A Near-Infrared Fluorescent Sensor for Detection of Cyanide in Aqueous Solution and Its Application for Bioimaging†

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Experimental Section

General methods Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. The starting material (2-aminoethyl)bis(2-pyridylmethyl)amine was prepared according to the previous report.[1] ^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker 250. Mass spectra were obtained using a JMS-HX 110A/110A Tandem Mass Spectrometer (JEOL). UV absorption spectra were obtained on UVIKON 933 Double Beam UV/VIS Spectrometer. Fluorescence emission spectra were obtained using RF-5301/PC Spectrofluorophotometer (Shimadzu).

Synthesis

N,N,N'-tris(pyridin-2-ylmethyl)ethane-1,2-diamine (2)

(2-aminoethyl)bis(2-pyridylmethyl)amine (1.70 g, 7.0 mmol) dissolved in 20 mL of dry methanol was added dropwise to a solution of picolinaldehyde (0.75 g, 7.0 mmol) in 20 mL of dry methanol. After the mixture was reflux for overnight, the resulting solution was concentrated under reduced pressure and sodium borohydride (1.06 g, 28.0 mmol) was slowly added. The resulting solution was stirred for 10 h at room temperature. After the solvent was evaporated, 15% NaOH solution (20 mL) was added to the residue, and then extracted with three 30 mL portions of CH_2Cl_2. The combined organic solvent was dried over anhydrous Na_2SO_4 and filtered, and the solvent was removed under reduced pressure to yield oil. The further purification was carried out by Al_2O_3 column using CH_2Cl_2/CH_3OH (100:0.5, v/v) as eluent and afford 0.82 g of product (yield 36%). ^{1}H NMR ($CDCl_3$, 250 MHz) δ (ppm): 8.43-8.46 (m,

3H), 7.52-7.60(m, 3H), 7.47 (d, 2H, *J* = 7.5 Hz), 7.24 (d, 1H, *J* = 7.5 Hz), 7.04-7.10 (m, 3H), 3.79 (s, 2H), 3.78 (s, 4H), 2.75 (s, 4H). ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ (ppm): 159.570, 159.459, 149.213, 148.939, 136.390, 122.961, 122.231, 121.933, 60.553, 54.832, 53.911, 46.641.

Compound 1

IR-780 (200 mg, 0.3 mmol) and compound **2** (400 mg,1.2 mmol) were dissolved in anhydrous N,N-dimethylformamide (DMF) (30 mL). The mixture was stirred at 85 ^{o}C for overnight under an argon atmosphere. The solvent was removed under reduced pressure, then the crude product was purified by silica gel chromatography using $CH_2Cl_2/CH_3OH/N(Et)_3$ (100:2:0.1, v/v/v) as eluent and Al_2O_3 column using CH_2Cl_2/CH_3OH (100:0.25, v/v) as eluent afford the desired product as a blue solid (42 mg, 15%). ^{1}H NMR ($CDCl_3$, 250 MHz) δ (ppm): 8.57 (d, 1H, *J* = 5.0 Hz), 8.43 (d, 2H, *J* = 5.0 Hz), 7.71 (dt, 1H, *J* = 7.5 Hz, 1.5 Hz), 7.52-7.58 (m, 4H), 7.20-7.33 (m, 7H), 7.09-7.12 (m, 5H), 6.95 (d, 2H, *J* = 7.5 Hz), 5.80 (d, 2H, *J* = 14 Hz), 4.73 (s, 2H), 3.87 (t, 4H, *J* = 14 Hz), 3.77 (s, 4H), 3.65 (t, 2H, J = 7.5 Hz), 2.98 (t, 2H, *J* = 7.5 Hz), 2.43 (t, 4H, *J* = 12.5 Hz), 1.78-1.83 (m, 6H), 1.41 (s, 12H), 0.99 (t, 6H, *J* = 7.5 Hz). ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ (ppm): 173.333, 169.486, 158.443, 156.570, 150.085, 149.109, 142.844, 142.730, 140.257, 137.559, 136.624, 128.545, 124.566, 123.851, 123.274, 123.184, 122.324, 122.046, 109.693, 96.974, 60.604, 60.187, 53.004, 52.412, 48.121, 45.281, 28.849, 24.868, 21.904, 20.434, 11.737. FAB MS m/z = 836.5383 [M + H]$^{+}$, calc. for $C_{56}H_{66}N_7$ = 836.5380.

Fluorescent study Stock solutions (10 mM) of sodium salts of CN^-, SCN^-, Pi, PPi, HSO_4^-, NO_3^-, HCO_3^-, ClO_4^-, CH_3COO^-, F^-, Cl^-, Br^- and I^- were prepared in double distilled water. Stock solutions of host (1 mM) were also prepared in CH_3CN by mixing 1 and $Cu(ClO_4)_2$ with the ratio of 1:1. Typically, test solutions were prepared by placing 15 μL of the probe stock solution into a test tube, adding an appropriate aliquot of each anion stock, and diluting the solution to 3 mL with HEPES (0.02 M, pH 7.4). Final concentration of the probe was 5 μM. For all measurements, excitation was at 680 nm.

C. elegans **and chemicals** The *C. elegans* wild type strain N2 was obtained from the *Caenorhabditis* Genetics Center (University of Minnesota). The nematodes were maintained at 25°C on NGM (nematode growth medium) agar plates (0.3% NaCl, 0.25% peptone, 2.5% agar, 5 mg/ml cholesterol, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 25 mM KH_2PO_4) previously seeded with *Escherichia coli* OP50 as described elsewhere.[2] All chemicals here used were purchased from Sigma-Aldrich, if not otherwise mentioned.

Exposure of ***C. elegans*** **to exogenous NaCN** For *C. elegans* to be exposed to NaCN on the plate, 3.5-cm-diameter NGM agar plates containing NaCN were prepared.[3,4] In details, 20 μL of different concentrations (1 μM - 1 mM) of NaCN were dropped onto the agar plates and dried at 25°C before transferring the nematodes on the plates. The N2 young adult (3 days old) nematodes that had grown by feeding *E. coli* strain OP50 as a food source on NGM plate[2] were collected and then washed

three times with NGM buffer (0.3% NaCl, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 25 mM KH_2PO_4). The worms were then moved onto NGM plate containing different concentrations (1 μM - 1 mM) of NaCN and incubated at 25°C for 4 h. After the incubation, the nematodes were washed three times with NGM buffer.

For *C. elegans* to be exposed to NaCN in the buffer solution, the nematodes were collected and incubated in 100 μL of NGM buffer containing different concentrations (1 μM - 1 mM) of NaCN at 25°C for 4 h. After the incubation, the worms were washed three times with NGM buffer.

Imaging of exogenous cyanide in *C. elegans* with the NIR sensor To obtain *in vivo* images of the cyanide accumulated in *C. elegans*, the worms were incubated with 100 μM NIR sensor in 100 μL of NGM buffer at 25°C for 20 min. After incubation, the worms were washed three times with NGM buffer and mounted onto a glass slide with Faramount™ aqueous mounting medium (Dako, Glostrup, Denmark). The fluorescent images of the mounted nematodes were obtained by using a confocal laser scanning microscope (CLSM LSM510, Carl Zeiss) with a 633 nm excitation and LP 650 nm emission filters at various magnifications (200 × to 1260 ×). The selectivity of NIR signal was confirmed by using an emission filter BP 700-750 nm.

***C. elegans* infection with a cyanogenic *P. aeruginosa* strain** A cyanogenic PA strain (PA14) expressing green fluorescent protein (GFP) was generously provided by Prof. You-Hee Cho (Seoul National University, Korea). The PA14 strain was cultured

overnight in LB broth with 100 μg/mL carbenicillin and 5 μL of the overnight culture was inoculated into 5 mL of LB carbenicillin in a 30-mL glass tube and incubated at 37°C in a shaker at 220 r.p.m. Growth was monitored by measuring optical density (O.D.) at 600 nm using a spectrophotometer (Biowave CO8000, WPA Ltd., Cambridge, England). For *C. elegans* to be infected with the PA14 strain, 20 μL of the culture (O.D. = 2.0) were dropped onto the NGM agar plates and dried at 25°C before transferring nematodes on the plates. After the incubation on the plates for 2 days, the nematodes were collected and transferred on the NGM plates previously seeded with *E. coli* OP50. After 2 h-incubation on the *E. coli* OP50 plates, the nematodes were washed three times with NGM buffer.

Imaging of biogenic cyanide in the infected *C. elegans* with the NIR sensor The infected nematodes were incubated with NIR sensor (100 μM) in 100 μL of NGM buffer at 25 °C for 20 min. After the incubation, the worms were washed again three times with NGM buffer. For the efficacy test of ceftazidime,[5] the nematodes that had been fed on the PA14 strain were collected at 2 days and incubated with ceftazidime (200 μg/mL) in 100 μL of NGM buffer for 2 h. After the incubation, they were collected, washed three times with NGM buffer and incubated with the NIR sensor (100 μM) in 100 μL NGM buffer at 25 °C for 20 min. The nematodes were washed again three times with NGM buffer and prepared for the observation. The infected nematodes with or without the antibiotic treatment were mounted onto a glass slide as described previously. The fluorescent images were obtained by using the CLSM with

a 488 nm excitation and 505-530 nm emission filters for GFP fluorescence and with 633 nm excitation and LP 650 nm emission filters for NIR fluorescence.

C. elegans **killing assays** The assays were conducted as described previously[2], with some modifications. PA14 was grown overnight in LB broth at 37 ^{o}C with aeration. 10 μL of cultures were spread onto NGM agar plates. The plates were dried at 25 ^{o}C and then immediately utilized for the assays. 10 nematodes (N2 strain) previously synchronized in the young adult stage were transferred to each plate, incubated for 24 h at 25 ^{o}C, and transferred to a new plate every 24 h to allow for the determination of the survival rates of the original worms without the presence of progeny. A worm was considered dead when it did not respond to being touched by a platinum wire pick. Each assay was conducted in triplicate and repeated three times. The time for 50% of the nematodes to die (TD_{50}) was calculated by using PRISM V.5 program (GraphPad Software, Inc., La Jolla, CA).[6]

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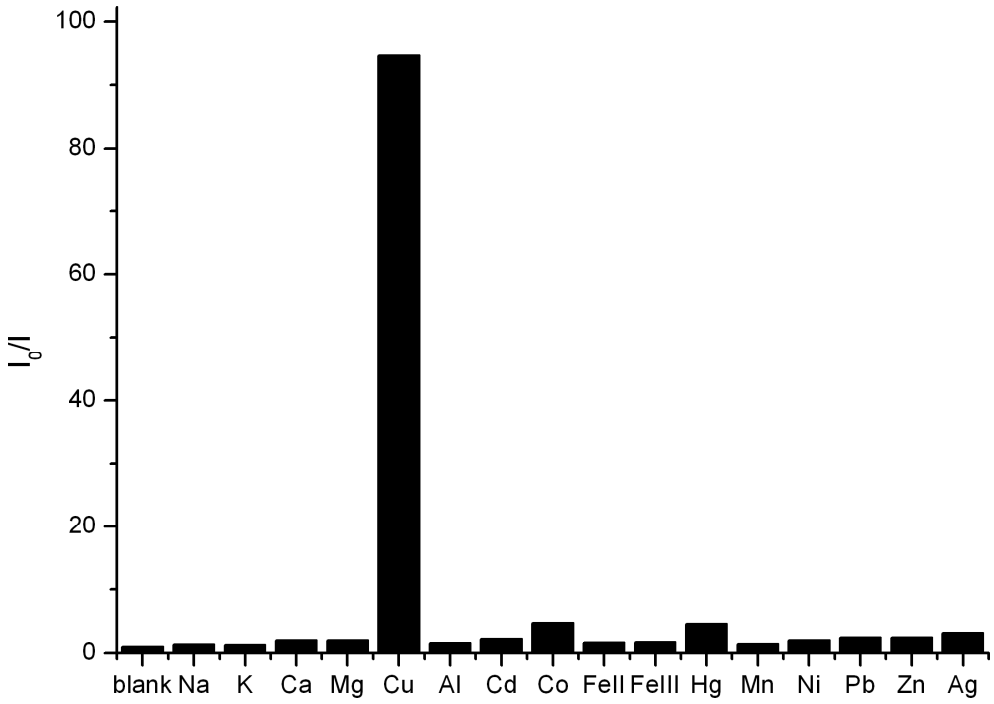

Figure S1. Fluorescence intensity at 748 nm in the absence of metal ions relative to that in the presence of various metal ions: [**1**] = 5 μM; [metal ion] = 50 μM; CH_3CN/HEPES (20 mM, pH 7.4) = 0.5:99.5, v/v; Ex: 680 nm.

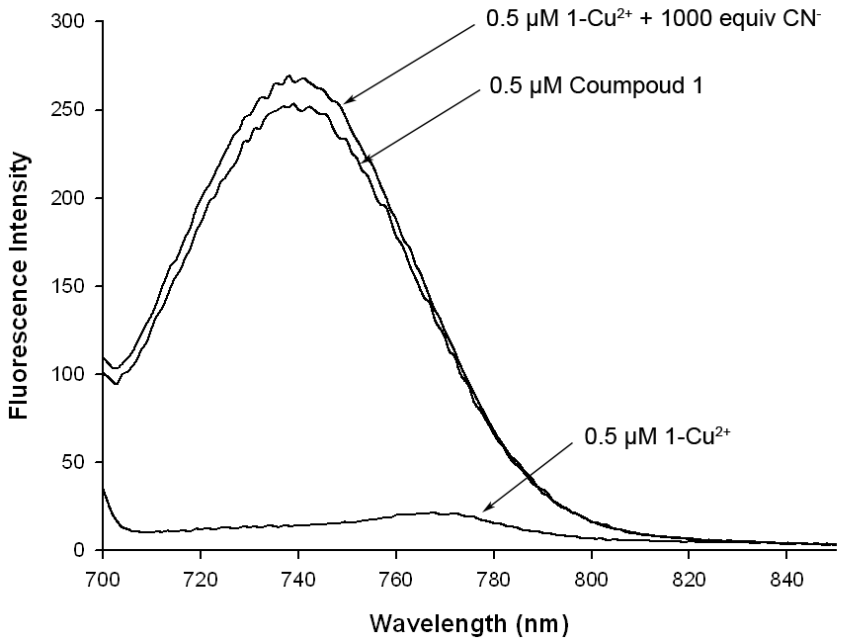

Figure S2. The fluorescent recovery of after exposure of **1-Cu**$^{2+}$ (0.5 μM) to excess cyanide.

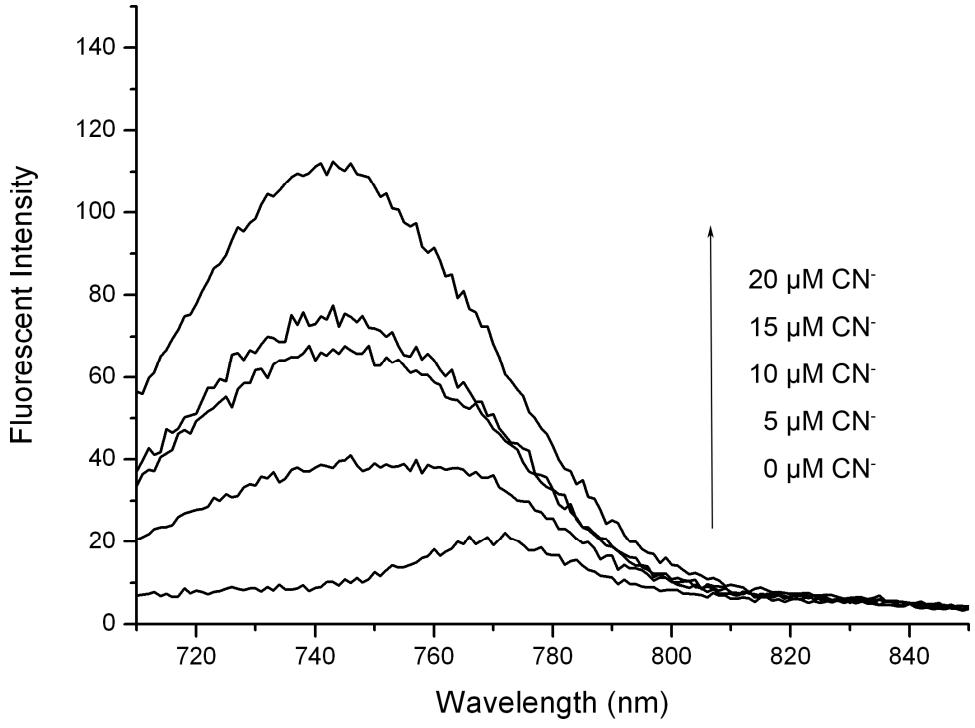

Figure S3. The fluorescent enhancement upon the addition of CN^- using low concentration of **1-Cu**$^{2+}$: [**1-Cu**$^{2+}$] = 0.5 μM; HEPES buffer (20 mM, pH 7.4); Ex: 680 nm.

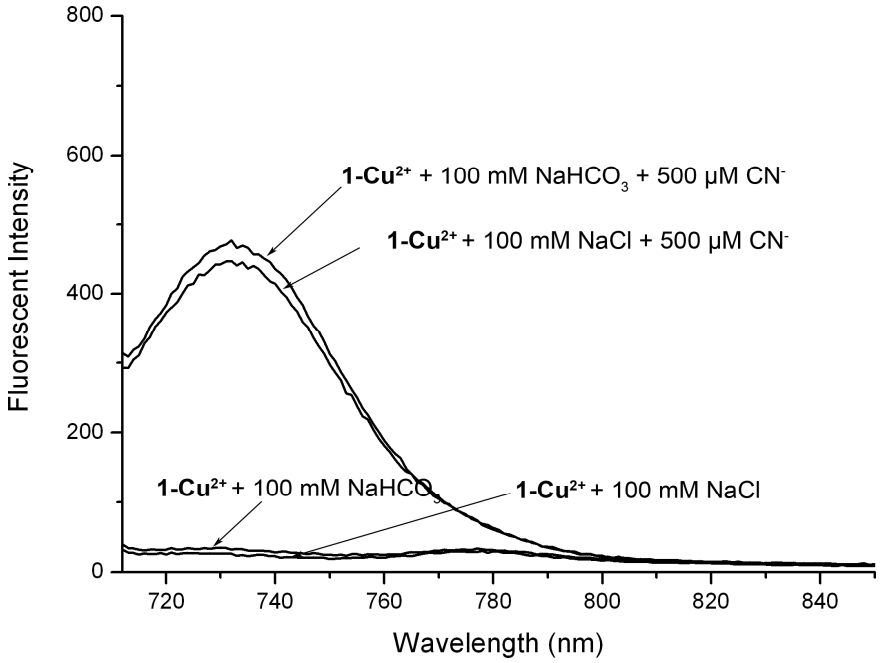

Figure S4. The fluorescence spectra of **1-Cu**$^{2+}$ (5 μM) with or without 100 equiv CN^- in the presence of NaCl/$NaHCO_3$ (100 mM).

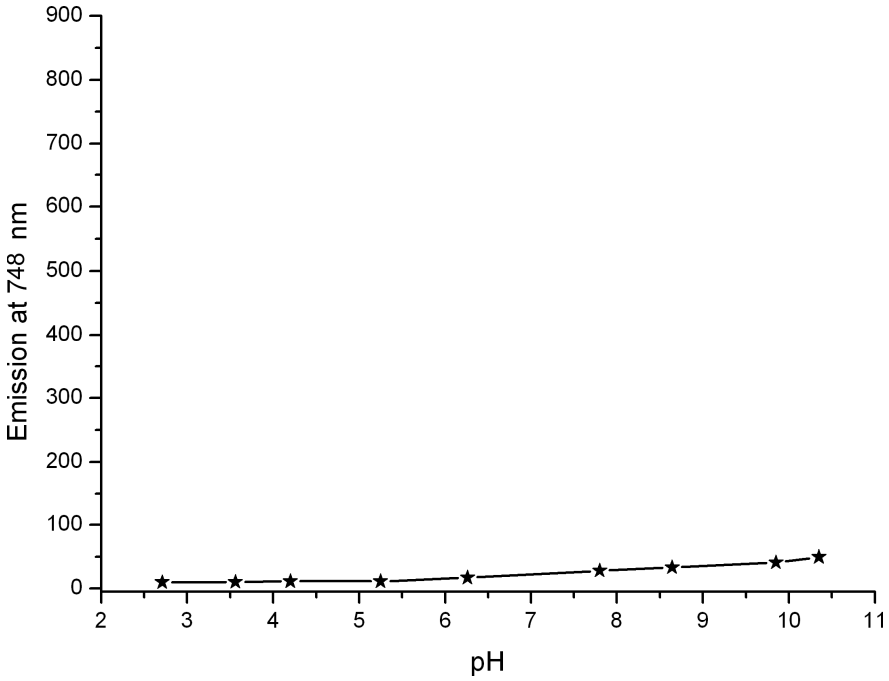

Figure S5. Fluorescent Intensity of **1-Cu**$^{2+}$ at 748 nm under various pH conditions: [**1-Cu**$^{2+}$] = 5 μM; Ex: 680 nm.

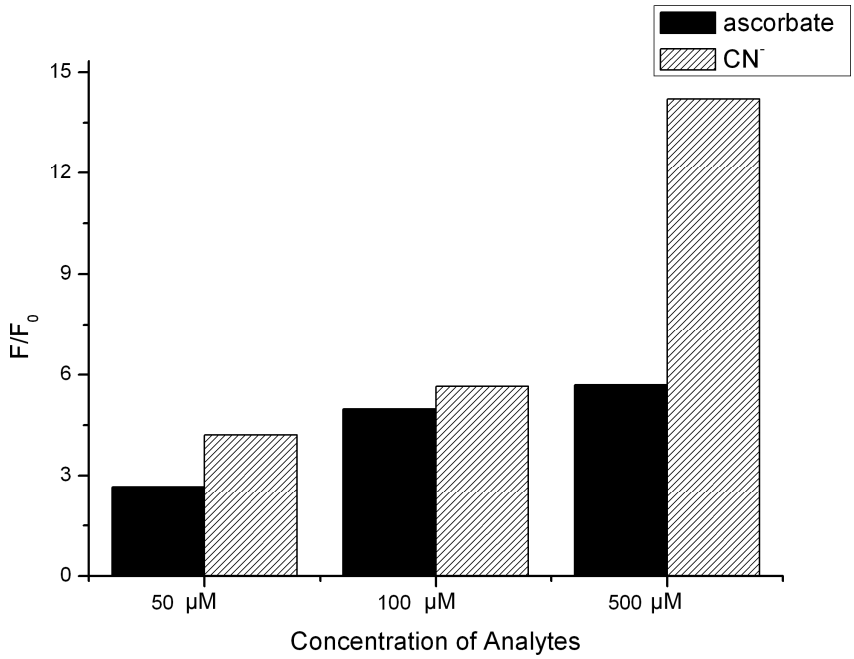

Figure S6. The fluorescent enhancement at 748 nm upon the addition of CN- and ascorbate: [**1-Cu**$^{2+}$] = 5 μM; CH_3CN/HEPES (20 mM, pH 7.4) = 0.5:99.5, v/v; Ex: 680 nm.

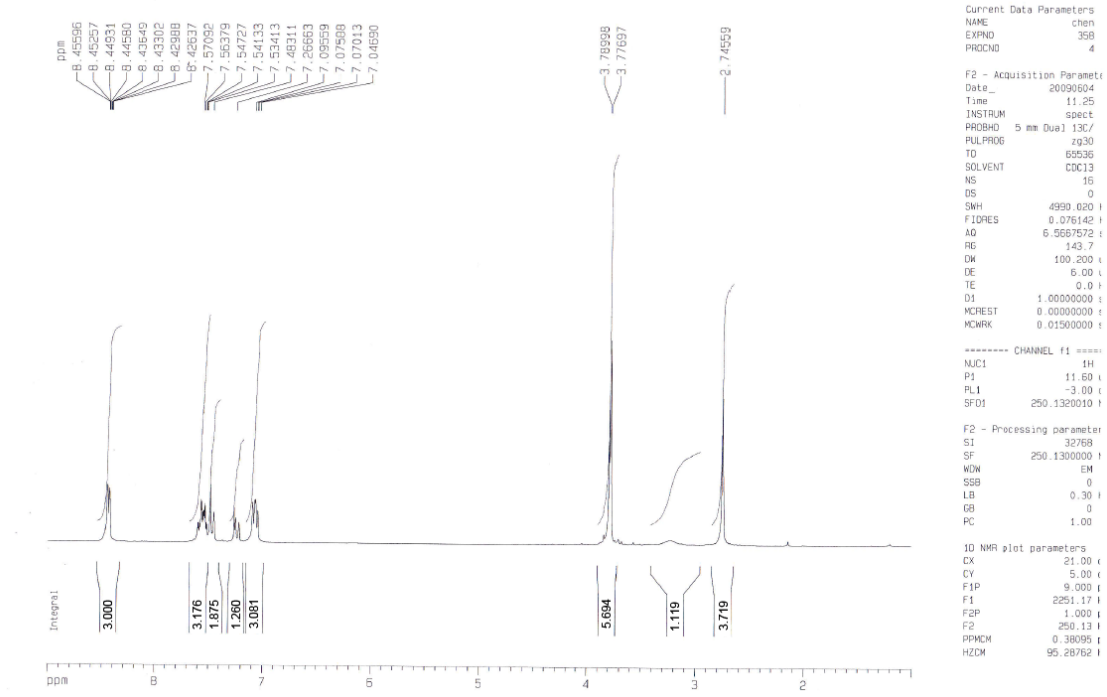

Figure S7. ^{1}H NMR (250 MHz) spectra of **2** in $CDCl_3$.

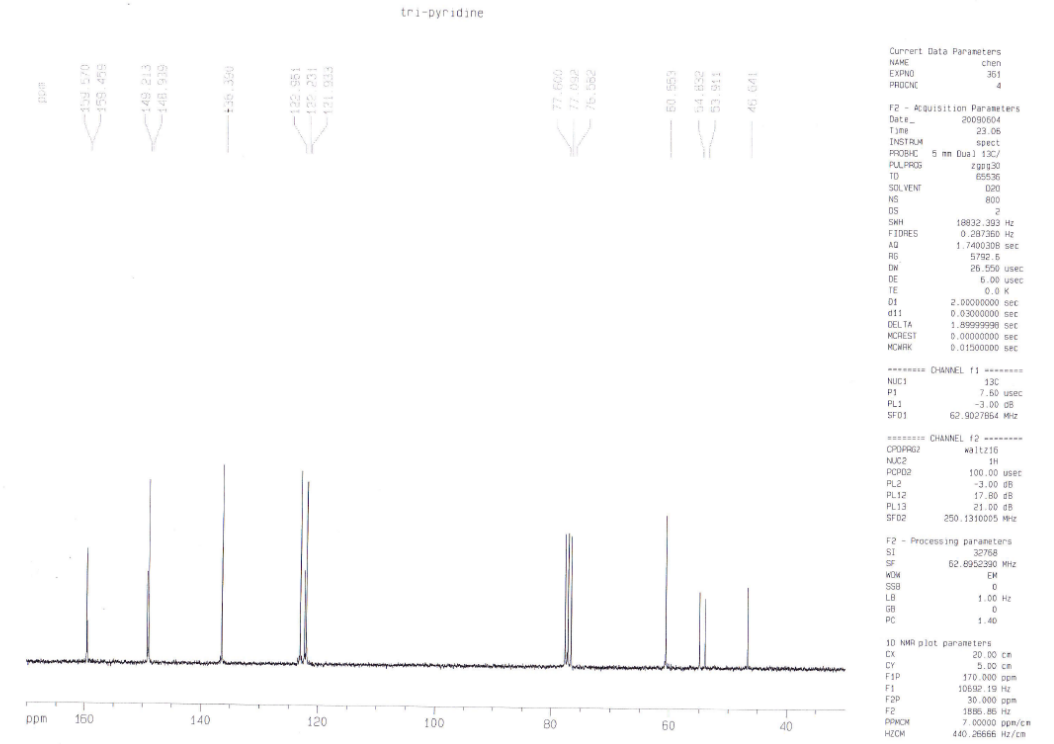

Figure S8. ^{13}C NMR (250 MHz) spectra of **2** in $CDCl_3$.

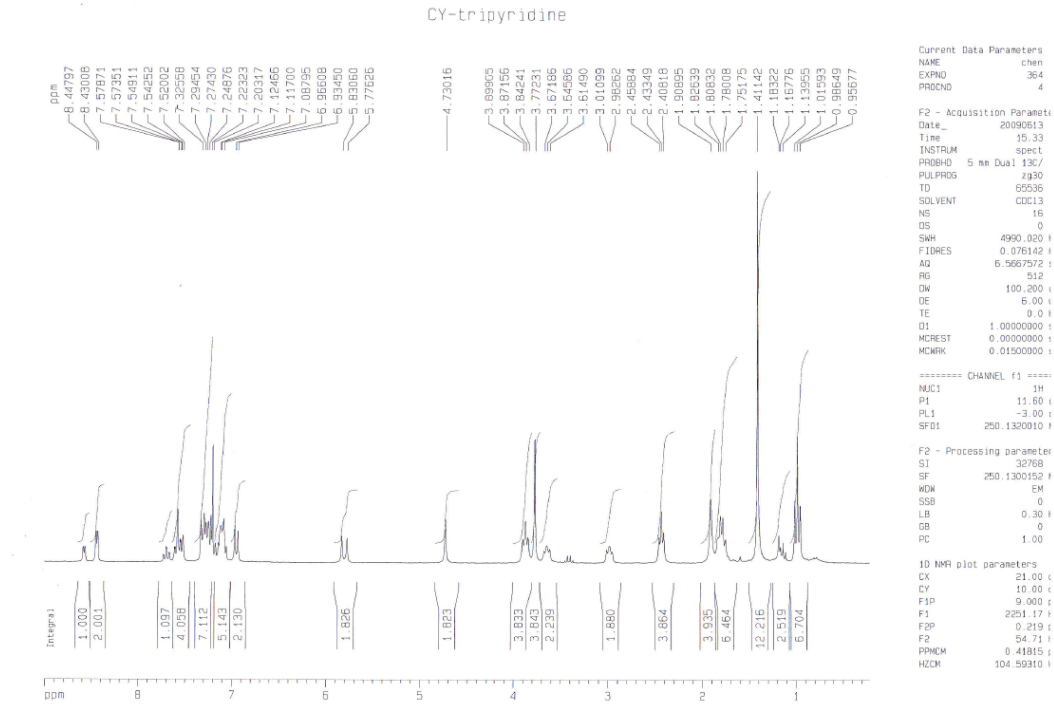

Figure S9. ^{1}H NMR (250 MHz) spectra of **1** in $CDCl_3$.

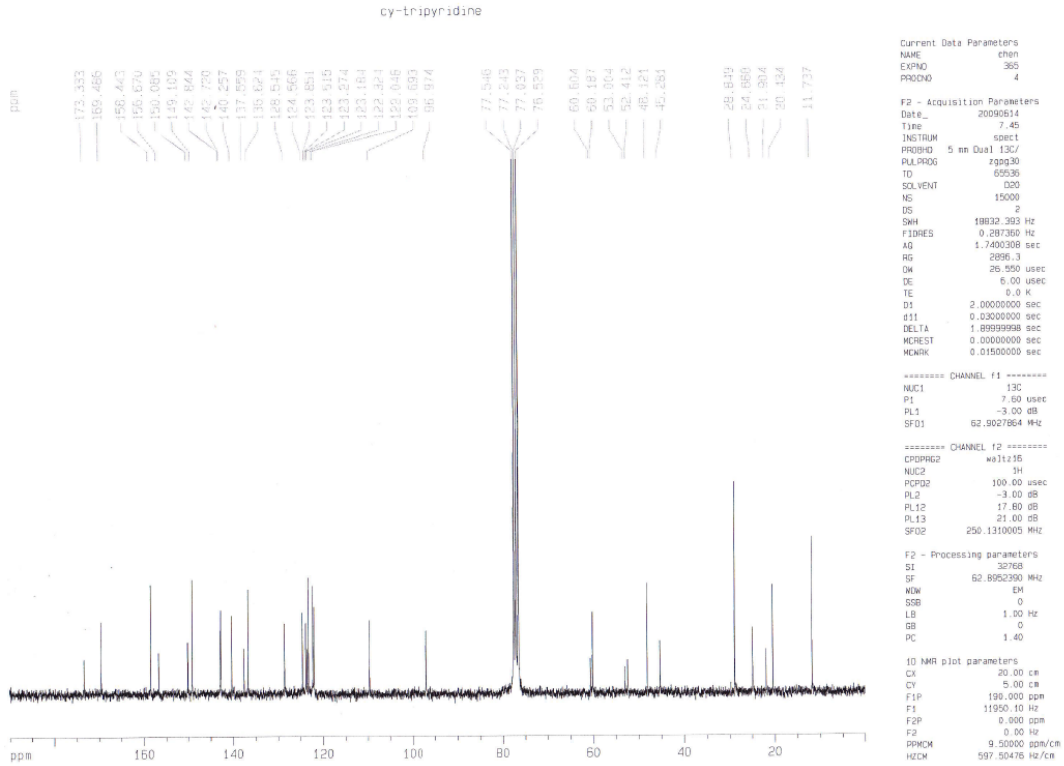

Figure S10. ^{1}H NMR (250 MHz) spectra of **1** in $CDCl_3$.

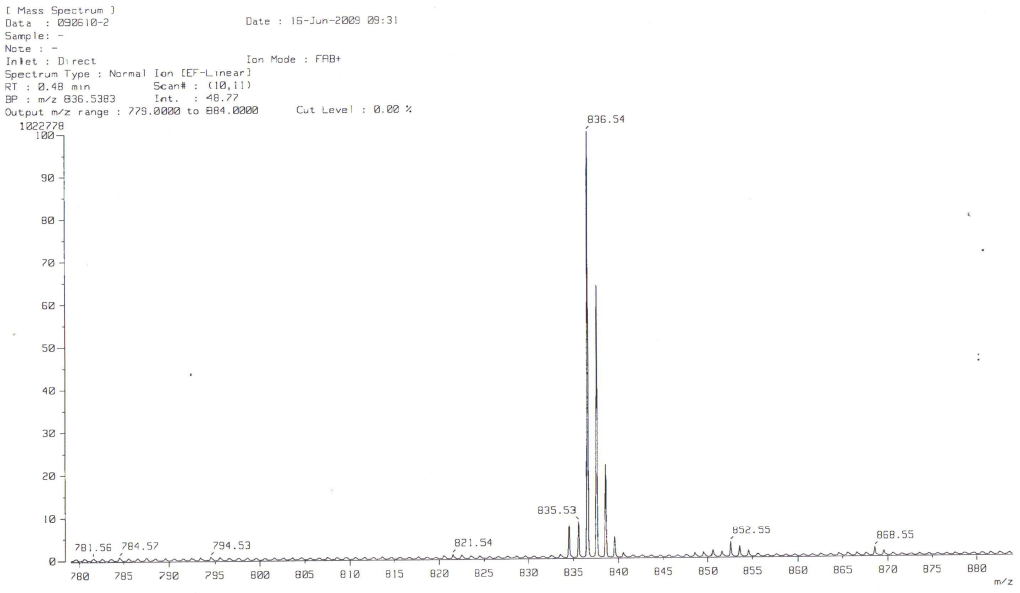

Figure S11. FAB mass spectrum of **1**.