Electronic Supplementary Material (ESI) for Analytical Methods

Supporting information

Facile synthesis of a water-soluble bidentate pyridine-acid ligand and the application in quantifying bovine serum albumin by fluorescence spectroscopy

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1.1 Materials and Instrumentation.

Methanol was dried with Na before use; other reagents with analytical grade were purchased from Sigma-Aldrich and used without further purification. Distilled deionized water (Easy Pure LF) was used for the preparation of all aqueous solutions. The products were purified by neutral column chromatography on silica gel (300-400 mesh). The structures of compounds were identified by ^{1}HNMR (Varian Mercury 300 NMR spectrometer), using TMS as an internal standard. Chemical shifts (δ) are given in parts per million and coupling constants are given as absolute values expressed in Hertz. Mass spectrawere obtained using LC/MS 1100 of Agilent Technology Corporation and Alltech ELSD 2000 instrument. Melting points were determined by X-4 digital microscopy apparatus. Fluorescence intensity and spectra were obtained using a CARY Eclipe Fluorescence Spectrophtometer of VARIAN. C, H, and N elemental analyses were taken on a Perkin–Elmer 240C elemental analyzer.

1.2 Synthesis.

Preparation of bidentate pyridine-acid compound (1)

2, 6-bis(bromomethyl)pyridine a: To a stirred mixture of 10.0 mmol of dimethyl-2,6-pyridine (1.2 mL) in 33 ml of dry benzene, benzoyl peroxide (0.3 g) and

N-bromosuccinimide (3.56 g, 20.0 mmol) were added with vigorous stirring. After the mixture was stirred ten minutes in room temperature, 0.6 mL acetic acid as protective agent was added to the mixture. Then the mixture was heated to reflux for 10 h under nitrogen. The reaction mixture was then cooled and washed by 1 M HCl. The mixture was extracted twice with ethyl acetate. The organic layer were washed with saturated brine twice and dried over anhydrous Na_2SO_4. The solvent was then removed under reduced pressure. The product was purified by column chromatography on silica gel (petroleum ether: EtOAc, 6:1) and then was obtained as white crystals, 30% yield, m. p. 82-83 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ(ppm) 4.539 (s, 4H), 7.375 (d, J=7.8, 2H), 7.706 (t, J=7.8, 1H).

β-Alanine Methyl Ester Hydrochloride b: The dropping funnel is charged with 2.3 mL of acetyl chloride. The flask is charged with 15 mL of methanol and cooled with an ice-water bath under nitrogen. Acetyl chloride is added dropwise over a period of 8 min. The solution is stirred for a further 5 min, then solid β-Alanine (1.01 g, 11.4 mmol), is added in one portion and the solution is slowly heated to reflux. The reflux is continued for 2 hr, then the solution is allowed to cool to room temperature and the solvent is removed under reduced pressure to give crude β-Alanine Methyl Ester Hydrochloride, 99% yield, which was used without further purification.

bidentate pyridine-acid compound (1): β-Alanine Methyl Ester Hydrochloride **b** (0.293 g, 2.1 mmol) was charged with 10 mL acetonitrile. Then Et_3N (0.42 mL, 3 mmol) was added to the mixture. To the vigorous stirring mixture, 2, 6-bis(bromomethyl)pyridine **a** (0.265 g, 1.0 mmol) in 10 ml acetonitrile were added. After the mixture was stirred 4 hours in room temperature, the solid was filtered off and the solvent was then removed under reduced pressure. The product was purified

by column chromatography on silica gel (chloroform: menthol: ammonium hydroxide, 25:5:1) and then was obtained as oil, 60% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ(ppm) 2.519-2.602 (m, 4H), 2.869-2.949 (m, 4H), 3.586-3.678 (m, 6H), 3.789 (s, 4H), 7.366 (d, J=7.8, 2H), 7.602-7.653 (m, 1H); MS (ESI): m/z 310.3 [$M+H^+$]; Elemental analysis calculated for $C_{15}H_{23}N_3O_4$: C, 58.24; H, 7.49; N, 13.58. Found: C, 58.19; H, 7.56; N, 13.50.

The oil (0.17 g, 0.55 mmol) was charged with 10 mL of ethyl alcohol. To the vigorous stirring mixture, K_2CO_3 (0.3 g, 2.2 mmol) in 10 ml water were added. After the mixture was stirred 12 hours in room temperature, the solution was acidification by addition of 1 M HCl to adjust pH 4. The water was then removed under reduced pressure to give bidentate pyridine-acid compound **1**, 99% yield, which was used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ(ppm) 2.824-3.055 (m, 2H), 3.282-3.574 (m, 2H), 4.689 (s, 4H), 5.891 (s, 4H), 7.506-7.573 (m, 2H), 7.869-7.917 (m, 1H); MS (ESI): m/z 282.1 [$M+H^+$]; Elemental analysis calculated for $C_{13}H_{19}N_3O_4$: C, 55.50; H, 6.81; N, 14.94. Found: C, 55.49; H, 6.89, N, 14.90.

Determination of Fluorescence quantum yields

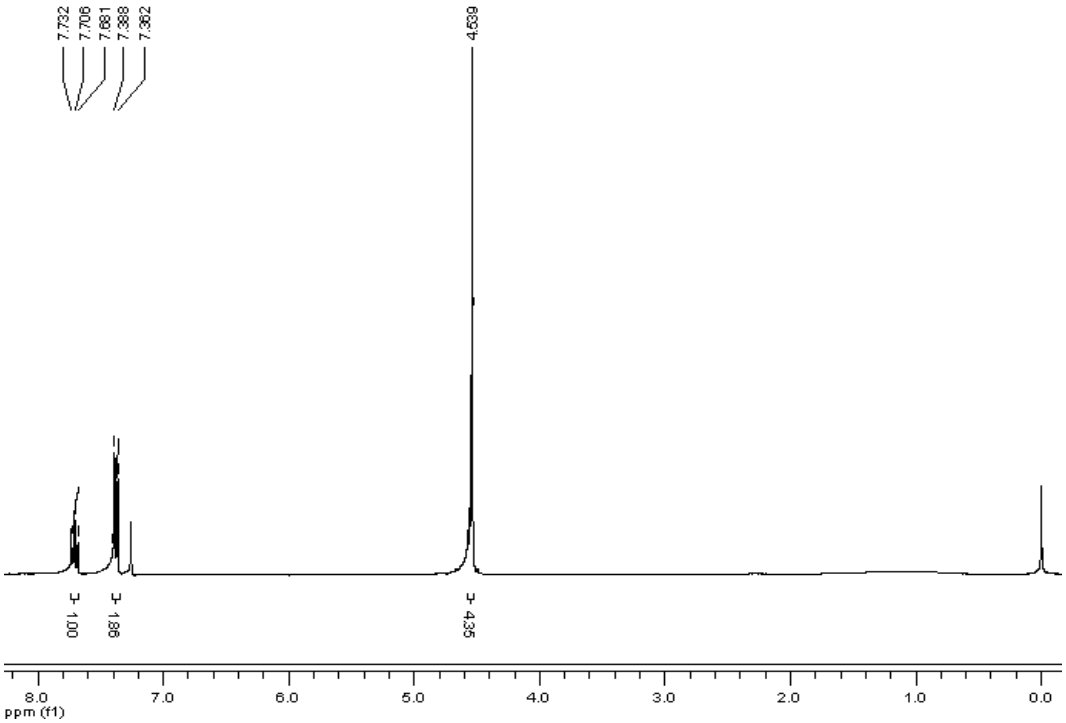
The method of choice for the determination of fluorescence quantum yields was to relate the quantum yield of the sample to that of a reference standard [S1]. Measurements were done in pH 6.3 Tris–HCl buffer solution. In order to avoid inner filter effects, the concentration of the samples was adjusted such that the absorbance was below 0.05 at the excitation wavelength. For all calculations the solvent background was subtracted from the sample. The equation used to relate these quantum yields is:

$$\Phi F_x = [(A_{std\ (\lambda exc)} / A_{x\ (\lambda exc)}) \times (F_x / F_{std})] \times \Phi F_{std}$$

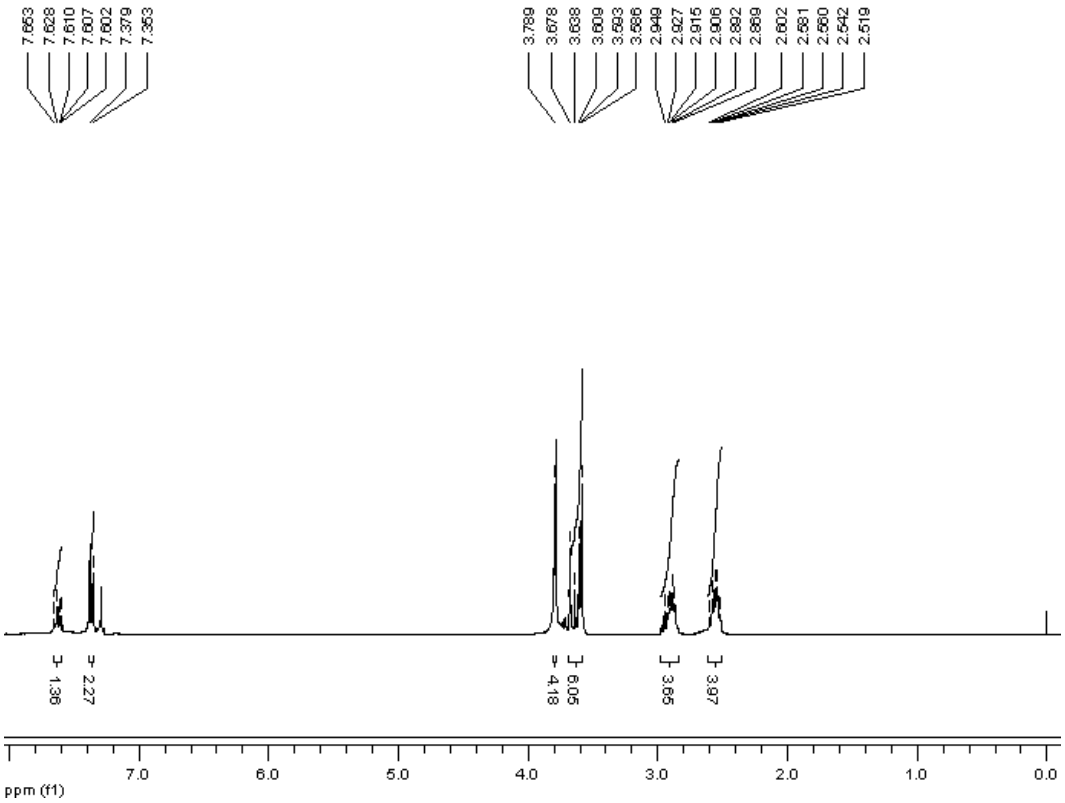
Where the subscript *x* refers to the unknown sample and the subscript *std* refers to the standard; ΦF is the fluorescence quantum yield, *F* is the integrated fluorescence intensity, *A* is the absorbance at the excitation wavelength. The standards were chosen such that the absorbance of the standard was overlapping with the absorbance of the sample. The reference standards used were quinine sulphate in 0.5M H_2SO_4 (ΦF = 0.54) [S2].

^{1}H NMR Spectra of Products

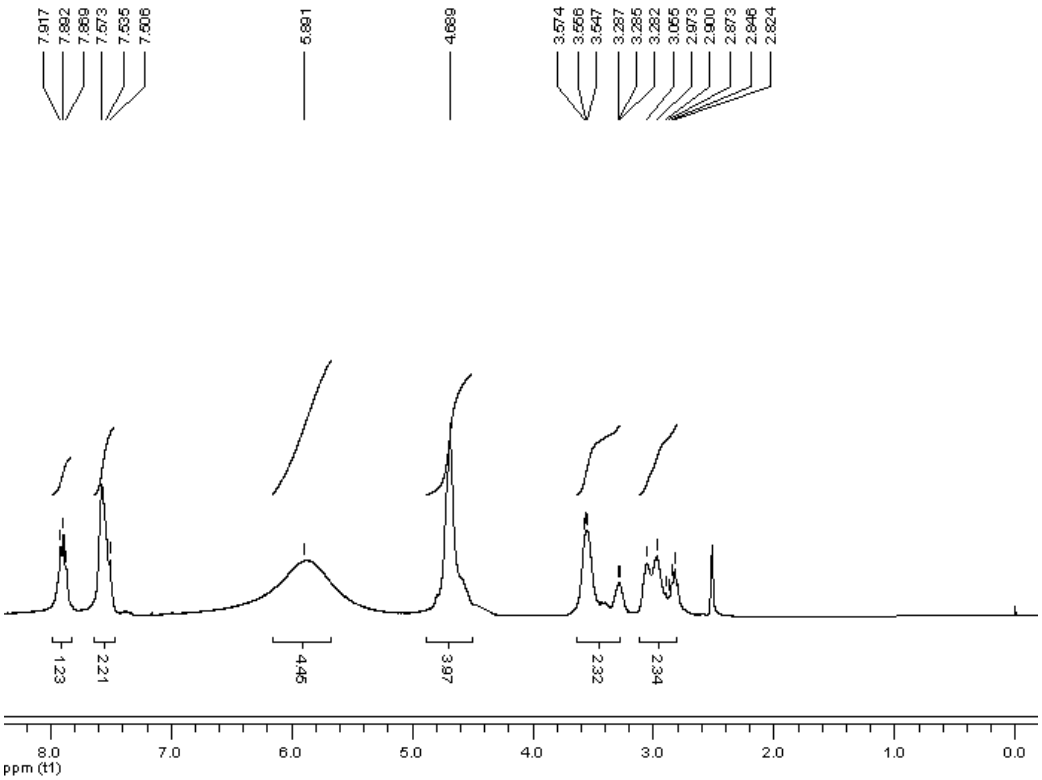
2, 6-bis(bromomethyl)pyridine (a):



Dimethyl-3,3'-(pyridine-2,6-diylbis(methylene))bis(azanediyl)dipropanoate:



bidentate pyridine-acid compound (1):



Spectral properties:

1.1 The Effect of pH on the fluorescence of ligand

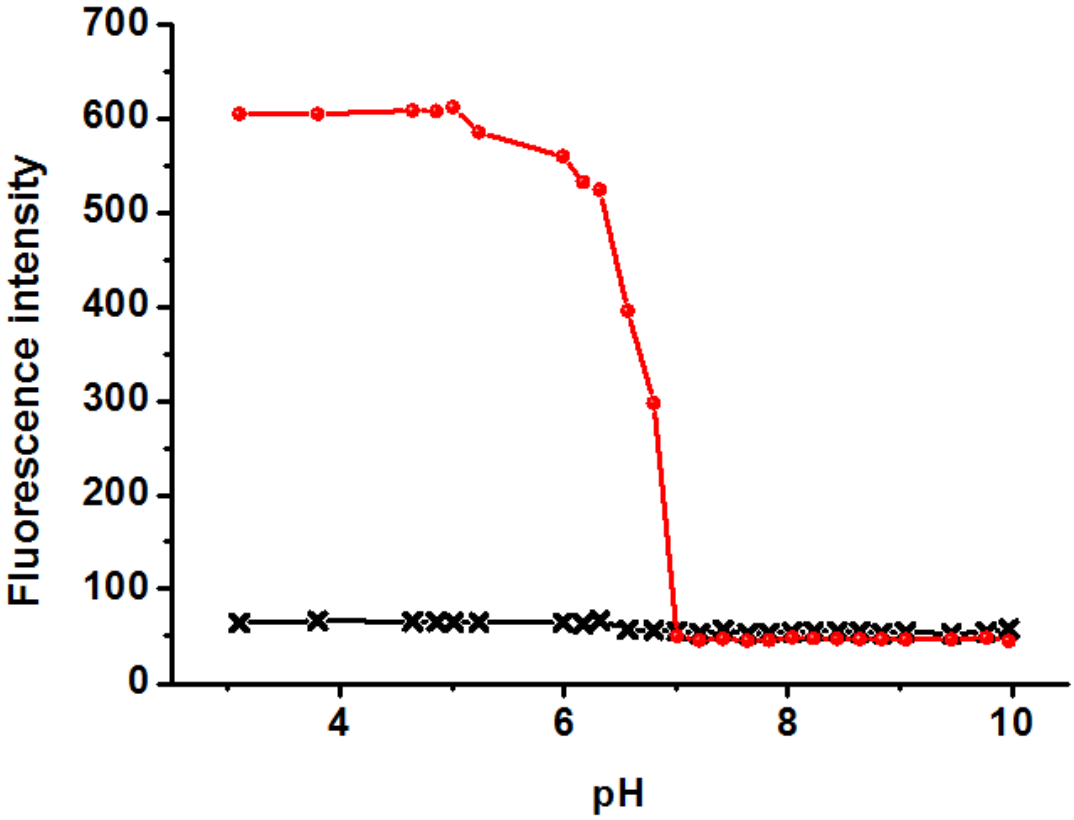


Fig. S1 The Effect of pH on the fluorescence of ligand

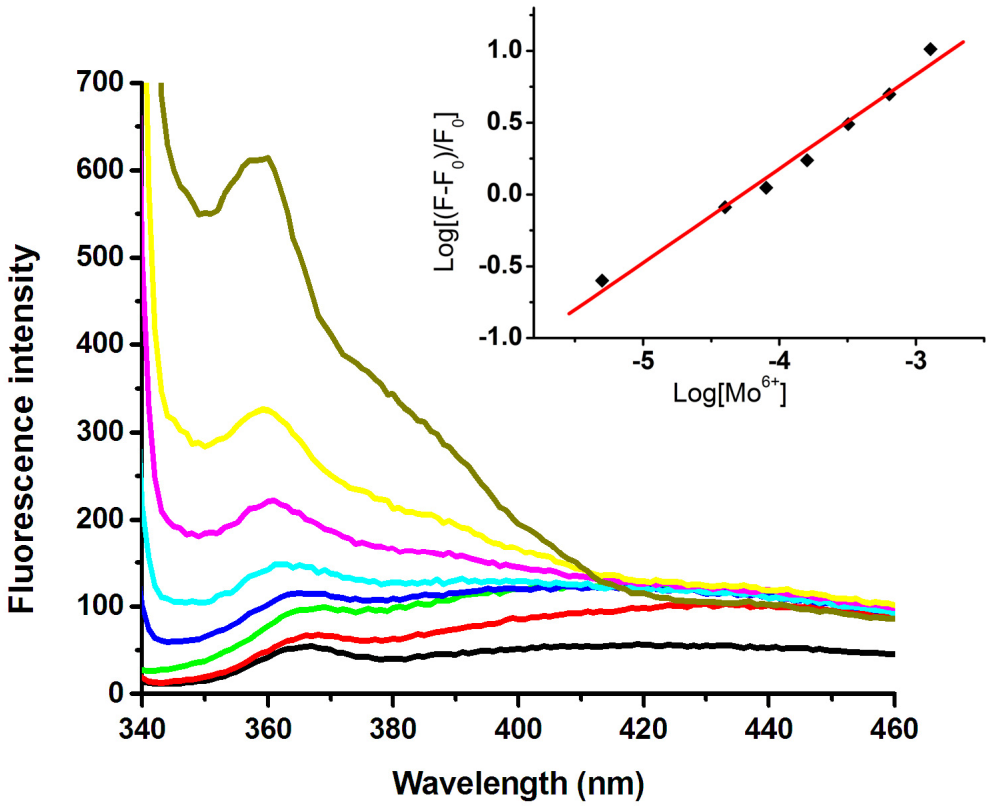


Fig. S2 Fluorescence emission of ligand (0.5 mM) with gradual addition of different amounts of Mo^{6+} (from bottom to top: 0, 5, 40, 80, 160, 320, 640and 1280 μM) and a plot of log[(F - F_0)/F_0] as a function of the log[Mo^{6+}] (inset).

References

S1 S. Fery-Forgues, D. Lavabre, *J. Chem. Ed.* 1999, ***9***, 1260.

S2 W. H. Melhuish, *J. Phys. Chem.*1961, **65**, 229.