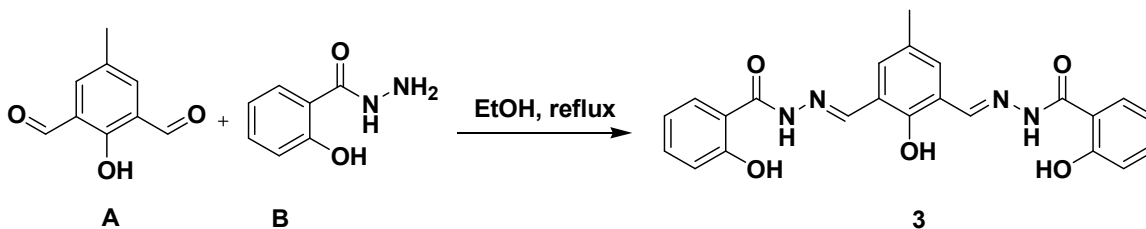


Supporting Information for

Binuclear Zn(II)–Zn(II) Complex from a 2-Hydroxybenzohydrazide-Derived Schiff Base for Selective Detection of Pyrophosphate Anion

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Synthesis of sensor 3. Intermediates **A** (80.0 mg)¹ and **B** (150.0 mg, from Acros Organics) were dissolved in EtOH, then the reaction mixture was refluxed for 2 hours. Dye **3** precipitated out as yellow solid and was purified by simple filtration in >90% yield. ¹H NMR (300 MHz, DMSO-*d*₆): 12.25 (2H, s), 12.05 (2H, br), 11.77 (2H, br), 8.73 (2H, s), 7.89 (2H, d, *J* = 7.5 Hz), 7.57 (2H, s), 7.45 (2H, tri, *J* = 7.2 Hz), 6.98 (2H, d, *J* = 8.7 Hz), 6.94 (2H, d, *J* = 8.4 Hz), 2.32 (3H, s); ¹H NMR (300 MHz, CD₃OD): 8.70 (2H, s), 7.94 (2H, d, *J* = 7.2 Hz), 7.67 (2H, s), 7.48 (2H, td, *J* = 1.8 Hz, *J* = 8.1 Hz), 7.01 (2H, d, *J* = 7.8 Hz), 6.98 (2H, d, *J* = 8.1 Hz), 2.37 (3H, s); ¹³C NMR (75 MHz, DMSO-*d*₆): 165.2, 159.5, 155.3, 147.4, 134.4, 131.1, 129.0, 128.8, 120.3, 119.4, 117.8, 116.1, 20.4; ESI (m/z): calcd for C₂₃H₁₉N₄O₅, [M-H]⁺, 431.1356, found, 431.1024.

Preparation for the Zinc complex. **3** was dissolved in DMSO at 10 mM as the stock solution and Zn(NO₃)₂ was dissolved in EtOH at 10 mM as the stock solution. 2 μL of **3** and 2 μL of Zn(NO₃)₂ stock solution were mixed to form the **3**-Zn²⁺ zinc complex. 2 μL of **3** and 4 μL of Zn(NO₃)₂ stock solution were mixed to form the **3**-2Zn²⁺ zinc complex, which was diluted to 2mL for UV-vis and fluorescent measurement.

Spectroscopic Measurements. NMR spectra were collected on a Varian 300 Gemini spectrometer. Mass spectrometric data were obtained on a HP1100LC/MSD mass spectrometry. HRMS data were performed on an ESI-TOF MS system (Waters, Milford, MA). UV-Vis spectra were acquired on a Hewlett-Packard 8453 diode-array spectrometer. Fluorescence spectra were obtained on a HORIBA Jobin Yvon NanoLog spectrometer.

Reference:

1. Q. Chu, D. A. Medvetz, M. J. Panzner, and Y. Pang, *Dalton Trans.*, 2010, **39**, 5254.

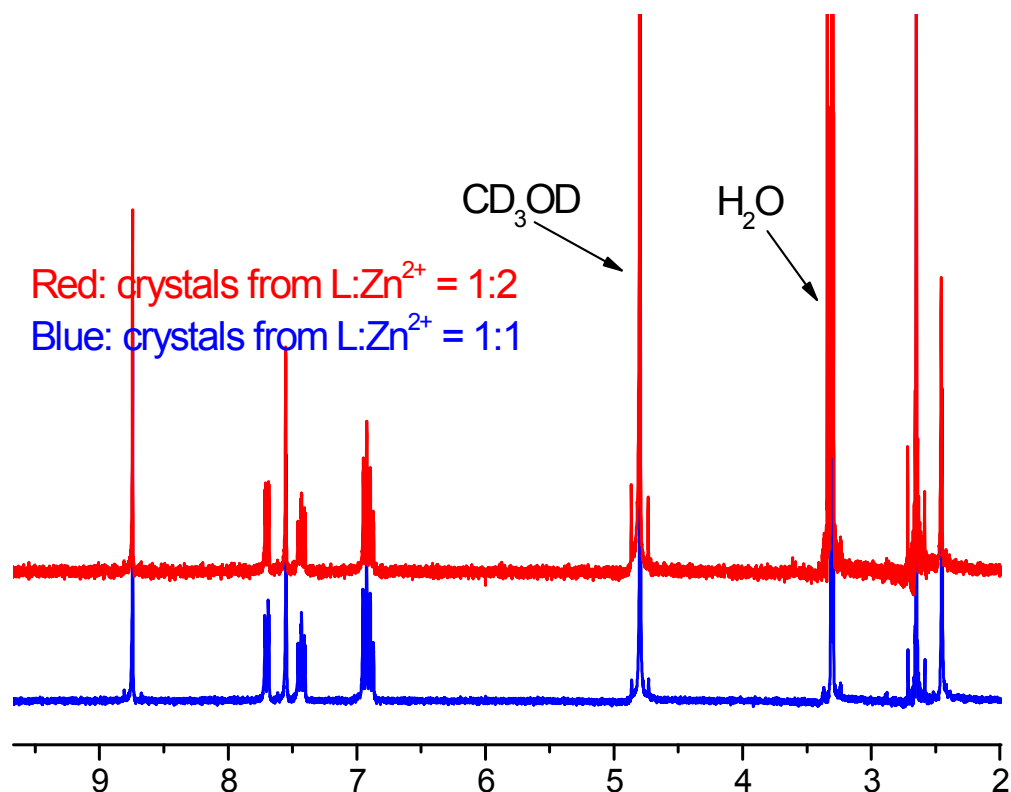


Fig. S1. ^1H NMR of **3**-Zn crystals **4** in CD_3OD : The crystalline products were obtained by mixing ligand **3** and $\text{Zn}(\text{NO}_3)_2$ in 1:1 and 1:2 ratio in DMSO/EtOH (5:1 by volume).

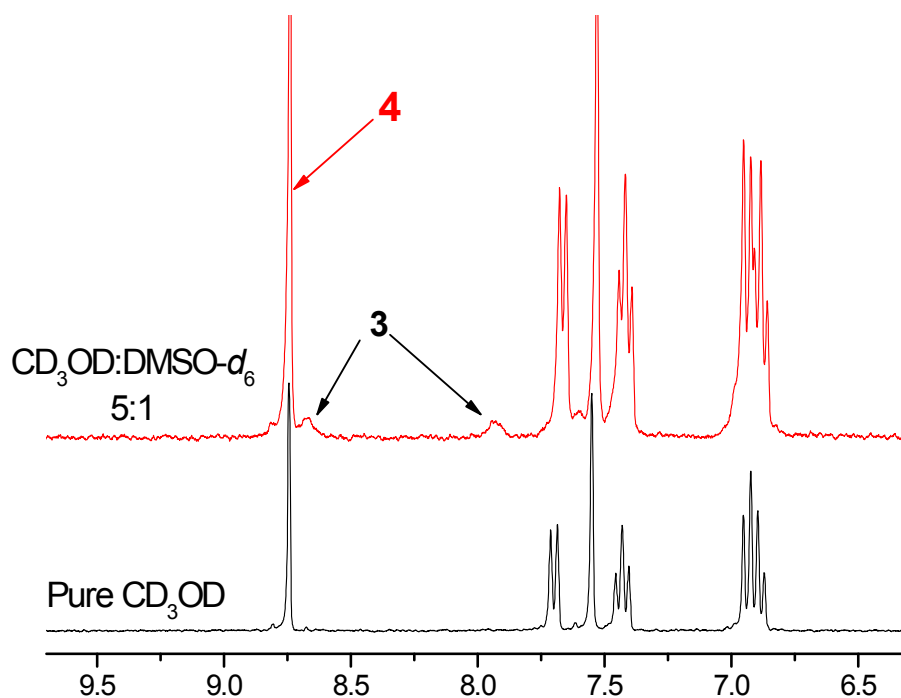


Fig. S2. ^1H NMR of crystals **4** in CD_3OD and the mixture solvent ($\text{CD}_3\text{OD}:\text{DMSO-}d_6 = 5:1$): This reveals that the crystals are stable in CD_3OD however led to partially decomposition upon addition of DMSO.

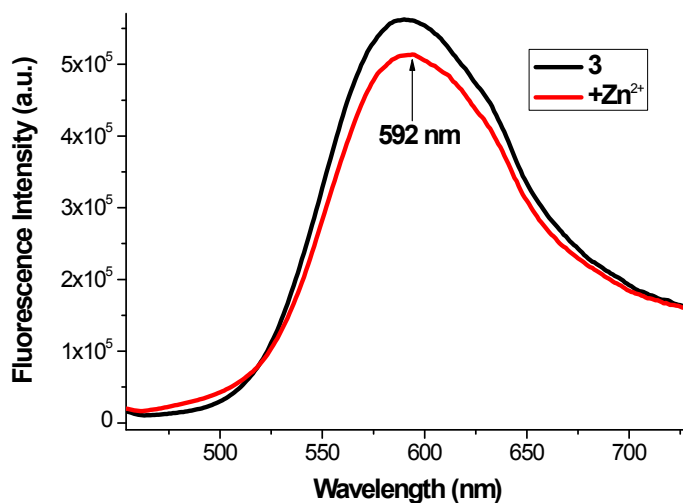


Figure S3a. Fluorescence spectra of **3** ($10\ \mu\text{M}$) in HEPES buffer ($10\ \text{mM}$, $\text{pH} = 7.2$), followed by addition of 2.0 equiv. of Zn^{2+} . (It tells the weak binding between **3** and Zn^{2+} in HEPES buffer.)

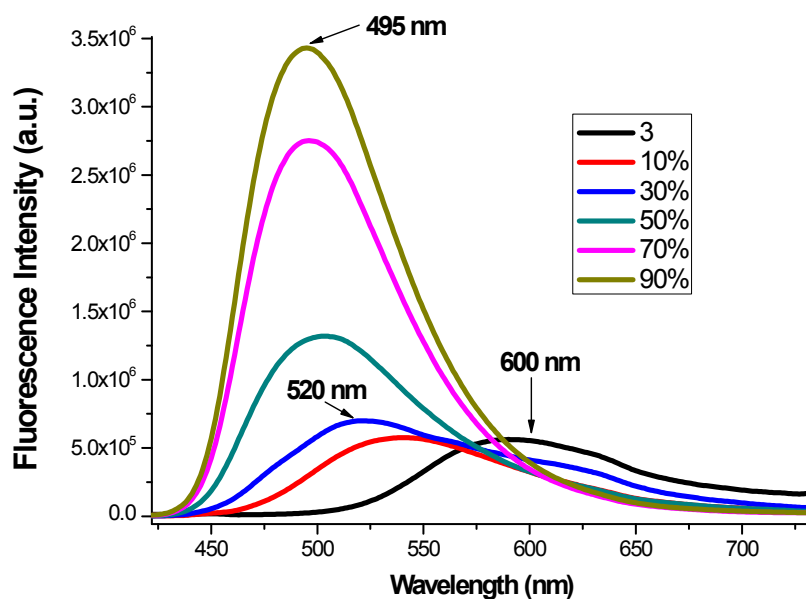


Figure S3b. Fluorescence spectra of **3** (10 μM) in HEPES buffer (10 mM, pH = 7.2) with different fractions of EtOH (% v/v), followed by addition of 2.0 equiv. of Zn²⁺. (It indicates that **3** binds Zn²⁺ strongly in EtOH, however very weak in HEPES buffer.)

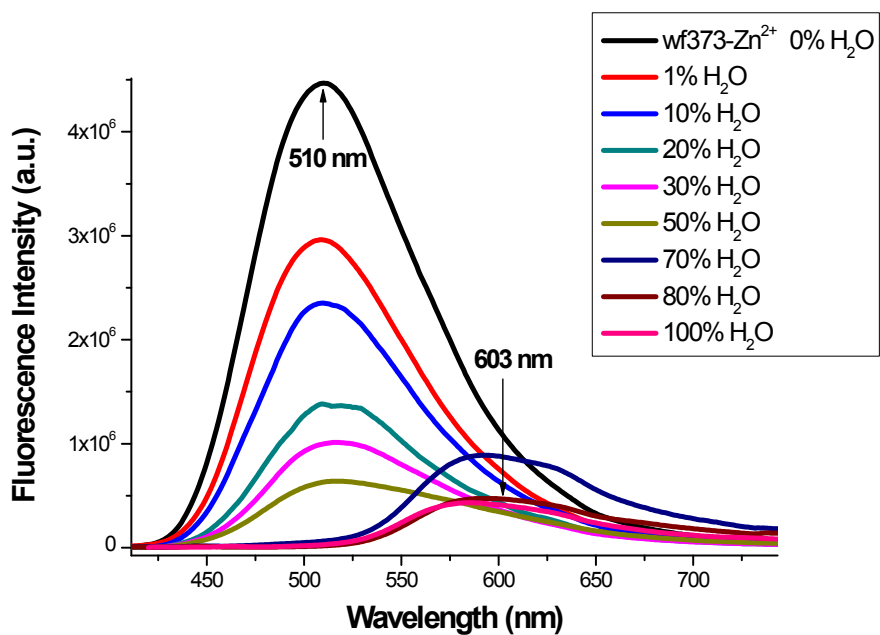


Figure 3c. Fluorescence spectra of [3-Zn²⁺] in the solvent of EtOH and H₂O (%). (It tells that **3** binds Zn²⁺ strongly in EtOH, however very weak in H₂O)

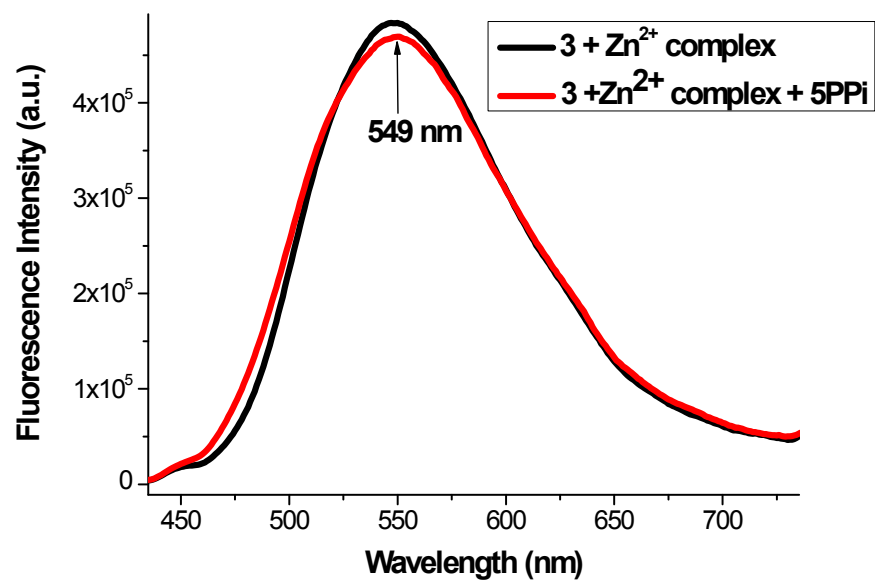


Figure S4. Fluorescence spectra of [**3** + **Zn**²⁺] complexes (10 μ M) in HEPES buffer (10 μ M, pH = 7.2) upon addition of 5.0 equiv. of PPI. It tells: a) zinc complexes decomposed in HEPES buffer; b) PPI did not response to [**3** + **Zn**²⁺] complexes in 100% buffer.

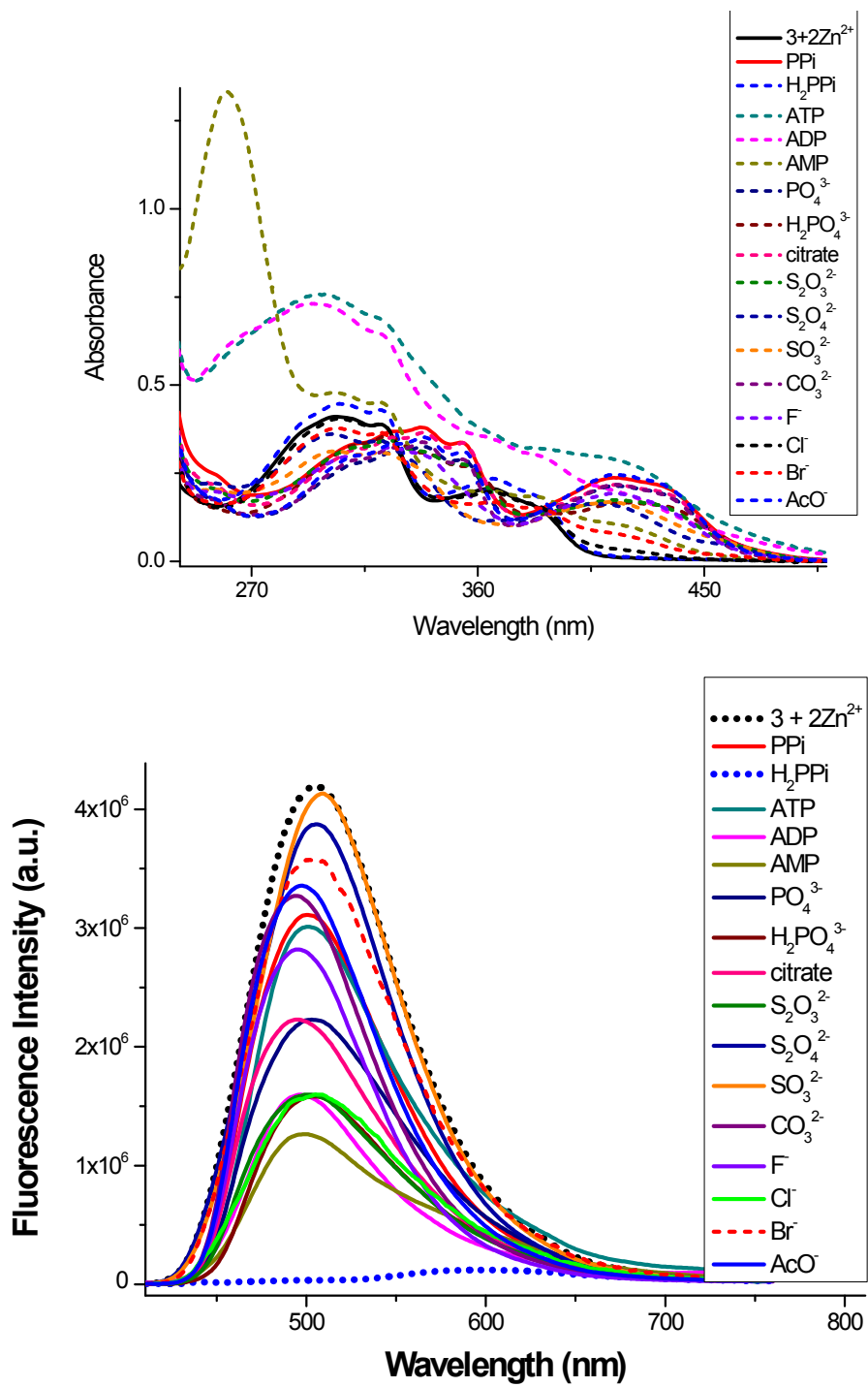


Figure S5. UV-vis (a) and fluorescence spectra (b) of $3+Zn^{2+}$ complex (10 μ M) in EtOH upon addition of 5.0 equivalent of different anions.

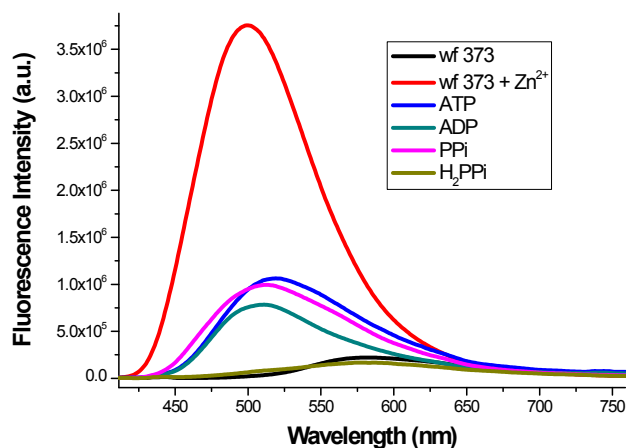


Figure S6. Fluorescence response of $3+Zn^{2+}$ complex ($10 \mu M$) in EtOH upon addition of 5.0 equivalent of different anions.

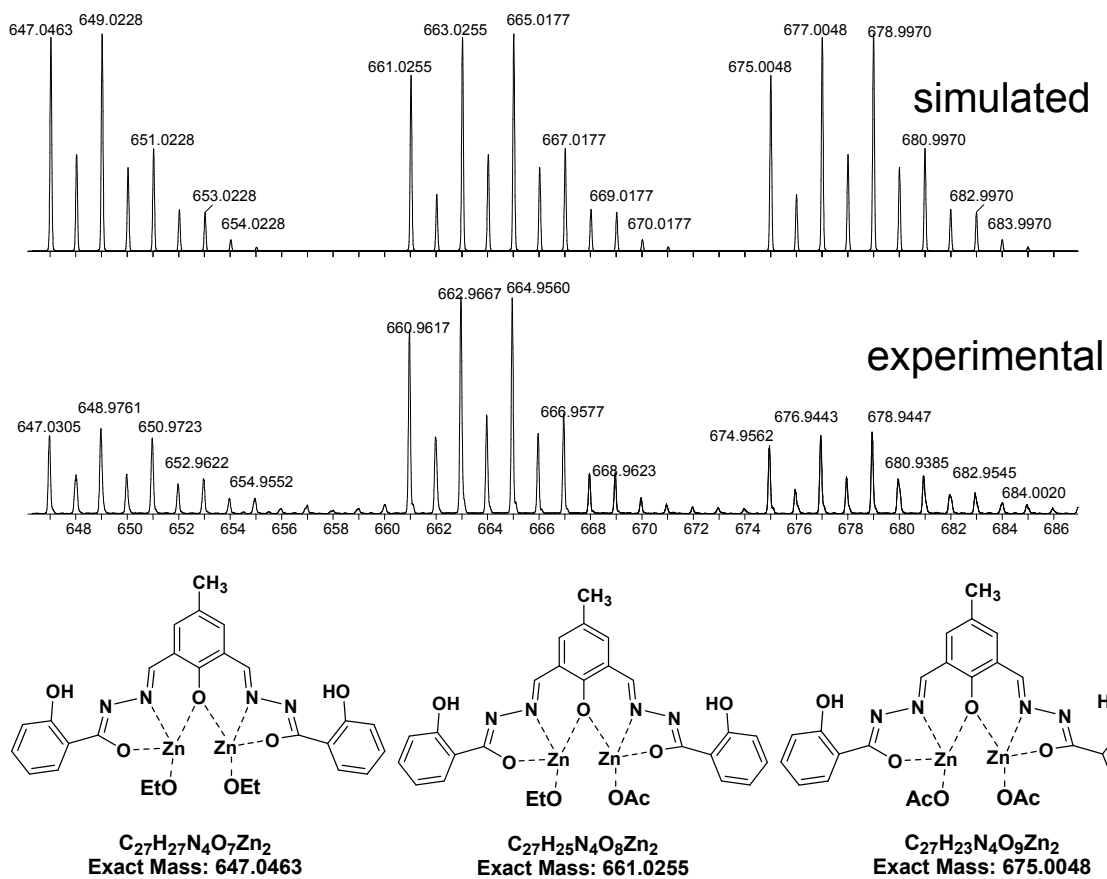


Figure S7. ESI Mass spectra of the zinc complexes of $3-Zn^{2+}$

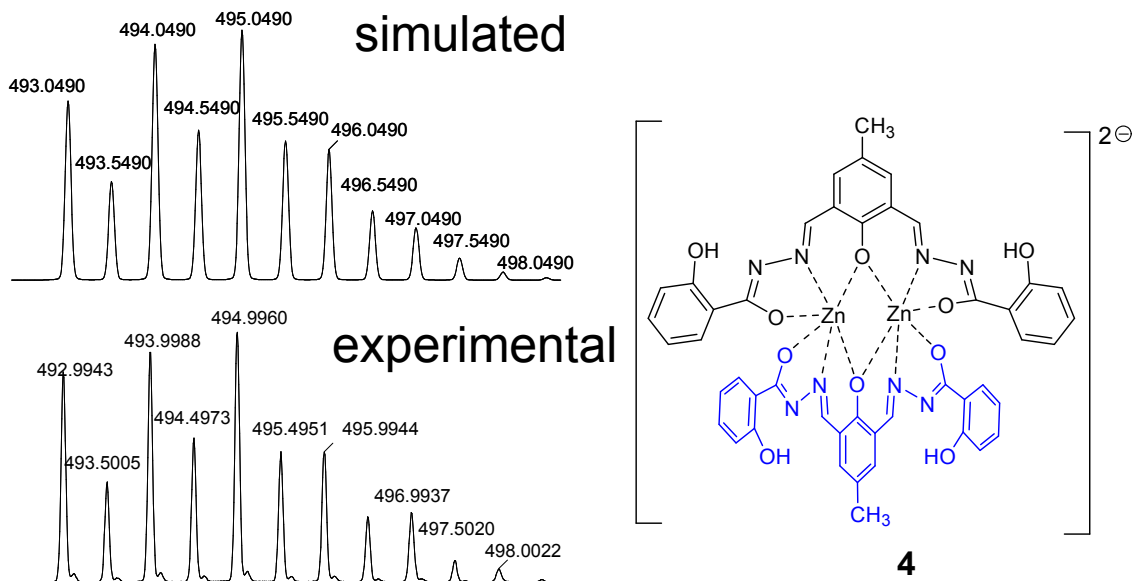


Figure S8. ESI Mass spectra of the dimer zinc complex **4**: 3_2Zn_2

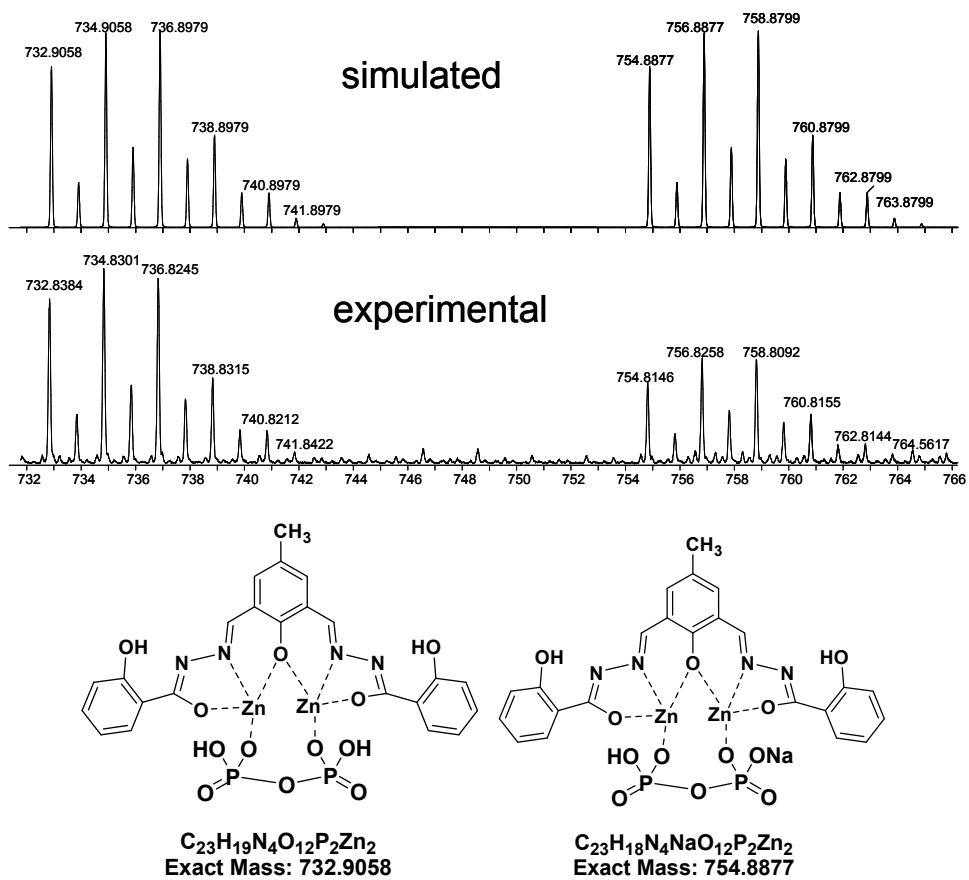


Figure S9. ESI Mass spectra of 3-Zn^{2+} -PPi adducts

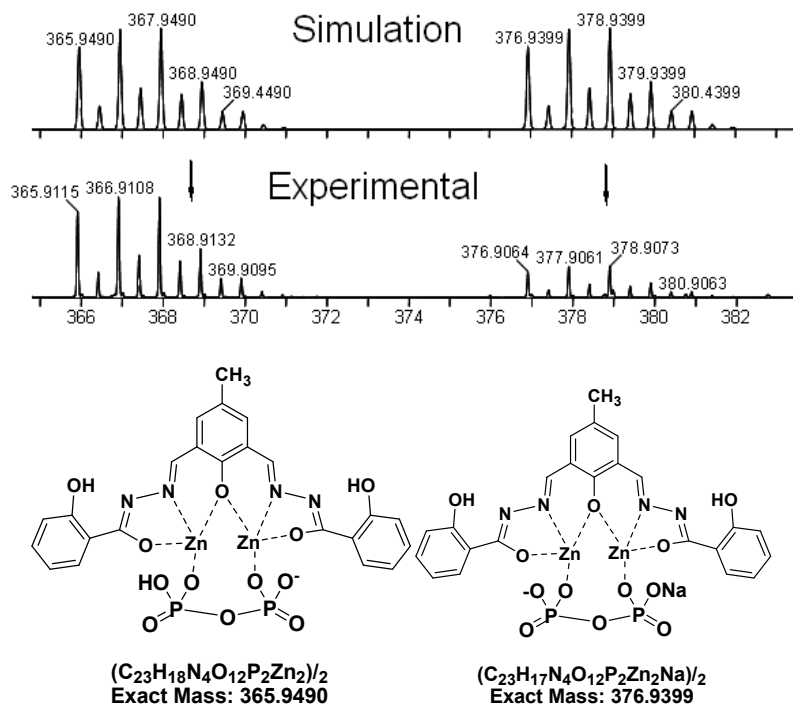


Figure S10. ESI Mass spectra of **3**-Zn²⁺-PPi adducts

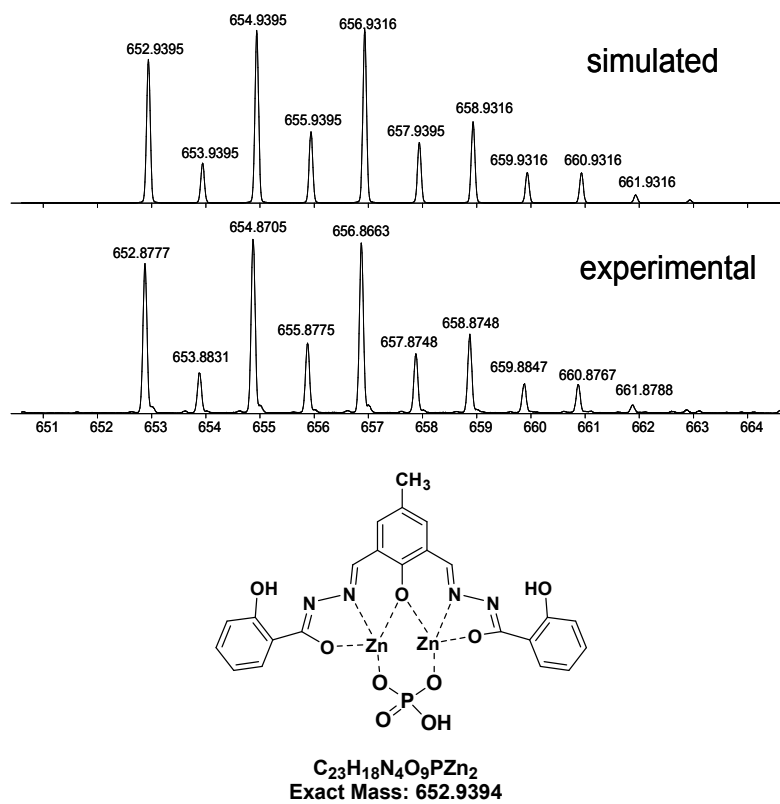


Figure S11. ESI Mass spectra of the key intermediate **8**

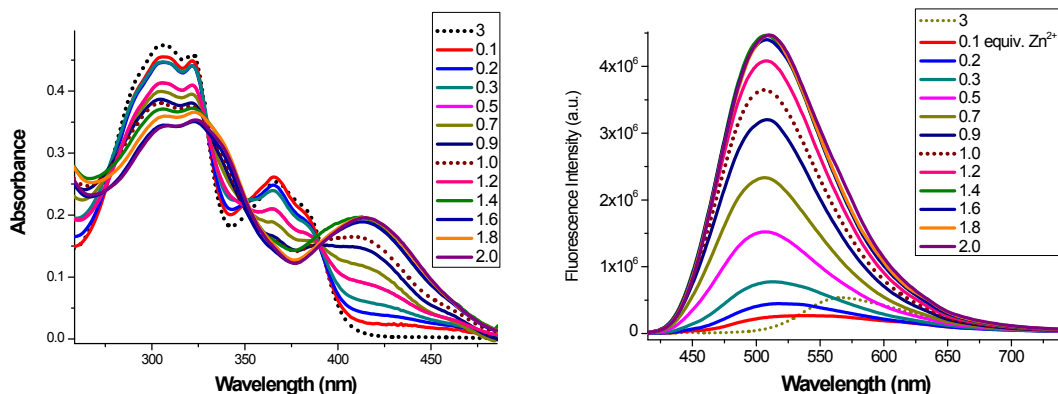


Figure S12a. UV-vis (a) and fluorescence spectra (b) of **3** (10 μ M) in EtOH upon addition of different equivalent of Zn(NO₃)₂. **3** (10 mM) was dissolved in DMSO as the stock solution and then diluted 1000 times into EtOH; Zn(NO₃)₂ was dissolved in EtOH (1 mM) as the stock solution.

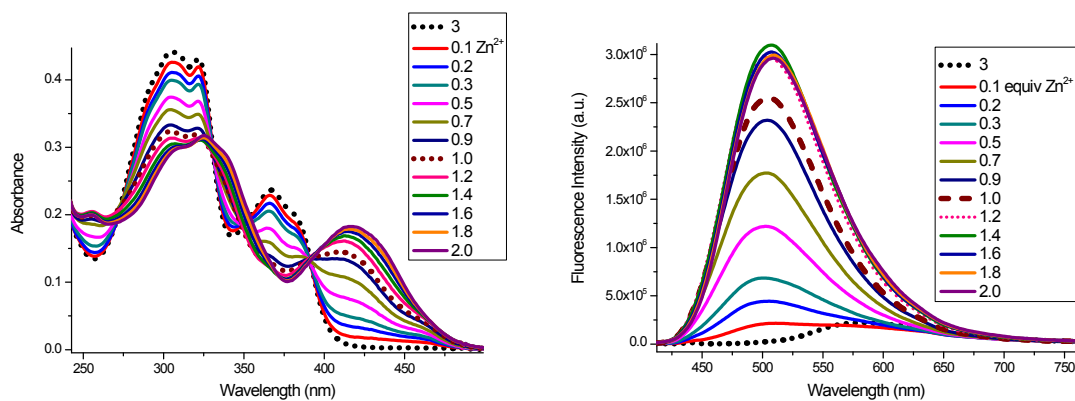


Figure S12b. UV-vis (a) and fluorescence spectra (b) of **3** (10 μ M) in EtOH upon addition of different equivalent of Zn(NO₃)₂. **3** (10 mM) was dissolved in DMSO as the stock solution and then diluted 1000 times into EtOH; Zn(NO₃)₂ was dissolved in H₂O (1 mM) as the stock solution (Very little water caused great fluorescence decrease).

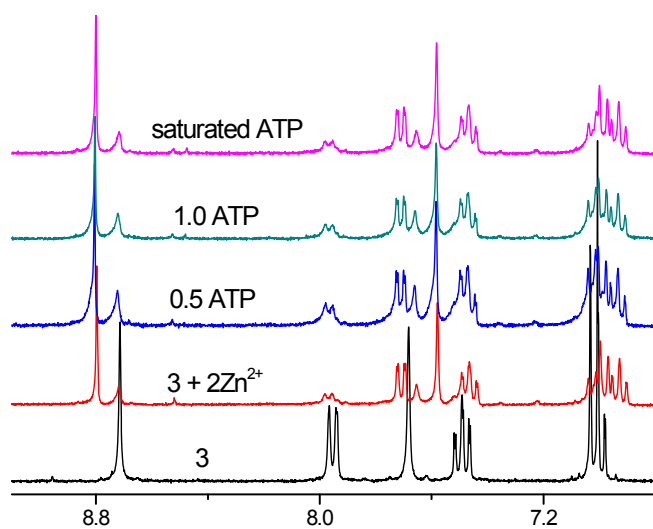


Figure S13. ^1H NMR titration of $[3 + 2\text{Zn}^{2+}]$ in CD_3OD with different equiv. of H_2ATP

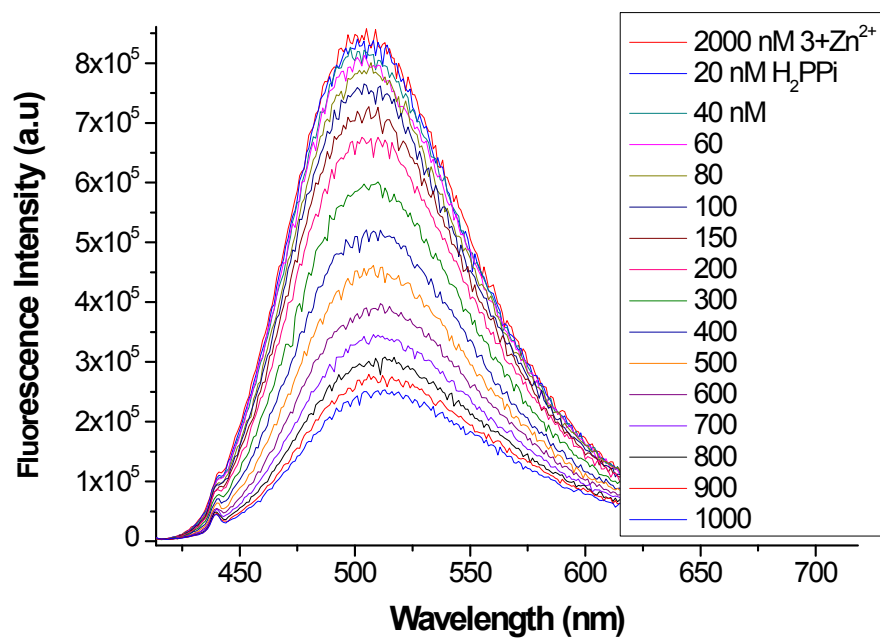


Fig S14. Emission of 3-Zn^{2+} ($2\ \mu\text{M}$) at different concentrations of H_2PPi (0-1000 nM) added in EtOH.

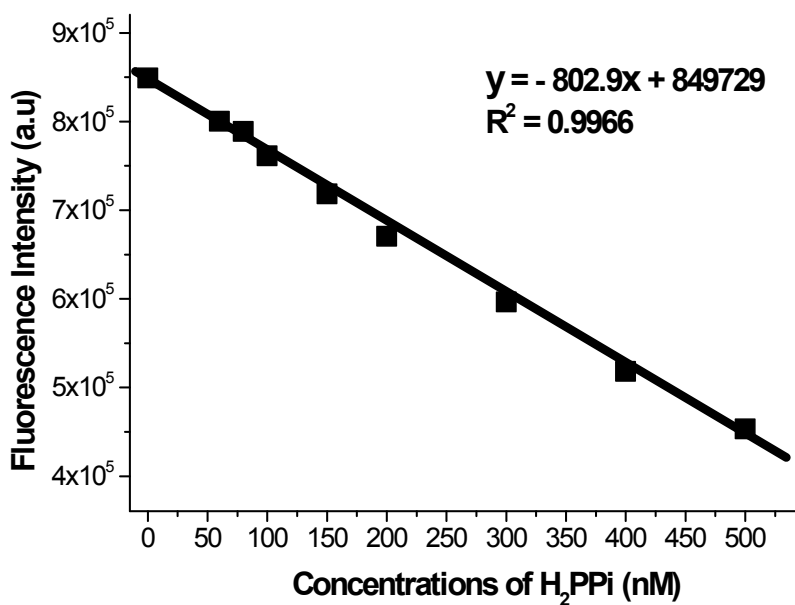


Fig S15. Emission of **3-Zn²⁺** (2 μ M) at different concentrations of H₂PPI added in EtOH.

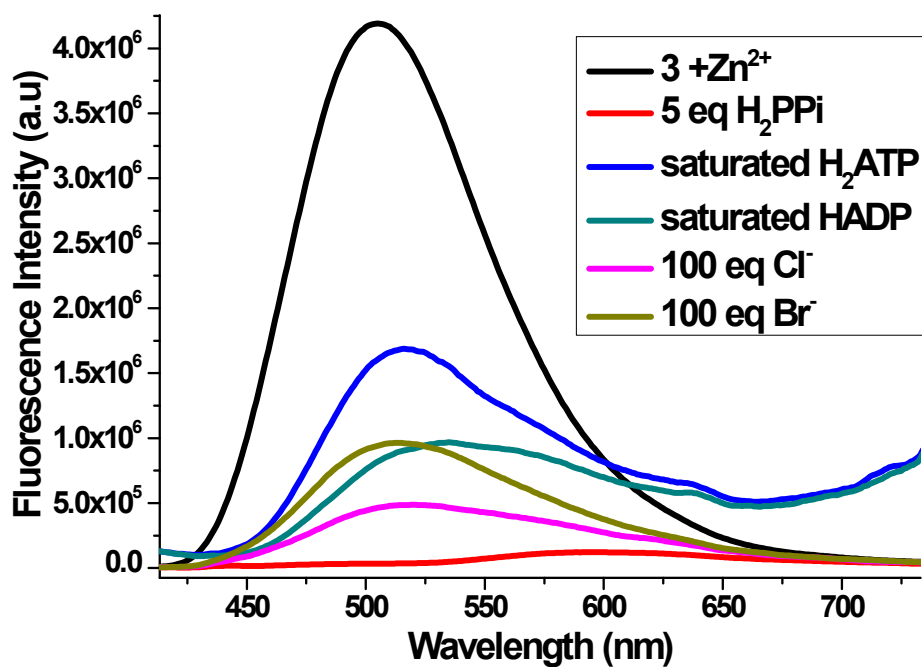


Figure S16. Fluorescence spectra of **3+Zn²⁺** complex (10 μ M) in EtOH upon addition of different anions. The large excess of H₂ATP, HADP and halides were used to illustrate that the other anions are much less effective in interaction with the zinc complex.

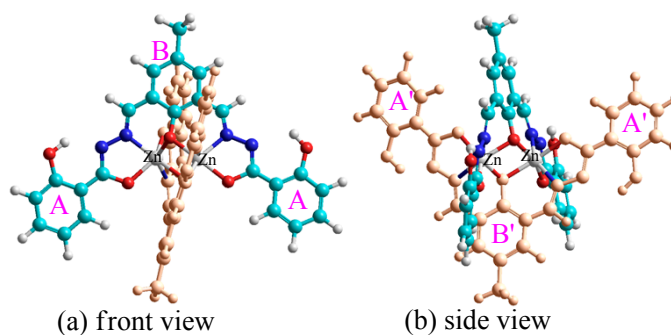
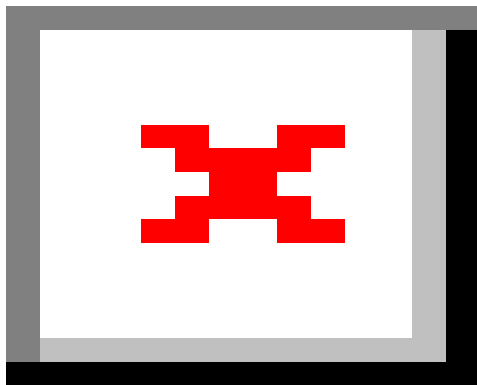
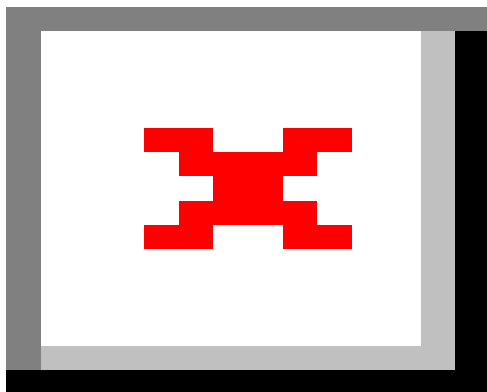


Figure S17. Geometry-optimized structure of zinc complex **4** by using HyperChem software (molecular mechanics force field MM+). The C, N, O and Zn atoms are in cyan, blue, red and grey colors, respectively. For clarity, one of the ligand is shown in orange color. The end and central phenyl rings are indicated by letters A and B, respectively.

¹HNMR of 3 in DMSO-D₆:



¹HNMR of 3 in CD₃OD:



^{13}C NMR of 3 in DMSO- D_6 :

