

Supporting Information

Determination of nitrite in real food and water samples by a novel Terbium-macrocyclic complex

Jinghua Yin,^{a,†} Zhixue Liu,^{a,†} Tong Zhao,^a Yingjin Jin,^{a, b} Xin Zhou^{*a, b} and Xue Wu^{*a}

^a Research Centre for Chemical Biology, Department of Chemistry, ^b Key Laboratory of Natural Resources of Changbai Mountain & Functional Molecules, Ministry of Education, Yanbian University, Yanji 133002, P. R. China.

† These authors contributed equally to this work.

* E-mail of corresponding author: hsinzh@yahoo.com; wuxue@ybu.edu.cn.

Experimental Section

Materials and Methods

All chemicals and solvents were of analytic grade and bought from commercial sources without further purification. The solutions of anions were prepared from their sodium salts. The **Tb-Ac** was dissolved in distilled water to prepare the stock solutions with a concentration of 1 mM. All spectroscopic measurements were performed in PBS (10 mM, pH 7.4) buffer. UV-vis spectra were recorded on Perkin Elmer Lambda 3500 UV-vis spectra with a 1.0 cm quartz cell. PL spectra were conducted on Fluorescence Spectrophotometer (RF-540). MALDI-TOF mass spectra were recorded on a Shimadzu MALDI AXIMA-CFR+ Spectrometer. FT-IR spectrometer was recorded on Nicolet 6700. Electrospray ionization mass spectra (ESI-MS) were acquired on an LCQ Fleet electrospray mass spectrometer. ¹H and ¹³C NMR spectra were recorded on a Bruker AV-300 (300 MHz) spectrometer with TMS as the reference.

Detection limit

The detection limit was calculated based on the fluorescence titration. To determine the S/N ratio, the emission intensity of Tb-Ac without NO₂⁻ was measured by 10 times and the standard deviation of blank measurements was determined. Under the present conditions, a good linear relationship between the fluorescence intensity and the NO₂⁻ concentration could be obtained in the 0 - 10 μM (R = 0.998). The detection limit is then calculated with the equation: detection limit = 3σ_{bi}/m, where σ_{bi} is the standard deviation of blank measurements; m is the slope between intensity versus sample concentration. The detection limit was measured to be 8.6 μM at S/N = 3.

Chromatographic conditions and sample preparation

The HPLC column was C18, 250 × 4.6 mm ID, with 5 μm particles (Thermo Scientific, USA) with a suitable guard-column. The mobile phase was comprised of a mixture of methanol and water (20:80 [v/v]) with HAc (0.01%). The elution programme was employed with a mobile phase flow-rate of 1 ml/ min. The column-oven and auto-sampler temperatures were set at 30 °C and 25 °C, respectively. The detection wavelength was at 254 nm. Samples were prepared as used for detection of

UV-Vis spectra and fluorescence spectra.

Assay of the nitrite content in food samples

Samples of pickled vegetables were purchased at local stores. The pretreatment was as follows: first, 5 g of the food sample was crushed into mash and mixed with 12.5 ml saturated borax solution. Then, 300 ml of 70 °C water were added and the mixture was heated at boiling for 15 min. To precipitate the proteins, 5 ml of 20% zinc acetate was introduced. After being cooled to room temperature, the mixture was diluted to 500 ml with water and then filtered. The resulting sample solution was stored at 4 °C in a refrigerator.

The nitrite content in samples was determined according to the standard addition method.¹ Standard nitrite solutions were added as internal standards after measurement of the sample solution. Thus, the concentration of nitrite in the real sample could be calculated.

Synthesis

Preparation of 5 and 4

The compounds **5** and **4** were prepared according to the reported methods.²

Preparation of 3

The 4-hydroxypyridine-2, 6-dicarboxylic acid dimethyl ester (80 mg) was dissolved in 500 mL refined methanol solution. With rapid stirring, the solution was added 100 mL refined methanol solution of triethylene tetramine rapidly. After being stirred at room temperature for 30 min, the solution was refluxed, and continued reacting for another 24 h with TLC track. Finally, the solution was span, dried and purified by column chromatography (MeOH), to give the product **3** in a yield of 32%. ¹H NMR (CDCl₃, 300MHz): δ 8.18 (s, 2H, CH), 4.81 (s, 2H, CH₂), 3.53 (s, 4H, CH₂), 2.96 (s, 4H, CH₂), 2.86 (s, 4H, CH₂).

Preparation of 2

Compound **3** (0.07g), K₂CO₃ (0.094g, 2.2 equiv.), and KI (0.011g, 0.3 equiv.) were

added to 20 mL refined acetonitrile solution. Then N, N-dimethylacetamide (0.083g, 2.2 equiv.) was added quickly under the condition of ice bath. After restored to room temperature, the reaction continued refluxing for one day under nitrogen atmosphere. The corresponding product was further purified by alumina column chromatography (methanol/ dichloromethane = 1: 100). The product **2** was obtained in a yield of 75%. ¹H NMR (CDCl₃, 300 MHz): δ 9.32 (s, 2H, NH), 8.25 (s, 2H, Ar-H), 4.86 (s, 2H, OCH₂), 3.49 (dd, J = 9.7, 4.7 Hz, 4H, CH₂), 3.40 (s, 4H, CH₂), 3.04 – 2.99 (m, 4H, CH₂), 2.97 (s, 6H, CH₃), 2.92 (s, 4H, CH₂), 2.89 (s, 6H, CH₃); ¹³C NMR (CDCl₃, 300 MHz): 170.26, 163.52, 154.67, 146.65, 121.07, 63.34, 54.91, 53.87, 51.44, 36.64, 35.53, 29.70; MS m/z: 478.8.

Preparation of **1**

Compound **2** and Et₃N (2 equiv.) were dissolved in dichloromethane under nitrogen atmosphere in ice bath, and then acryloylchloride (2 equiv.) was added. After being restored to room temperature, the solution was further stirred for 12 h. Then the reaction was quenched with saturated NaHCO₃ and washed three times with H₂O. The organic layers were combined and then dried by anhydrous MgSO₄. After being concentrated, it is subjected to alumina column chromatography (DCM: EtOH = 500:1) to give the product in a yield of 75%. ¹H NMR(CDCl₃, 300 MHz): δ 9.36 (s, 2H), 8.23 (s, 2H), 7.28 (s, 2H), 6.54 (d, J = 17.4 Hz, 1H), 6.25 (dd, J = 17.4, 10.3 Hz, 1H), 5.96 (d, J = 10.3 Hz, 1H), 5.36 (s, 2H), 3.50 (s, 4H), 3.39 (s, 4H), 3.03 (s, 4H), 2.97 (d, J = 2.8 Hz, 8H), 2.90 (d, J = 3.5 Hz, 8H). MS m/z: 532.6.

Preparation and characterization of Tb-Ac

After ligands **1** and TbCl₃ • 6H₂O were dissolved in refined acetonitrile (1:1), the solution was stirred at reflux for about two days which accompanied with lots of white solid. Then filtered the white solid and dissolved in the minimum amount of methanol. The ether was added to precipitate white solid, then filtered and washed with acetonitrile and dried in vacuum. MS (ESI) m/z: 688.2.

Table S1 Data from detecting the content of NO_2^- in the groundwater by using spectrophotometry

Sample volume (ml)	Absorbance (A)	Nitrite content (mg/kg)
50	0.984	1.33

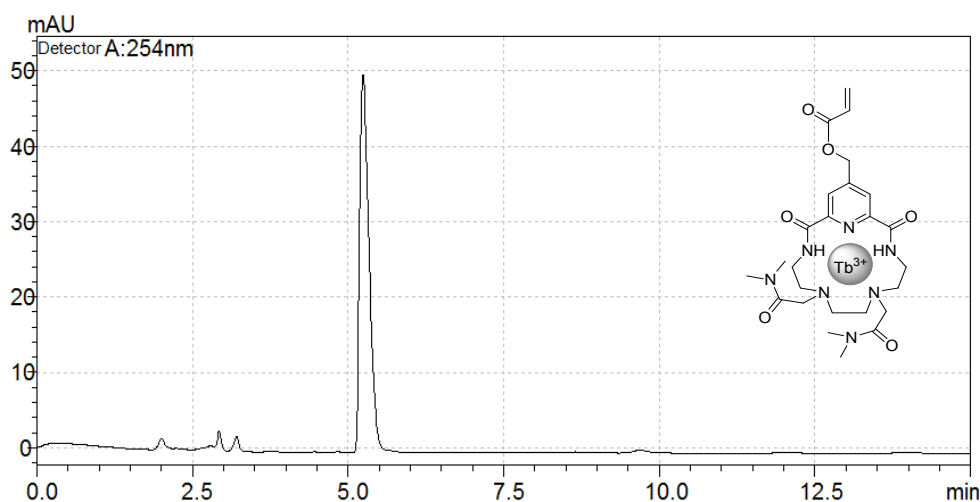


Fig. S1 HPLC chromatogram of Tb-Ac. The wavelength was 518 nm for detecting with the retention time of 5.2 min under the condition of mobile phase (MeOH: H₂O=20: 80).

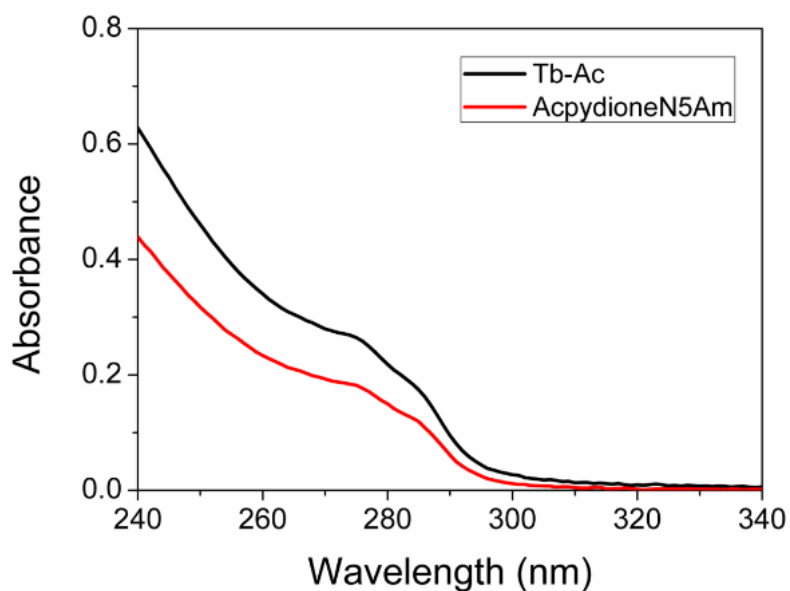


Fig. S2 UV-vis absorption spectra of the ligand AcpydioneN₅Am and Tb-Ac.

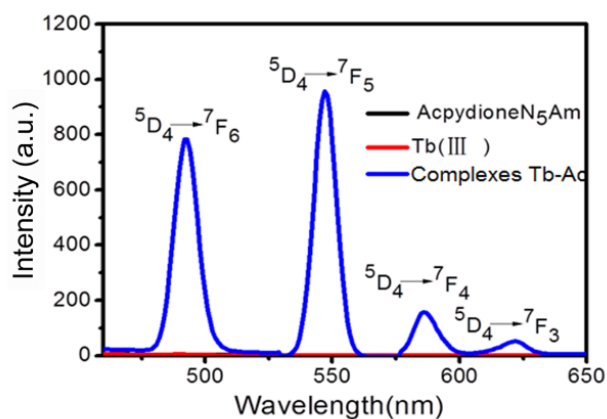


Fig. S3 Fluorescence spectra of ligand AcpydioneN₅Am, Tb and Tb-Ac complexes (10 μM) in HEPES buffer solution (pH = 7.4). ($\lambda_{\text{ex}} = 280 \text{ nm}$)

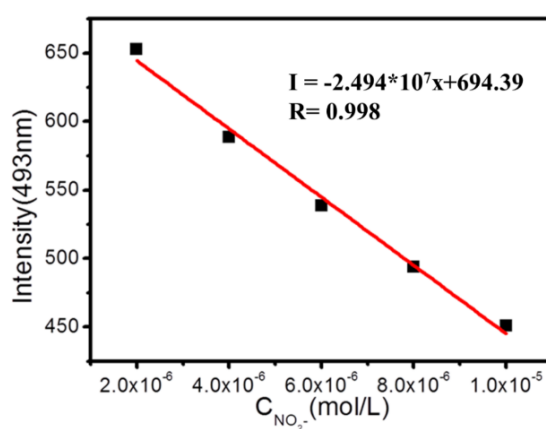


Fig S4. The line relationship between the fluorescent intensity ratio of the Tb-Ac (10 μM) and the concentration of the NO_2^- in HEPES buffer solution (pH = 7.4). ($\lambda_{\text{ex}} = 280 \text{ nm}$)

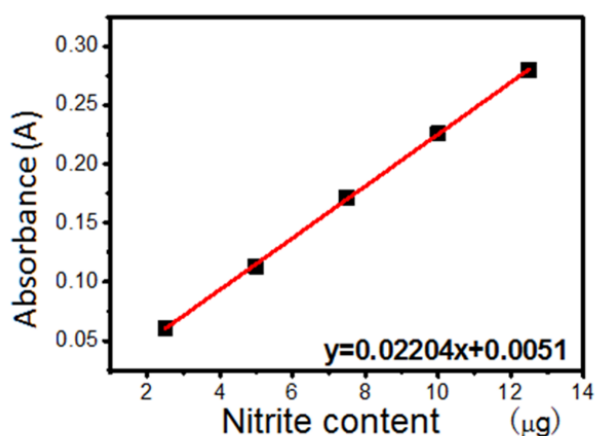


Fig. S5 Standard curve for sodium nitrite measurement by using hydrochloric acid naphthylethylenediamine spectrophotometry.

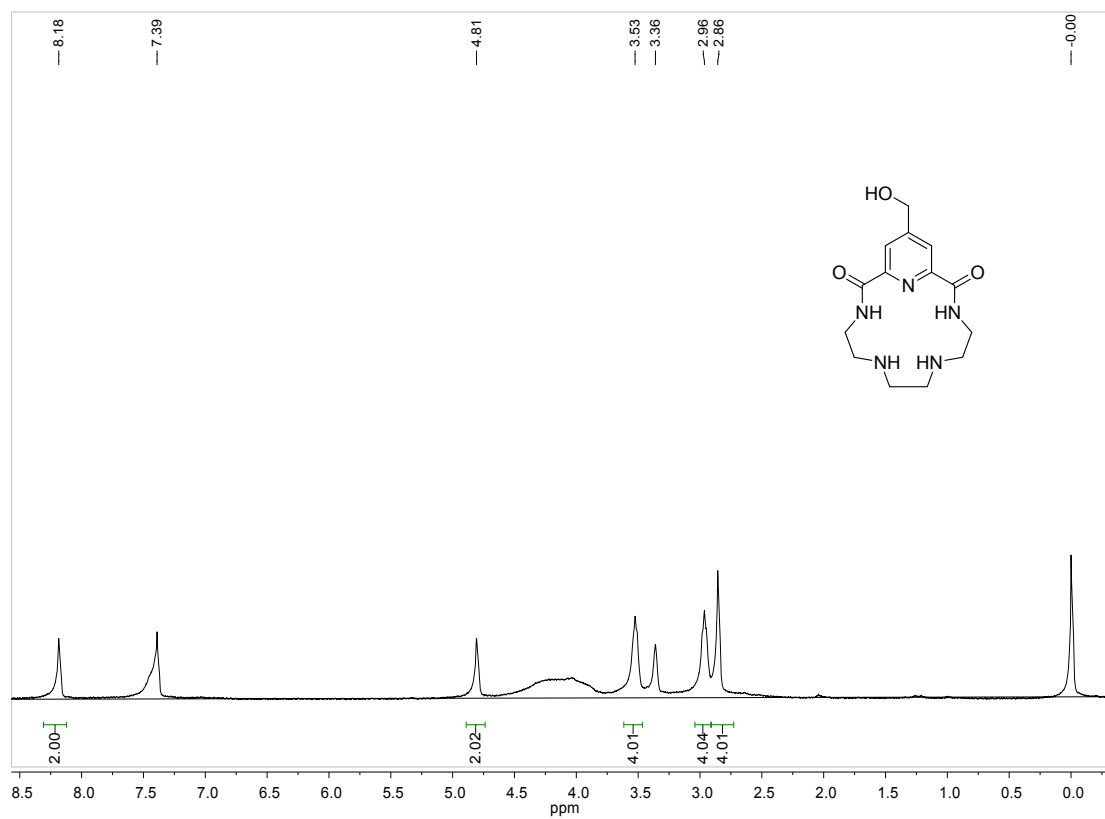


Fig. S6 ^1H NMR spectrum of 3.

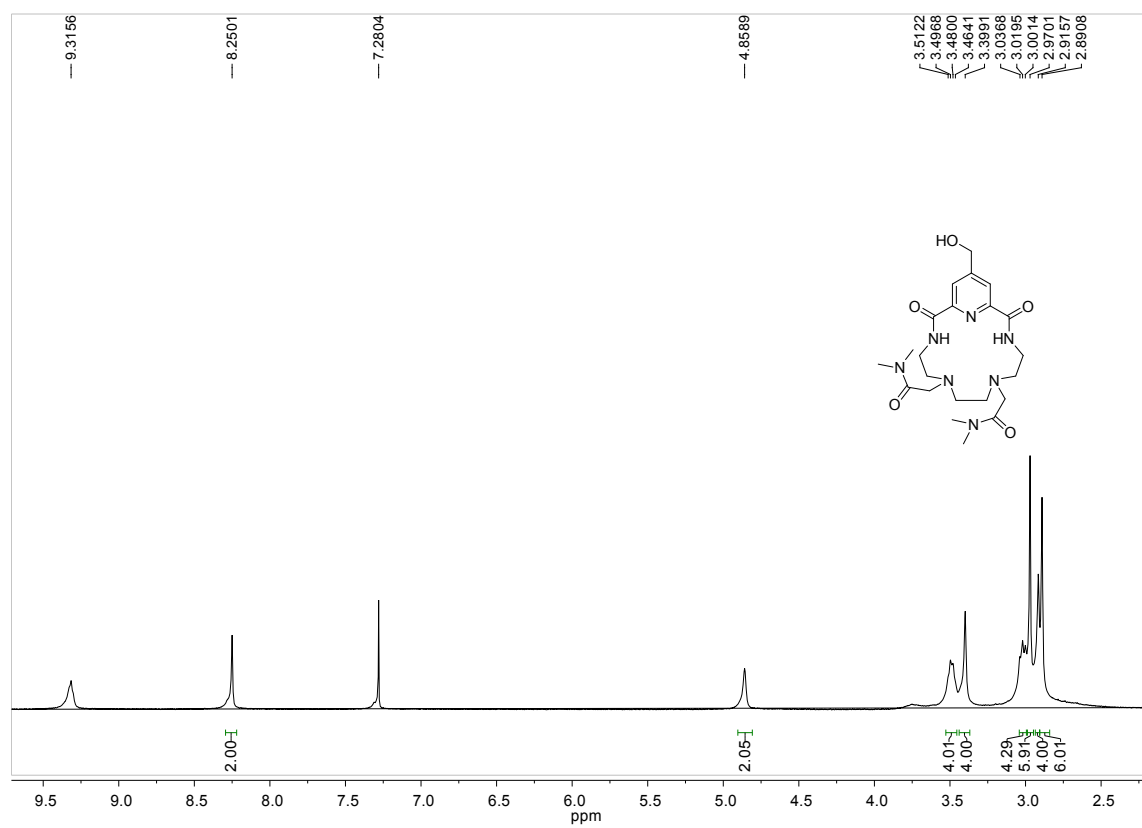


Fig. S7 ^1H NMR spectrum of 2.

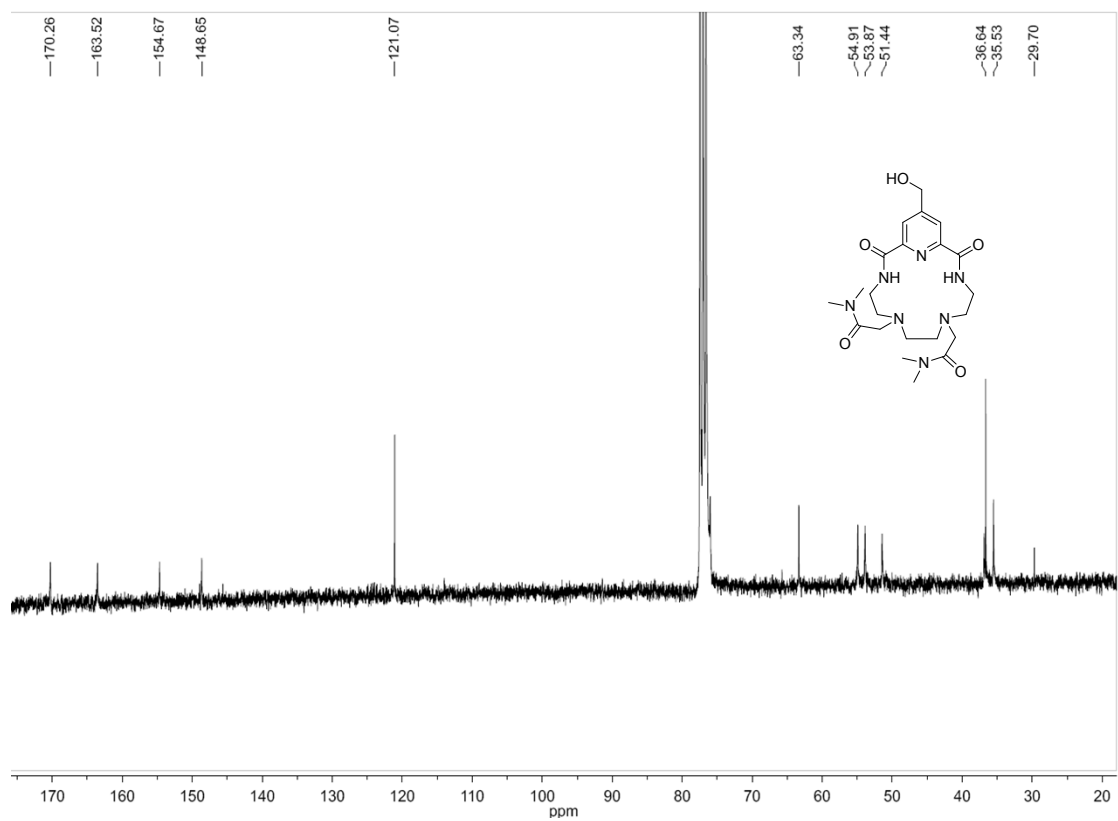


Fig. S8 ^{13}C NMR spectrum of 2.

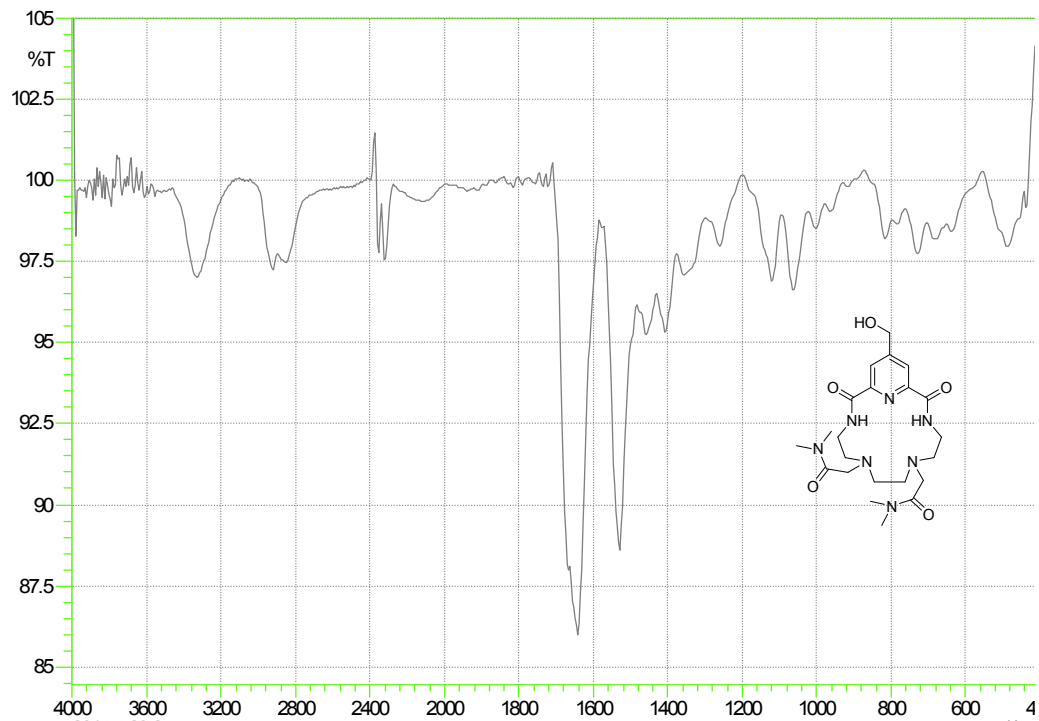


Fig. S9 IR spectra of 2.

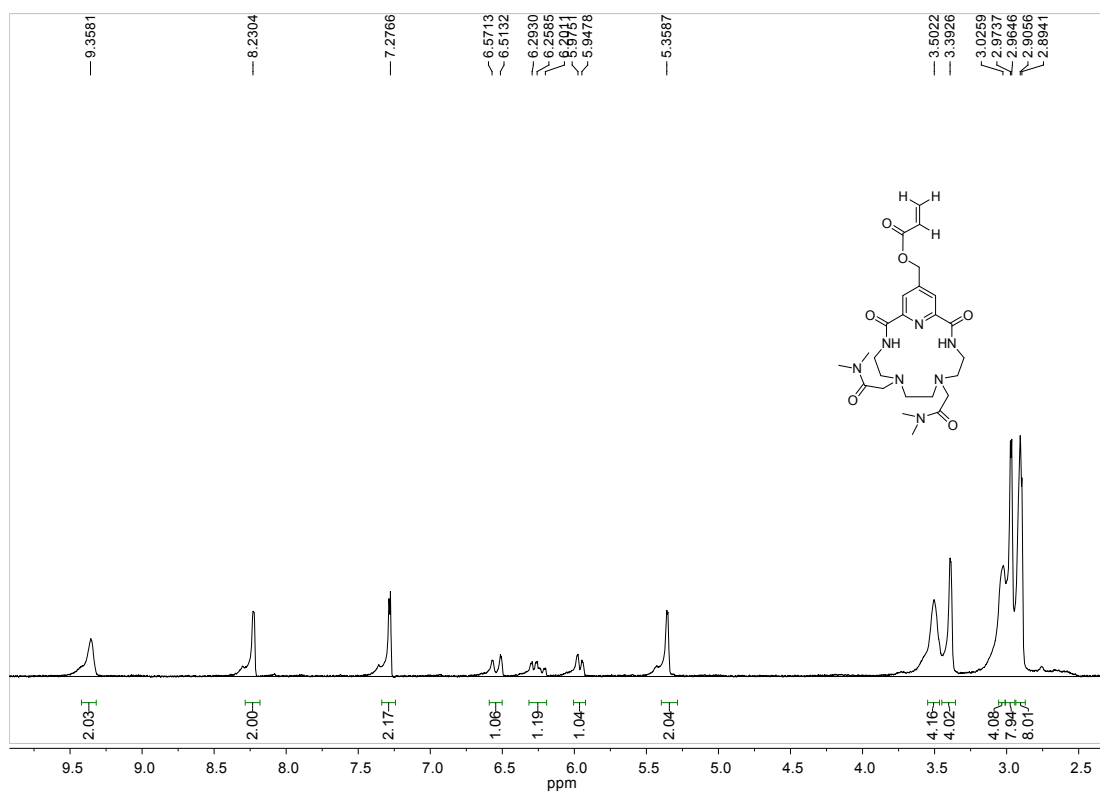


Fig. S10 ¹H NMR spectrum of 1.

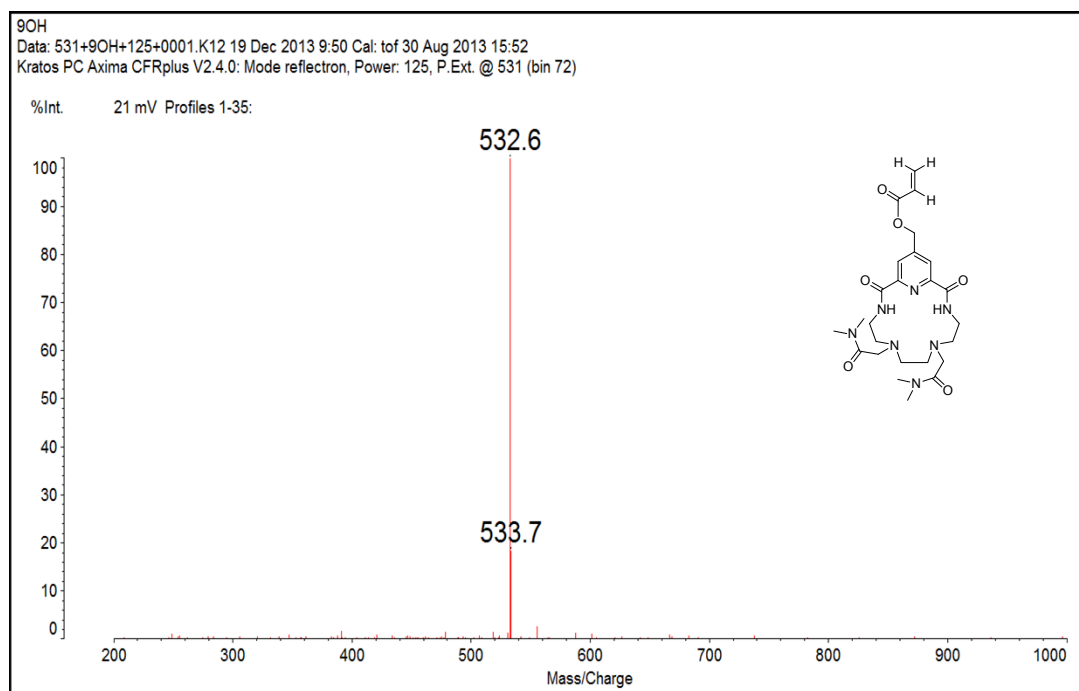


Fig. S11 TOF-MS spectra of 1.

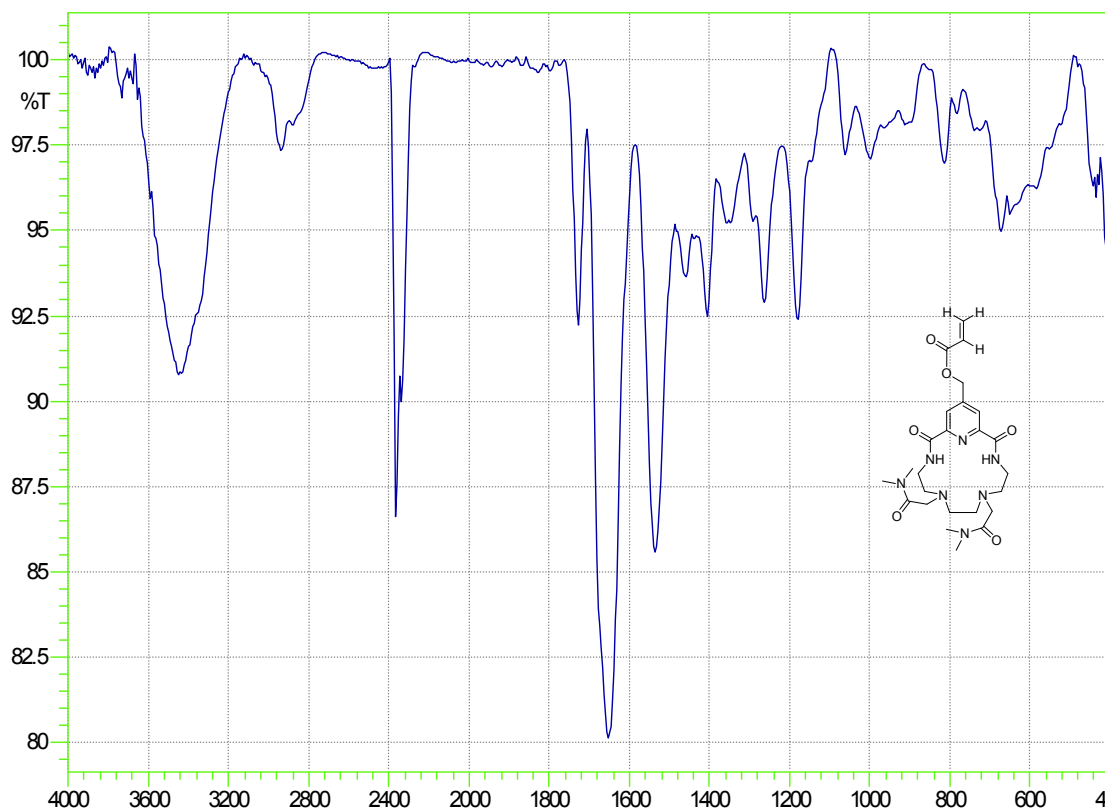


Fig. S12 IR spectra of 1.

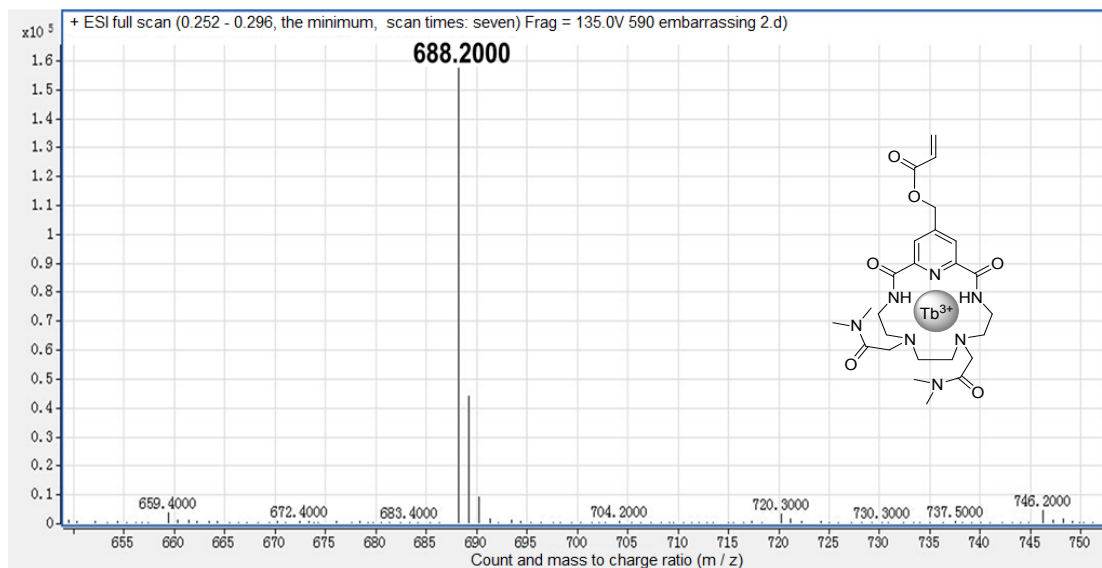


Fig. S13 ESI-MS of Tb-Ac

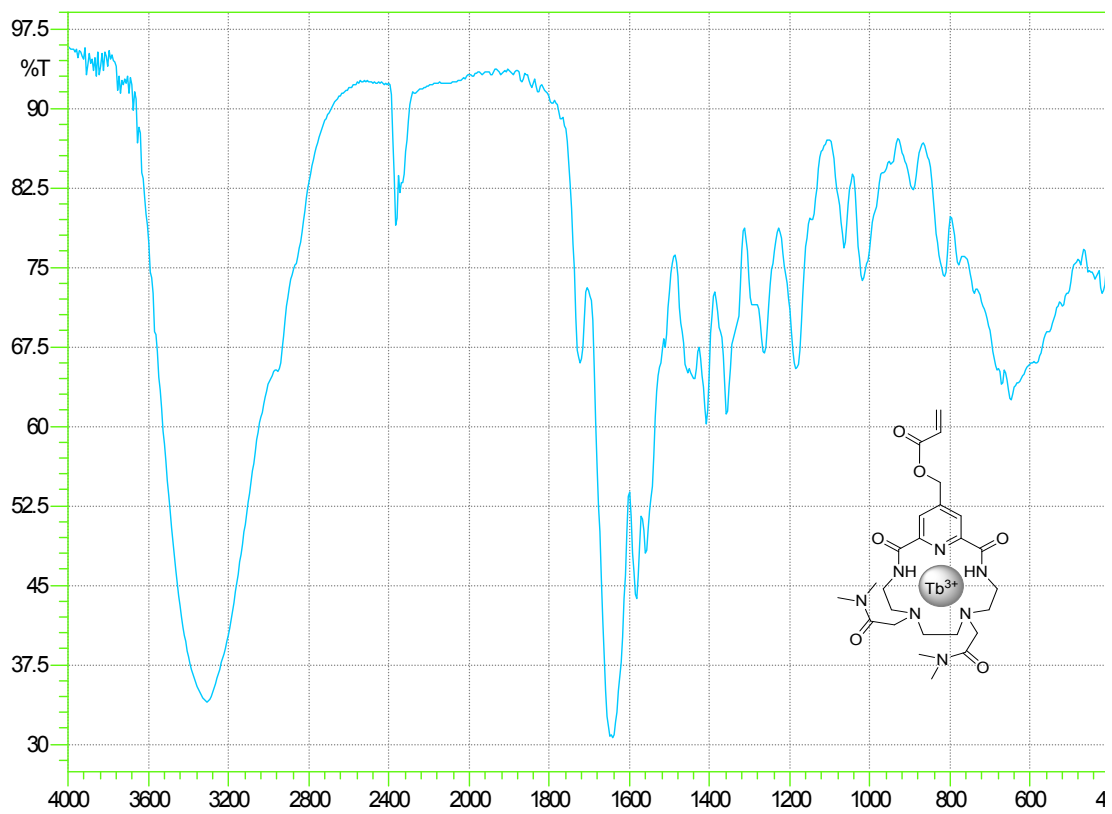


Fig. S14 IR spectra of Tb-Ac.

Reference

1. P. K. Tarafder and D. P. S. Rathore, *The Analyst*, 1988, **113**, 1073.
2. G. Labbe, A. P. Krismanich, S. de Groot, T. Rasmusson, M. Shang, M. D. Brown, G. I. Dmitrienko and J. G. Guillemette, *Journal of inorganic biochemistry*, 2012, **112**, 49-58.