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Fluorescence turn-on of salicylaldimine ligands by co-ordination with magnesium and amines.

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Materials and Methods.

Reagents.

3-aminopropyltriethoxysilane (APTES), tris(hydroxymethyl) aminomethane (Tris), methanamide, n-dimethylacetamide, nitromethane and p-nitrophenol were purchased from Sigma Aldrich (Shanghai, China). Magnesium methoxide solution (7–8 wt.% in methanol) was obtained from Shanghai Aladdin, Ltd. (Shanghai, China). All metal salts ($BaCl_2 \cdot 2H_2O$, $Pb(NO_3)_2$, $HgCl_2$, $FeCl_3 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, $CoCl_2 \cdot 6H_2O$, $CdCl_2 \cdot 2½H_2O$, $ZnCl_2$, $MnCl_2 \cdot 4H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2$), and 2-aminoethanol, diethylenetriamine, acrylamide and ethylenediamine were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Magnesium Assay Kit(Xylene amine Blue-I) was obtained from Biosino Bio-Technology and Science Inc. The Human Serum–Pooled was purchased from Beijing Wolcavi Co., Ltd. The other two human blood samples were collected from healthy adult volunteers at Peking University Third Hospital. All reagents were of analytical grade, and were used without further purification.

Synthesis of ligand L: The synthetic procedure for salicylaldimine ligands followed the method previously described in the literature. APTES (0.4 mmol) was dissolved in a round-bottom flask containing 20 ml absolute ethanol. Hydroxybenzaldehyde (0.4 mmol) was added dropwise into the flask within 5 min, and stirred at room temperature for 5 h. The solvent was removed under reduced pressure yielding the product, ligand **L**, as a yellow oil, which was stored under vacuum. Other salicylaldimine ligands (**L**^{a,b,c}) were synthesized following the aforementioned method *via* the condensation of aldehydes and imines at the same stoichiometric ratio (1:1).

General procedure for Mg^{2+} detection

A 2.0×10^{-3} M stock solution of **L** was prepared in absolute ethanol. To a 2 mL centrifuge tube containing different amounts of Mg^{2+} ions, 10 μL of the **L** stock solution and 0.5 ml of a Tris/ethanol solution (2 mM) were added directly using a micropipette. The solutions were diluted with ethanol to 2 mL and mixed. Absorption and fluorescence spectra were run after stirring the solutions for 3 h.

Fluorescence spectroscopy: general procedure for different metal ions

A series of 1.0×10^{-3} M stock solutions of different metal ions ($BaCl_2 \cdot 2H_2O$, $Pb(NO_3)_2$, $HgCl_2$, $FeCl_3 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, $CoCl_2 \cdot 6H_2O$, $CdCl_2 \cdot 2\frac{1}{2}H_2O$, $ZnCl_2$, $MnCl_2 \cdot 4H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2$) were prepared in absolute ethanol. The metal ion stock solution (40 μL) and the **L** stock solution (20 μL) were added into 2 mL centrifuge tubes. Each **L**-metal solution group was added to 0.5 ml of a Tris/ethanol solution (2 mM). The solutions were diluted with ethanol to 2 mL and mixed. Absorption and fluorescence spectra were run after stirring the solutions for 3 h.

Fluorescence spectroscopy: general procedure for different nitrogenous compounds

A series of 1.0×10^{-2} M stock solutions of different nitrogenous compounds (0. Ck, 1. Tris, 2. 2-aminoethanol, 3. ethylenediamine, 4. diethylenetriamine, 5. acrylamide, 6. methanamide, 7. n,n-dimethylacetamide 8. nitromethane, 9. p-nitrophenol) were prepared in absolute ethanol. 20 μL of the **L** stock solution and 40 μL of the Mg^{2+} stock solution were added into a 2 mL centrifuge tube. Thereafter, 40 μL of the stock solution containing different nitrogenous compounds was added and diluted with ethanol to 2 mL. The fluorescence spectra were run after stirring the solutions for 3 h.

Fluorescence determination of magnesium in human blood serum

The blood samples were pretreated to eliminate any protein interference and to improve the recovery. A 3 mL portion of trichloroacetic acid (quality fraction 15%) was added to 1 mL serum to destroy the activity of proteins in the serum and to precipitate them from the solution. The mixture was centrifuged at 10 000 rpm for 10 min after vigorous shaking for 15 min. The supernatant was obtained and modulated to pH 7.0 utilizing NaOH solution. The treated serum samples were diluted 50 times using ethanol. The spiked samples were prepared by adding different Zn solutions to the diluted serum samples then the spiked serum samples were analyzed according to the developed protocol. Standard addition and recovery experiment was carried out in the Human Serum-Pooled, and quantitative detection of magnesium was completed in all three blood samples.

Fluorescence intensity of L+Mg(II) in different pH

Emission intensity of a 20 μM ligand **L** solution upon the addition of one equivalent of Mg(II) was studied as a function of pH (Fig S6). Fluorescent intensity was found to be highest at pH 7. Increasing the pH above pH 8 results in the transformation of Mg(II) into $Mg(OH)_2$ and the dissociation from **L**, thus decreasing the emission intensity. Similarly, below pH 7, Mg(II) is thought to be replaced by H^+ from the **L**-Mg(II) complex, which also results in a decrease of fluorescence intensity.

Instrumentation and Characterization

All the nuclear magnetic resonance spectra were recorded at room temperature on a Bruker DPX (Karlsruhe, Germany) instrument, operating at 300 MHz for ^{1}HNMR and 75 MHz for ^{13}CNMR. The sample (8 mg) was dissolved in 0.6 mL DMSO-D6 for ^{1}HNMR and $CDCl_3$-D1 for ^{13}CNMR respectively, and tetramethylsilane was used as an internal reference.

FT-IR spectra were recorded on a Nicolet NEXUS-470 Spectrometer (Madison, USA) from KBr pellets at room temperature, using an accumulation of 32 scans and a resolution of 4 cm^{-1}, in the range of 4000~500 cm^{-1}. Samples (2 mg) were thoroughly ground with KBr and pelletized using a hydraulic press under a pressure of 600 kg/cm^2.

Fluorescence spectra were recorded on an F-7000 fluorescence spectrophotometer (Hitachi, Tokyo, Japan). All fluorescence intensity measurements were performed under the same conditions: the excitation and emission slit widths were both 10 nm, and the excitation wavelength was set at 365 nm, with a recording emission range of 380~630 nm. The photomultiplier tube voltage was set at 400 V. All ultraviolet−visible (UV-Vis) spectra were recorded on a WTF UV-2102PC UV-Vis spectrophotometer (UNICO Shanghai Instrument Co., Ltd., China). Quantitative determination of magnesium in blood samples were recorded on an atomic absorption spectrometer (PERSEE TAS-990, Beijing, China).

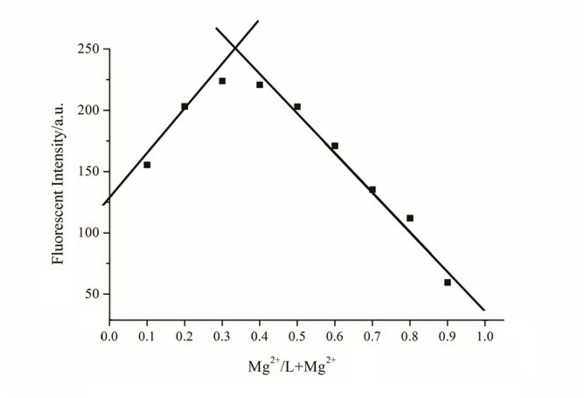

Fig S1. Plots according to the method for continuous variations, indicating the 2:1 stoichiometric ratio of the **L**-Mg(II) complex in the absence of Tris. The total concentration of **L** and Mg(II) is 100 μM.

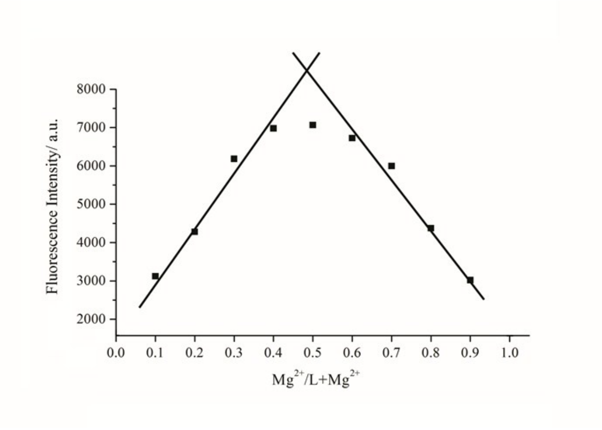

Fig S2. Plots according to the method for continuous variations, indicating the 1:1 stoichiometric ratio of the **L**-Mg(II) complex in the presence of Tris (5 mM). The total concentration of **L** and Mg(II) is 100 μM.

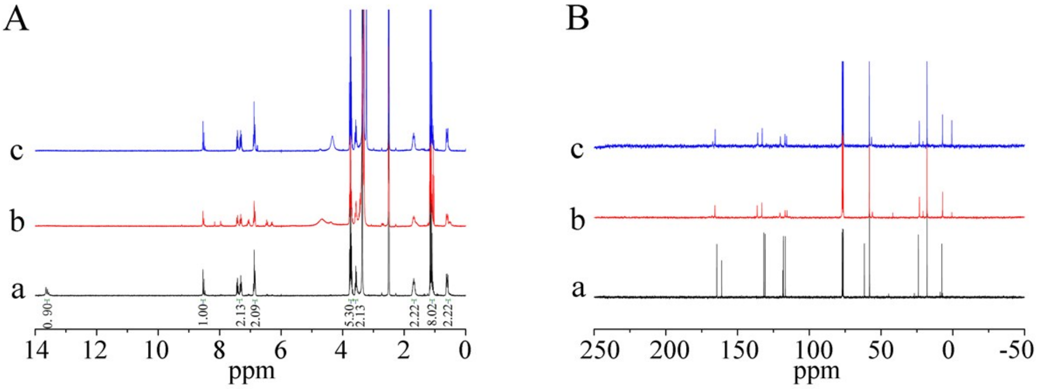

Fig S3. A, ^{1}HNMR spectra of: a) **L**, b) **L**+Mg (1:1) and c) **L**+Mg+Tris (1:1:1) in DMSO-D6. B, ^{13}CNMR of: a) **L**, b) **L**+Mg (1:1) and c) **L**+Mg+Tris (1:1:1) in CDCl$_3$-D1.

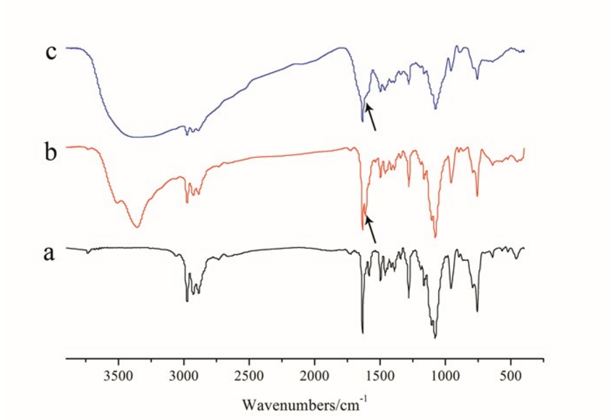

Fig S4. FT-IR spectra of: a) **L** (100 μM), b) **L**+Mg (1:1, 100 μM) and c) **L**+Mg+Tris (1:1:1, 100 μM).

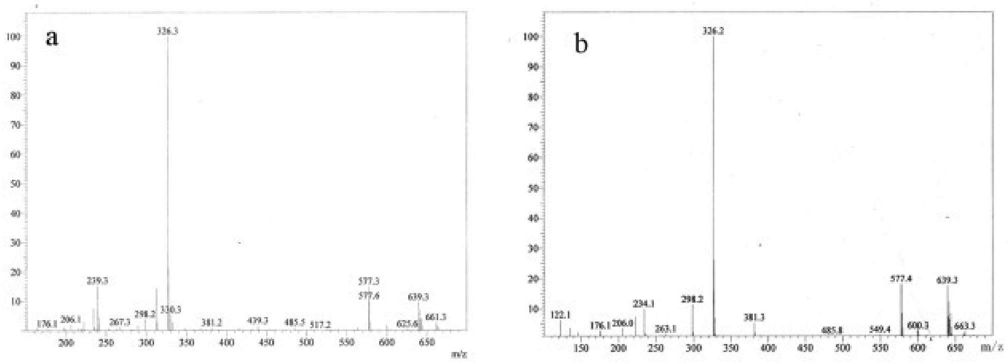

Fig S5. Electrospray ionization-mass spectrometry of: a) **L**+Mg (1:1, 200 μM) and b) **L**+Mg+Tris (1:1:1, 200 μM).

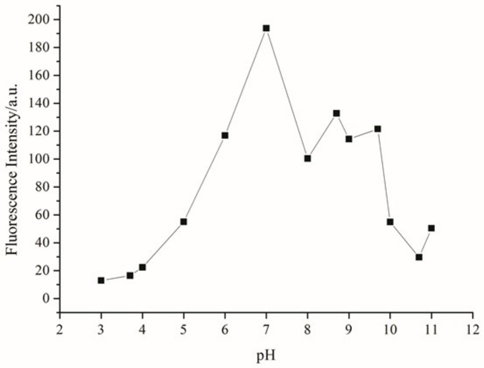

Fig S6. Fluorescent intensity at 460 nm of **L** (20 μM) and Mg(II) (20 μM) in ethanol at pH 3~11 (λ_{ex} = 365 nm).

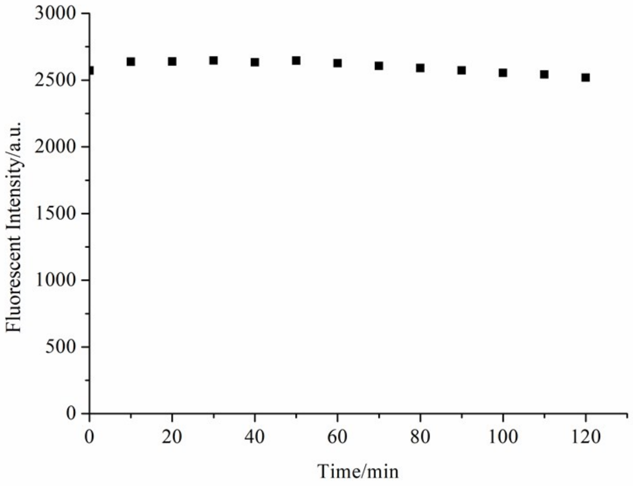

Fig S7. Fluorescent intensity variation of the **L**+Mg+Tris(20 μM, 20 μM, 5 mM) as a function of illumination time.

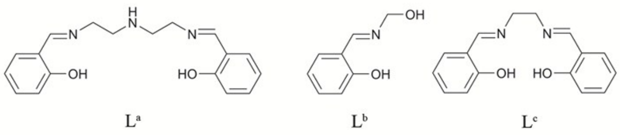

Fig S8. Three salicylaldimine ligands.

Table S1. Fluorescence quantum yield of four salicylaldimine ligands.

	L	L^a	L^b	L^c
L* only	0.30%	4.71%	2.03%	7.54%
L*+Mg	5.11%	11.26%	16.24%	36.12%
L*+Mg+Tris	31.18%	26.69%	34.26%	25.86%

Table S2 Determination of Mg(Ⅱ) in human serum samples

plasma samples	**Added Mg(μM)**	**Found Mg(μM)**	**Recovery(%)**	**RSD(n=3,%)**
1	50	43.88	87	22.71
2	100	94.76	94	9.75
3	500	498.84	99	11.61
4	1000	1079.58	107	3.89