acetate, perchlorate, hydrogen phosphate, and carbonate ions as their sodium salts. The fluorescence titration toward each of the anions was carried out using an ensemble system containing 2.0 μM each of Zn(II) complex **3** and eosin Y in an aqueous solution buffered at pH 7.0 (HEPES, 10 mM) at room temperature. The results are summarized in Figure 4.

The ensemble system shows the highest selectivity for IP_3 among the anions including mono- and diphosphate anions. Clearly, the tripodal nature of ensemble system discriminates monophosphate compounds such as AMP and HPO_4^{2-} as well as all the nonphosphate anions, exhibiting slight fluorescence response. Also, the ensemble system has decreased sensitivity toward ATP and ADP, showing relative fluorescence intensity of 58% and 48% with respect to that of IP_3, respectively. This lower selectivity seems to be originated from the tripodal nature of the ensemble system, of which geometry does not match with the linear tri- or diphosphates. Interestingly, PPi shows the next best analyte

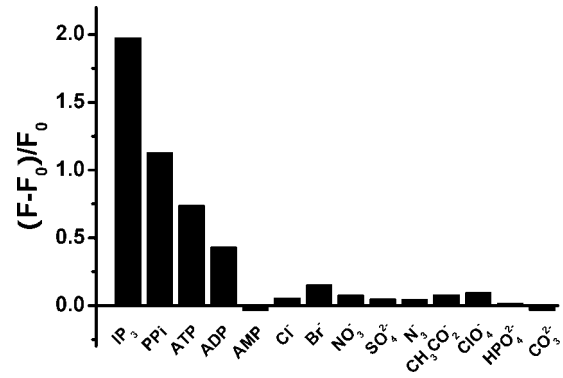


Figure 4. Comparison of the fluorescence intensity (peak height at 536 nm) of the ensemble system (2.0 μM eosin Y and 2.0 μM **3**) toward various anions (2.0 μM). F_0 is the intensity of the ensemble itself.

for the ensemble system. A higher selectivity of PPi over ATP or ADP suggests that steric bulkiness of the latter phosphates may influence the binding negatively. Regarding to the binding modes of such linear di- and triphosphate anions, not a 1:1 but a complex binding mode is expected. Indeed, ITC titrations of Zn(II) complex **3** with PPi gave a complex binding isotherm (Figure S7).

In summary, we have devised a novel chemosensing ensemble system for IP_3 based on a new tripodal receptor containing three Zn(II)-dipicolylamine ligands. The addition of IP_3 to the chemosensing ensemble system, a mixture of the Zn(II) complex and eosin Y, in an aqueous medium at pH 7 resulted in the restoration of fluorescence of eosin Y as it is expelled by the anion added. The ensemble system shows the maximum fluorescence enhancement in the case of IP_3, while it shows reduced changes in the cases of PPi, ATP, ADP, and monovalent anions (HPO_4^{2-}, CH_3CO_2-, CO_3^{2-}, Cl−, Br−, ClO_4-, N_3-, and NO_3-). The present chemical ensemble system is structurally simple, yet it can be used for fluorescence sensing of IP_3 in aqueous medium of physiological pH. A further exploration of the new tripodal platform in chemical sensing of other analytes is under investigation.

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Supporting Information Available: Details for the synthesis of Zn(II) complex **3** and Figures S1−S7. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) McCleskey, S. C.; Metzger, A.; Simmons, C. S.; Anslyn, E. V. *Tetrahedron* **2002**, *58*, 621.