Electronic Supplementary Information (ESI):

A novel near-infrared fluorescent probe for selectively sensing nitroreductase (NTR) in an aqueous medium

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Contents

I. Chemicals and apparatus

All chemicals for synthesis were purchased from commercial suppliers and were used as received. Nitroreductase (NTR) expressed in *Escherichia coli* and NADH were purchased from Sigma-Aldrich. Fluorescence spectra measurements were performed on a HITACHI F-7000 spectrofluorimeter equipped with a xenon discharge lamp using 1 ml Fluor Micro Cell. IR spectra were measured using the method of KBr pellets and recorded on PerkinElmer FT-IR spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on a JEOL JNM-ECA 300 spectrometer using tetramethylsilane (TMS) in the solvent of DMSO as an internal standard (^{1}H NMR: TMS at 0.00 ppm, DMSO at 2.50 ppm; ^{13}C NMR: DMSO at 39.51 ppm). ESI mass spectra and HRESI mass spectra were obtained using a ESI-LTQ MS spectrometer and a Bruker Apex IV FTMS spectrometer, respectively. All measurements were performed in 50 mM PBS (pH 7.4). Deionized water was used to prepare all aqueous solutions. The probe (**5**) and compound (**3**) were synthesized according to a modified literature procedure.[1]

II. Synthesis of compounds

1. Synthesis of 4-(4-nitrobenzyloxy) isophthalaldehyde (3)

To a solution of Compound (**1**) (0.15 g, 1 mmol) in DMF (5 ml) at -5℃ was added K_2CO_3 (0.28 g, 2 mmol). After a 5-min stirring at this temperature, (**2**) (0.32 g, 1.5 mmol) was added. The mixture was stirred at room temperature for 12 hours and then water was added. The precipitate was filtered, dried under vacuum and recrystallizaed from EtOAc to afford the compound (**3**) as a light yellow solid (0.23 g, 79% yield). ^{1}H NMR (300MHz, DMSO-d_6): δ = 10.49 (s, 1H), 9.98 (s, 1H), 8.30-8.28 (m, 3H), 8.20

(d, 1H, *J*=8.6 Hz), 7.84 (d, 2H, *J*=8.6 Hz), 7.52 (d, 1H, *J*=8.6 Hz), 5.60 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 191.34, 188.75, 163.96, 147.19, 143.65, 136.51, 130.47, 129.64, 128.27, 124.61, 123.72, 114.62, 69.23. IR (KBr pellet): 3116, 3080, 2929, 2873, 1743, 1694, 1682, 1603, 1579, 1520, 1494, 1434, 1381, 1350, 1263, 1251, 1213, 1165, 1099, 1034, 951, 845, 736, 613, 555 cm^{-1}. MS (ESI): m/z calc. for $C_{15}H_{11}NO_5$: 285.06 ; found: 308.11 $[M+Na]^+$.

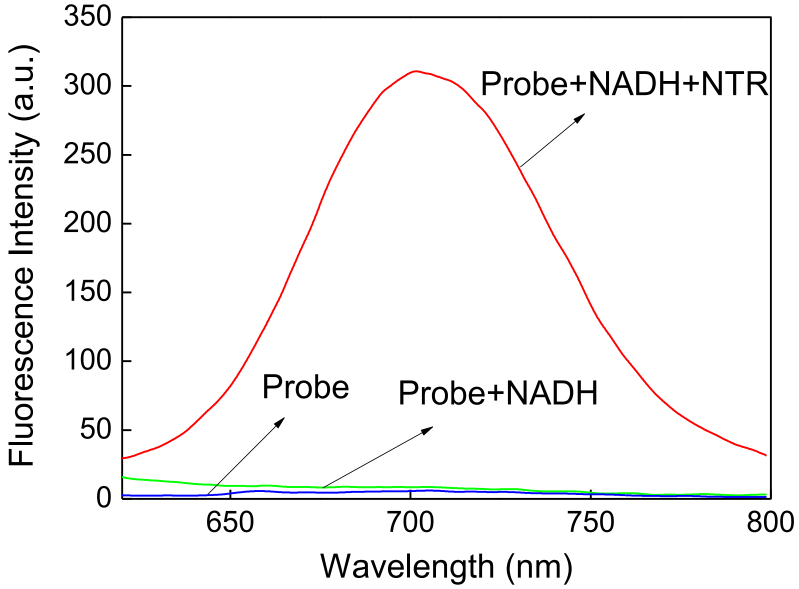
2. Synthesis of 2,3,3-trimethyl-1-(3-sulfopropyl)-3H-indolinium (4)

The solution of 2,3,3-trimethylindolenine (0.16 g, 1 mmol) and 1,3-propane sultone (0.15 g, 1.2 mmol) in toluene (5 mL) was heated under reflux for 24h. The resulting solution was cooled to room temperature and filtered. The filtered product was washed with diethyl ether and dried in vacuo resulting in compound (**4**) (0.25 g, 89% yield) as a pink solid. ^{1}H NMR (300MHz, DMSO-d_6): δ = 8.07-8.04 (m, 1H), 7.84-7.82 (m, 1H), 7.63-7.60 (m, 2H), 4.67 (t, 2H, *J*=7.7 Hz), 2.84 (s, 3H), 2.63 (t, 2H, *J*=6.6 Hz), 2.21-2.11 (m, 2H), 1.54 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 196.55, 141.93, 141.19, 129.31, 128.93, 123.42, 115.45, 54.12, 48.59, 47.37, 46.58, 23.74, 22.03, 13.79. IR (KBr pellet): 3027, 2985, 2929, 2157, 1659, 1627, 1609, 1480, 1461, 1418, 1371, 1336,1201, 1162,1038, 929, 783, 725, 617, 572, 532 cm^{-1}. MS (ESI): m/z calc. for $C_{14}H_{19}NO_3S$: 281.11 ; found: 280.27 $[M-H]^-$.

3. Synthesis of the fluorescent probe (5)

A mixture of compound (**3**) (0.14 g, 0.5 mmol), NaOAc (0.091 g, 1.1 mmol), and compound (**4**) (0.31g, 1.1 mmol) in Ac_2O (8 ml) was stirred overnight at 80℃ under an Ar atmosphere. After completion, the reaction mixture was cooled to room temperature and concentrated under vacuum. The resultant product was purified by preparative thin-layer chromatography (PTLC) to afford probe (**5**) (0.12 g, 30%) as an orange solid. ^{1}H NMR (300MHz, DMSO-d_6): δ = 9.28 (s, 1H), 8.68-8.55 (m, 3H), 8.35 (d, 2H, *J*=8.9 Hz), 8.22 (d, 1H, *J*=16 Hz), 8.11-8.03 (m, 3H), 7.92-7.84 (m, 4H), 7.70-7.59 (m, 4H), 7.54 (d, 1H, *J*=8.9 Hz), 5.69 (s, 2H), 5.03-4.90 (m, 4H), 2.71-2.68 (m, 4H), 2.28-2.25 (m, 4H), 1.86 (s, 6H), 1.78 (s, 6H). IR (KBr pellet): 2930, 2873, 1591, 1524, 1472, 1345, 1264, 1174, 1122, 1042, 841, 739, 525 cm^{-1}. HRMS (ESI): m/z calc. for $C_{43}H_{46}N_3NaO_9S_2$: 834.2489, $[M + Na]^+$; found: 834.2480.

III. Fluorescence spectra analysis of Probe (5).



Fluorescence spectra of 20 µM Probe (5) and NTR (10 µg/mL) with or without 5 mM NADH in PBS buffer (pH 7.4) at 37 ℃ after an incubation time of 20 min.

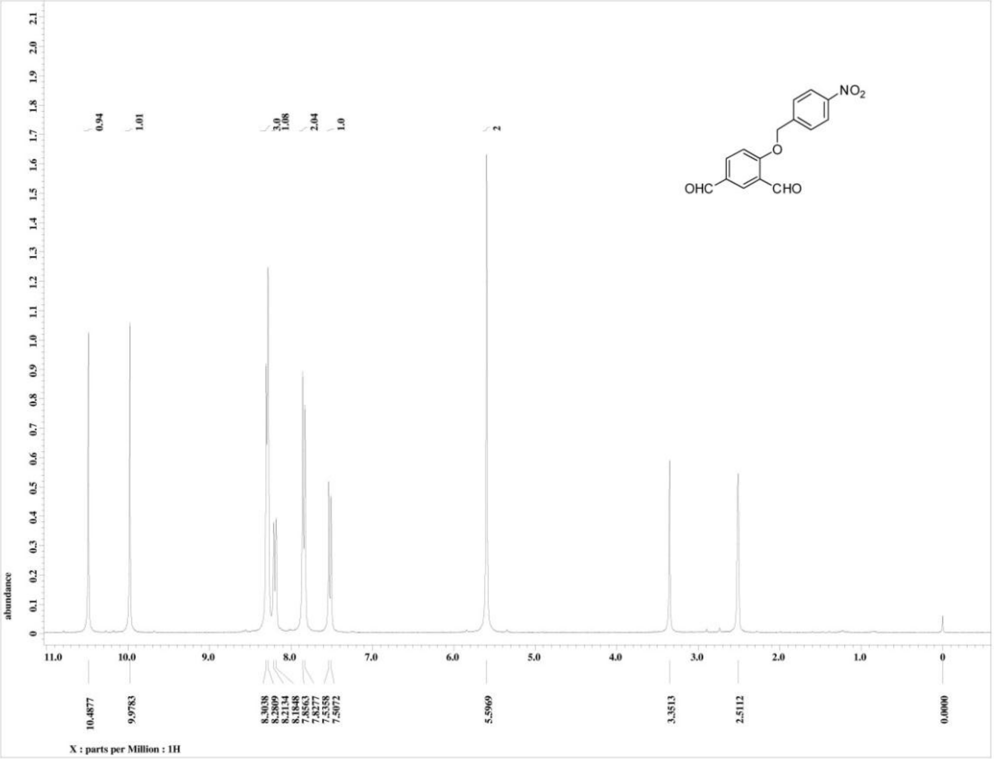
IV. Kinetic parameters determination

All kinetic measurements were performed in 0.05 M phosphate buffer (pH 7.4 at 37℃) containing 10 µg/mL NTR and 0.5 mM of NADH as an electron donor. Excitation and emission wavelengths were 590nm and 708nm. The kinetics rate of (**7**) released from probe (**5**) in the presence of NTR (10 µg/mL) and NADH (0.5 mM) was measured by adding varied concentrations of probe (**5**) (0-50 µM) to the reaction system and recorded the fluorescence change at a wavelength of 708 nm. The fluorescence calibration curve for (**7**) was used to calculate the rate. The parameters of the kinetic enzymic reaction, Michaelis-Menten constant (K_m), maximum rate (V_{max}) and catalytic rate constant (k_{cat}) were investigated from Lineweaver-Burk plot. The results reported here are the average values of three experiments conducted in triplicate in all analytical determinations.

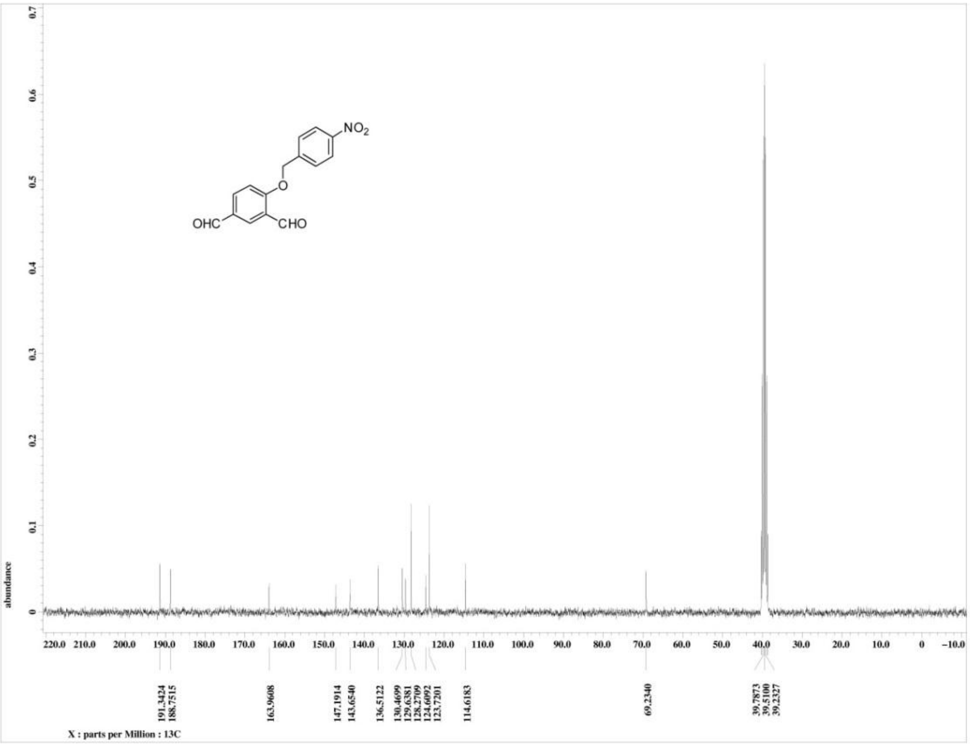
V. Fluorescent response assays by the biological reductants

Stock solutions (10 mM) of biological reductants in aqueous solutions (50 mM PBS, pH 7.4) were prepared. The analytical determinations were carried out by adding 10 µL of the biological reductants stock solutions to the test solutions in the presence of probe (5) with NTR (10 µg/mL) and with or without NADH (5 mM). The final concentration of probe (5) and biological reductants in a 1 mL quartz cuvette were 10 µM and 1 mM, respectively. For all measurements, the fluorescence spectra were obtained by excitation at 590 nm. Both the excitation and emission slit widths were 5 nm, respectively. The reactions were carried out at 37 ℃ for 15min and then fluorescence intensities were recorded at the wavelength of 708 nm.

VI. ^{1}H NMR and ^{1}C NMR spectra of compound (3)

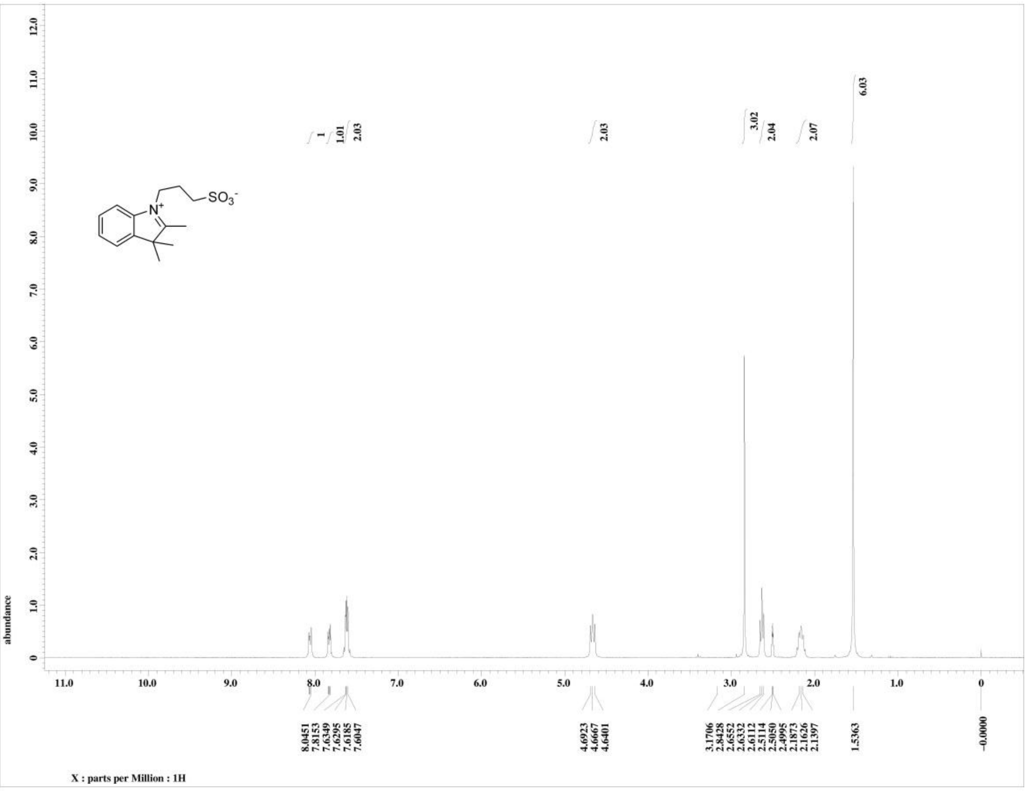


^{1}H NMR spectrum of compound (**3**)



^{1}C NMR spectrum of compound (**3**)

VII. ^{1}H NMR and ^{1}C NMR spectra of compound (4)



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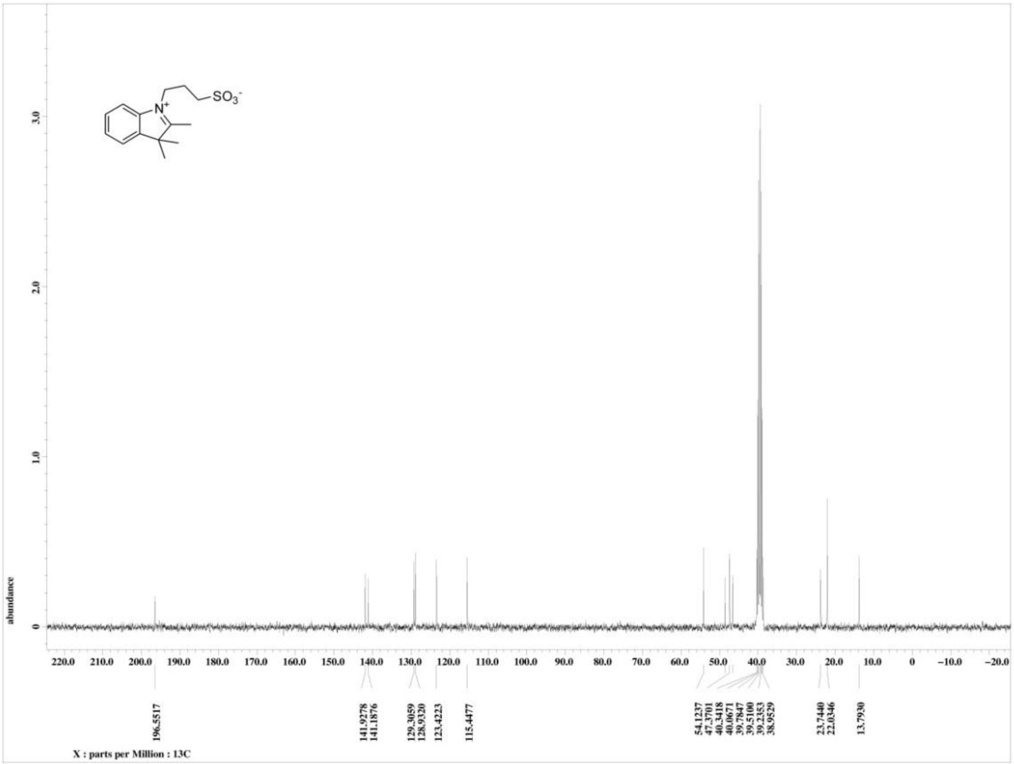
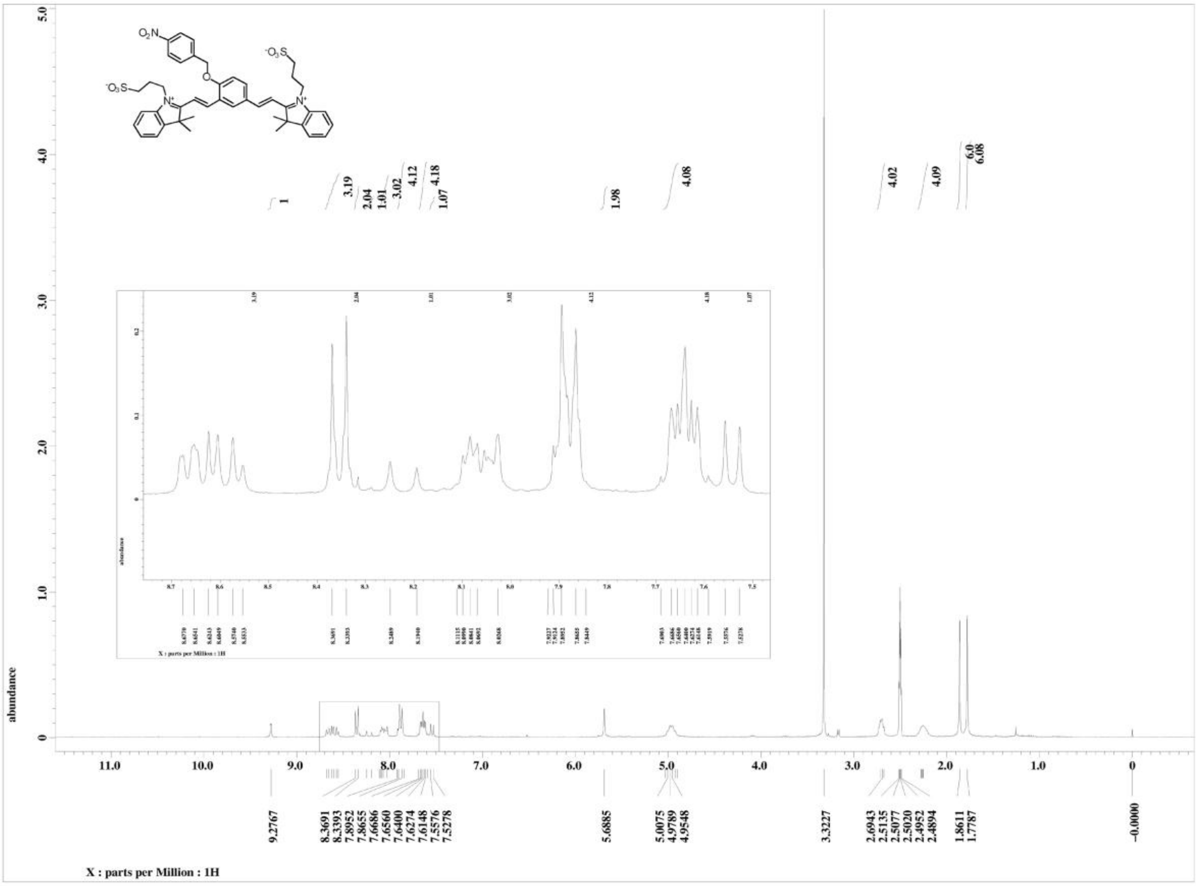
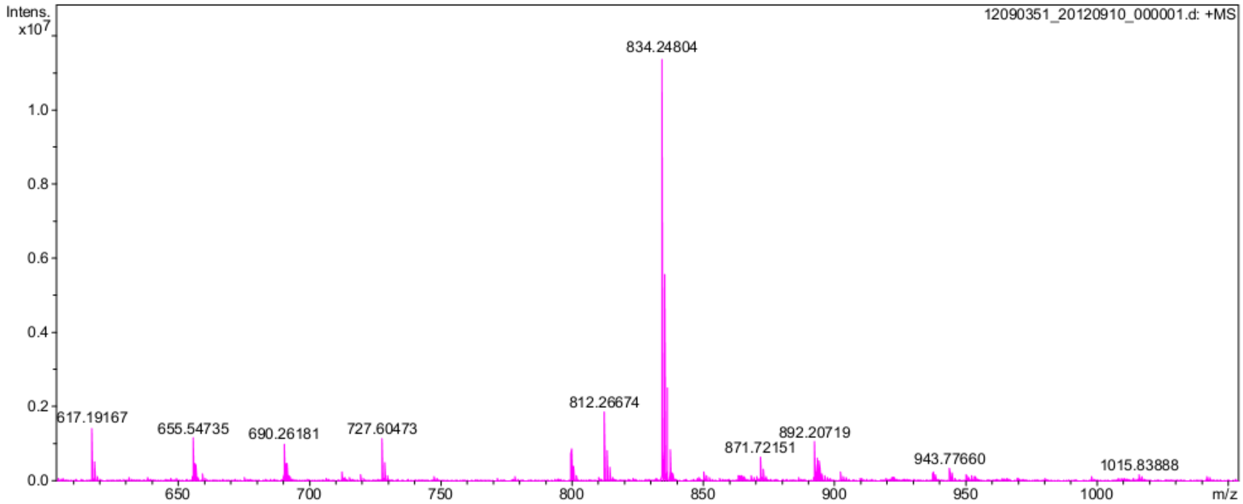


^{1}C NMR spectrum of compound (**4**)

VIII. ^{1}H NMR and HRMS (ESI) spectra of probe (5)



^{1}H NMR spectrum of probe (**5**)



HRMS (ESI) spectrum of probe (**5**)

Reference:

1. N. Karton-Lifshin, E. Segal, L. Omer, M. Portnoy, R. Satchi-Fainaro and D. Shabat, *J. Am. Chem. Soc.*, 2011, **133**, 10960.