

Supporting Information to

Cell Membrane Permeable Fluorescent Ca²⁺ Probe Based on Bis-BODIPY with Branched PEG

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1. General Information

Materials

Ionomycin, bis(pinacolato)diboron were purchased from Jiangsu Sukailu Chemical Co., Ltd; 3-(N-morpholino)propanesulfonic acid (MOPS) , ethyleneglycol tetraacetic acid (EGTA), phosphorus oxychloride and trifluoroacetic acid (TFA) were purchased from J&K Company; 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ) were purchased from Sinopharm Chemical Reagent Co., Ltd; 2,4-dimethylpyrrole were purchased from Tianjin Kemiou Chemical Reagent Co., Ltd; N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDCI) and 1-Hydroxybenzotriazole were purchased from Shanghai Medpep Co. Ltd; Hank's Balanced Salts Solution (HBSS) were purchased from Life Technologies Co., Ltd, and used without any further purification. Solvents were either employed as purchased or dried according to procedures described in the literature. Deionized water was obtained from a Milli-Q water purification system (Millipore).

The ^1H -NMR spectra were recorded at 20 °C on 600 MHz NMR spectrometer (Bruker). The ^{13}C -NMR spectra were recorded at 20 °C on 150 MHz NMR spectrometer (Bruker). Chemical shifts are reported in ppm at room temperature using CDCl_3 and DMSO-d_6 as solvent, tetramethylsilane as internal standard unless indicated otherwise. Abbreviations used for splitting patterns are s = singlet, d = dublett, t = triplet, qui = quintet, m = multiplet. Mass spectra were carried out using MALDI-TOF/TOF matrix assisted laser desorption ionization mass spectrometry with autoflexIII smartbeam (Bruker Daltonics Inc). UV/Vis spectra were recorded with a Shimadzu WV-2550 spectrophotometer. Fluorescence spectra were recorded on a Shimadzu RF-5301 fluorescence spectrophotometer. The concentration of the solution for the calculation of quantum yields was 10^{-5} mol/L. Fluorescein in 0.1M NaOH aq was used as reference ($\Phi_r = 0.85$). Recycling preparative GPC purifications were carried out on a Shimadzu HPLC system, which consisted of a model SPD-20A tunable absorbance detector, a model RID-10A differential refractometer, an in-line degasser, a model LC-6AD Pump, a model CBM-20A controller, and a Shodex KF-802 preparative GPC column. The Shimadzu model

LC-6AD pump was fitted with a 1.0 mL loop and a three directional recycling manifold that allowed for the product to be cycled back onto the column. All products were cycled over the column two times before separation using THF as a solvent at a flow rate of 5.0 mL min⁻¹.

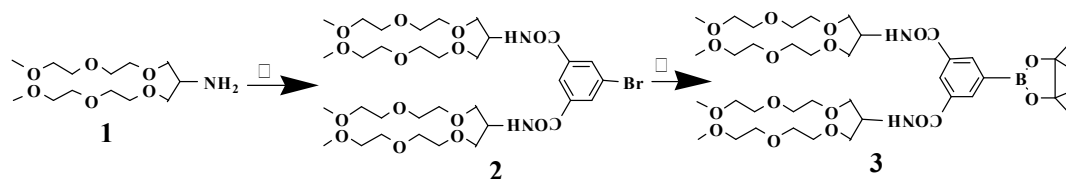
Preparation of Cell Cultures

HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM medium, Invitrogen Corp) supplemented with 10% FBS, penicillin (100 units/ml), and streptomycin (100 ug/ml). All cells were maintained in a humidified atmosphere of 5/95 (v/v) of CO₂/air at 37 C. The cells were passed for plated on 35 mm glass bottom poly-D-lysine coated Petri-dish for at least 24 h to enable adherence to the bottom.

Live cell imaging

HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM medium, Gibco) in a 35 mm glass bottom poly-D-lysine coated Petri-dish for at least 24 h to enable adherence to the bottom. The 200 µl of 100 µM Hanks' balanced salt solutions (HBSS) of MPFCP was added to the dish (final concentration of MPFCP is 20µM). After incubation at 37°C for 30 min, the cells were washed three times with PBS solutions. **CLSM** images were obtained using Olympus confocal laser scanning microscopy (Olympus Fluoview FV1000).

2. Synthesis and characterization of MPFCP



Scheme S1 synthetic Scheme for **Compound 3**

(i) 5-Bromoisophthalic acid, DMF, 0 °C, EDCI, 0.5 h; compound 1, HOBt, rt, 24 h; (ii) [Pd(dppf)₂Cl₂], KOAc, bis(pinacolato)diboron, DMF, 90 °C, overnight.

5-bromo-N¹,N³-di(2,5,8,12,15,18-hexaoxonadecan-10-yl)isophthalamide(2)

5-Bromoisophthalic acid (774 mg, 3.161 mmol) was dissolved in 100 mL of dry N,N-dimethylformamide (DMF), EDCI (1.5 g, 7.586 mmol) was added, and the mixture was stirred at 0°C for 30 min. Compound **1** (2.3 g, 7.651 mmol), HOBt (1.2 g, 7.586 mmol) were added and the mixture was stirred at room temperature for 24 hours. The solvents were removed by reduced pressure, water was added to the reaction mixture, followed by extraction with CH₂Cl₂. The combined organic phase was washed with water, dried over Na₂SO₄ and evaporated. The mixture was dried in a vacuum. The crude product was purified by silica gel column chromatography with CH₂Cl₂/CH₃CH₂OH (25:1) as eluent. After the solvent was removed by rotary evaporation, compound **2** was obtained as an oil liquid (1.39 g, 60%). The ¹H-NMR spectrum of compound **2** is shown in Fig.S6. ¹H-NMR (600MHz,CDCl₃): δ 8.21(s, 1H), 8.15(s, 2H), 7.25(d, J=7.8Hz, 2H), 4.47 (m, 2H), 3.76 (m, 4H), 3.71-3.64 (m, 28H), 3.54 (s, 8H), 3.30(s, 12H).

N¹,N³-di(2,5,8,12,15,18-hexaoxonadecan-10-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isophthalamide (3)

A mixture of **2** (1.20 g, 1.610 mmol), KOAc (838.2 mg, 8.540 mmol), and bis(pinacolato)diboron (614.4 mg, 2.420 mmol) in dry DMF (23 mL) was placed in a 100 mL flask. After the mixture was stirred for 10 min, Pd(dppf)₂Cl₂ (114.3mg, 0.140mmol) was added quickly. The mixture was stirred overnight at 90°C. After cooling to room temperature, the mixture was poured into water and extracted with dichloromethane (3 × 60 mL). The organic layer was washed with water (3 × 100 mL) and then dried over anhydrous Na₂SO₄. After the solvent was removed, the residue was purified by silica gel column chromatography (dichloromethane/ethanol = 20:1) to give compound **3** as a oil liquid (0.90 g, 66%).

67.4, 66.7, 61.2, 60.7, 53.7, 53.5, 25.6, 20.9, 14.0; Electrospray ionization mass spectrum is shown in Fig.S8. m/z Calcd for $C_{32}H_{43}N_2O_{11}$:631.28614, found:631.28590[M+H]⁺.

Compound 5b

Compound **4b** (3.00g, 5.10mmol) was dissolved with stirring in 45 mL of dry DMF, The mixture was cooled in an ice bath and phosphorus oxychloride (11 mL) was added dropwise. The reaction mixture turned black almost immediately. Keep the room temperature for 30 minutes, The reaction mixture was stirred at 45 °C for 20 h. After cooling to room temperature, The reaction mixture was dissolved in 20 mL dichloromethane and poured onto crushed ice mixed with aqueous $NaHCO_3$. The aqueous layer was extracted with dichloromethane (5×20 mL). The combined organic extracted with anhydrous Na_2SO_4 , filtered and avaporated. The residue was purified by silica gel column chromatography (cyclohexane/ethyl acetate = 3:1) to give Compound **5b** as a white solid (2.59 g, 79%). The 1H -NMR spectrum of **5b** is shown in Fig.S9. 1H -NMR(600MHz, $CDCl_3$): δ 9.82(s, 2H), 7.42(d, $J=8.4$ Hz, 2H), 7.39(s, 2H), 6.80(d, $J=8.4$ Hz, 2H), 4.34(s, 4H), 4.24(s, 8H), 4.10(m, 8H), 1.18(t, $J=6.6$ Hz, 12H); Electrospray ionization mass spectrum is shown in Fig.S10. m/z Calcd for $C_{32}H_{41}N_2O_{12}$:645.26540, found:645.26523[M+H]⁺.

Compound 6a

To a solution of **5a** (1.20g, 1.90mmol) and 2,4-Dimethylpyrrole (543mg, 5.71mmol) in dry dichloromethane (DCM) (318 mL), 3 drop of trifluoroacetic acid (TFA) was added. The reaction mixture was stirred at room temperature under nitrogen for 12 h. DDQ (520 mg) was added in the mixture was stirred for 40 min at room temperature under nitrogen. Then triethylamine (10.0 mL) and borontrifluoride etherate ($BF_3 \cdot Et_2O$) (10.0 mL) were added and the mixture was stirred for 40 additional min. Water was added and the product extracted with CH_2Cl_2 , the organic phase was dried over Na_2SO_4 and the solvent was evaporated. The crude product was purified by silica gel column chromatography (dichloromethane/methanol = 75:1-50:1) to give compound **6a** as a drab solid (0.64 g, 40%). The 1H -NMR spectrum of **6a** is shown in Fig.S11. 1H -NMR (600MHz, $CDCl_3$): δ 6.91(d, $J=7.8$ Hz,

1H), 6.81(t, J=4.8Hz, 3H), 6.71(t, J=8.4Hz, 2H), 6.00(s, 2H), 4.29(m, 4H), 4.23(s, 4H), 4.15(t, J=7.2Hz, 4H), 4.11(s, 4H), 4.10(t, J=7.2Hz, 4H), 2.57(s, 6H), 2.26(s, 3H), 1.51(s, 6H), 1.23-1.19(m, 12H); The ¹³C-NMR spectrum of **6a** is shown in Fig.S12. ¹³C-NMR(150MHz,CDCl₃):δ 171.4, 171.3, 155.3, 150.8, 150.3, 143.1, 141.6, 140.2, 137.1, 132.3, 131.7, 128.1, 122.3, 121.2, 121.1, 119.8, 119.1, 115.4, 113.2, 67.6, 67.2, 60.9, 60.5, 53.7, 53.6, 20.9, 14.6, 14.2, 14.1; MALDI-TOF spectrum is shown in Fig.S13. MALDI-TOF MS m/z Calcd for C₄₄H₅₅BF₂N₄O₁₀:848.40, found:848.70[M+H]⁺.

Compound 6b

Prepared analogously to **6a**, The residue was purified by silica gel column chromatography (dichloromethane/methanol = 150:1-100:1) to give compound **6b** as a brown solid (0.11 g, 5%). The ¹H-NMR spectrum of **6b** is shown in Fig.S14. ¹H-NMR(600MHz,CDCl₃):δ 6.93(d, J=8.4Hz, 2H), 6.83(d, J=7.8Hz, 2H), 6.81(s, 2H), 5.99(s, 4H), 4.26(s, 4H), 4.19(s, 8H), 4.12(m, 8H), 2.57(s, 12H), 1.48(s, 12H), 1.22(t, J=7.2Hz, 12H); The ¹³C-NMR spectrum of **6b** is shown in Fig.S15. ¹³C-NMR(150MHz,CDCl₃):δ 171.0, 155.4, 150.8, 143.0, 141.3, 140.4, 131.6, 128.3, 121.6, 121.1, 119.5, 114.0, 67.6, 60.8, 53.8, 14.6, 14.5, 14.2; MALDI-TOF spectrum is shown in Fig.S16. MALDI-TOF MS m/z Calcd for C₅₆H₆₆B₂F₂N₆O₁₀:1080.50, found:1080.50[M+H]⁺.

Compound 7a

To a solution of **6a** (600mg, 0.71mmol) in methanol/DCM (75 mL/25 mL), iodine monochloride (1.4 mL, 1M) in dichloromethane was added dropwise. The reaction mixture was stirred at room temperature for 5 min. After confirming the consumption of starting material by TLC, water was added and the product was extracted with DCM. The organic layer was washed with water and saturated NaCl aq., dried over Na₂SO₄ and the solvent was evaporated. The residue was purified by silica gel column chromatography (dichloromethane/methanol = 160:1-120:1) to give compound **7a** as a red solid (778 mg, 91%). The ¹H-NMR spectrum of **7a** is shown in Fig.S17. ¹H-NMR (600MHz, CDCl₃): δ 6.92(d, J=8.4Hz, 1H), 6.89(d, J=8.4Hz, 1H), 6.76(s, 2H), 6.73(t, J=8.4Hz, 2H), 4.31(d, J=4.8Hz, 2H), 4.27(d, J=4.8Hz, 2H), 4.24(s, 4H), 4.16(s, 4H), 4.14-4.08(m, 8H), 2.65(s, 6H), 2.28(s,

3H), 1.51(s, 6H), 1.23(m, 12H); The ^{13}C -NMR spectrum of **7a** is shown in Fig.S18. ^{13}C -NMR(150MHz,CDCl₃): δ 171.2, 171.1, 156.6, 150.9, 150.2, 145.4, 141.3, 140.7, 136.9, 132.5, 131.6, 127.5, 122.4, 121.0, 119.9, 119.2, 115.4, 113.0, 85.5, 67.6, 67.2, 62.8, 61.0, 60.6, 53.7, 20.9, 17.2, 16.0, 14.2, 14.1; MALDI-TOF spectrum is shown in Fig.S19. MALDI-TOF MS m/z Calcd for C₄₄H₅₃BF₂I₂N₄O₁₀:1100.19, found:1110.50[M+H]⁺.

Compound 7b

Prepared analogously to compound **7a** as a red solid (compound **7b**)(50 mg, 73%). The ^1H -NMR spectrum of **7b** is shown in Fig.S20. ^1H -NMR(600MHz, CDCl₃): δ 6.95(d, J=7.8Hz, 2H), 6.79(d, J=8.4Hz, 4H), 4.26(s, 4H), 4.20(s, 8H), 4.15(m, 8H), 2.66(s, 12H), 1.51(s, 12H), 1.24(t, J=7.2Hz, 12H); The ^{13}C -NMR spectrum of **7b** is shown in Fig.S21. ^{13}C -NMR(150MHz,CDCl₃): δ 170.9, 156.7, 151.0, 145.3, 141.1, 140.9, 131.6, 127.8, 121.5, 119.7, 113.7, 85.6, 67.8, 60.9, 53.8, 17.2, 16.0, 14.2; MALDI-TOF spectrum is shown in Fig.S22. MALDI-TOF MS m/z Calcd for C₅₈H₇₇BrN₂O₂₀:1584.08, found:1607.00[M+Na]⁺.

Compound 8a

In a 100 mL flask, **7a** (60mg, 0.0545mmol), **3** (125mg, 0.1636mmol) and Pd(PPh₃)₄ (2 mg) were dissolved in freshly distilled toluene (30 ml). An aqueous solution of K₂CO₃ (1.5 ml, 2M), ethanol(1.5 ml) was added and the mixture was heated at 65°C under nitrogen for 15 h. After cooling to room temperature, water was added and the product was extracted with CH₂Cl₂, the organic phase was dried over Na₂SO₄ and the solvent was evaporated. The residue was purified by column chromatography on silica gel (dichloromethane/methanol = 35:1) to give compound **8a** as a red viscous liquid (124 mg, 80%). The ^1H -NMR spectrum of **8a** is shown in Fig.S23. ^1H -NMR(600MHz, CDCl₃): δ 8.24(s, 2H), 7.84(s, 4H), 7.26(d, J=8.4Hz, 4H) 6.93(d, J=7.8Hz, 1H), 6.88(t, J=9.6Hz, 2H), 6.78(d, J=8.4Hz, 1H), 6.69(s, 2H), 4.49(m, 4H), 4.30(d, J=6.6Hz, 4H), 4.20(s, 4H), 4.10(s, 4H), 4.09(m, 8H), 3.77(m, 8H), 3.70-3.64(m, 56H), 3.52(t, J=4.8Hz, 16H), 3.33(s, 24H), 2.53(s, 6H), 2.24(s, 3H), 1.44(s, 6H), 1.19(m, 12H); The ^{13}C -NMR spectrum of **8a** is shown in Fig.S24. ^{13}C -NMR(150MHz, CDCl₃): δ 171.1, 166.4,

166.1, 154.0, 151.0, 150.3, 142.7, 140.4, 139.8, 135.0, 134.7, 132.2, 132.1, 131.6, 130.3, 128.7, 127.9, 125.5, 124.1, 122.2, 121.1, 119.9, 119.3, 115.3, 113.0, 71.9, 70.6, 70.5, 69.5, 67.7, 67.3, 60.9, 60.6, 58.9, 49.4, 49.3, 29.7, 20.9, 14.2, 14.1, 13.3, 13.1; MALDI-TOF spectrum is shown in Fig.S25. MALDI-TOF MS m/z Calcd for $C_{112}H_{171}BF_2N_8O_{38}$:2285.18, found:2308.10[M+Na]⁺.

Compound 8b

Prepared analogously to compound **8a**, The residue was purified by Recycling preparative GPC with tetrahydrofuran (THF) as mobile phase to give a red viscous liquid (compound **8b**) (78 mg, 70%). The ¹H-NMR spectrum of **8b** is shown in Fig.S26. ¹H-NMR(600MHz, CDCl₃):δ 8.17(s, 4H), 7.82(s, 8H), 7.16(d, J=7.8Hz, 8H), 6.92(d, J=8.4Hz, 2H), 6.87(d, J=10.2Hz, 4H), 4.47(m, 8H), 4.28(s, 4H), 4.16(s, 8H), 4.07(m, 8H), 3.77-3.74(m, 16H), 3.70-3.63(m, 112H), 3.52(t, J=4.8Hz, 32H), 3.32(d, J=2.4Hz, 48H), 2.52(s, 12H), 1.45(s, 12H), 1.17(t, J=7.2Hz, 12H); The ¹³C-NMR spectrum of **8b** is shown in Fig.S27. ¹³C-NMR(150MHz, CDCl₃):δ 170.9, 166.2, 154.1, 151.0, 142.6, 140.4, 139.8, 134.9, 134.6, 132.2, 131.6, 127.9, 124.0, 107.9, 107.6, 106.3, 71.8, 70.6, 70.4, 69.5, 68.0, 60.8, 58.9, 49.3, 29.1, 25.6, 23.9, 14.2, 13.3, 13.1; MALDI-TOF spectrum is shown in Fig.S28. MALDI-TOF MS m/z Calcd for $C_{192}H_{298}B_2F_4N_{14}O_{66}$:3956.09, found:3975.94[M+Na]⁺

MPFCP-1

To a solution of **8a** (40 mg, 17.6 μmol) in methanol (4 mL), 0.1 M KOH aq. (12 mL) was added. The reaction mixture was stirred at room temperature for 12 h. The produce was dialyzed for three day against ultrapure water using Spectrumlabs dialysis membrane (molecular weight cutoff = 1000, Spectrum), then eluted through an Dowex® 50WX8-200 ion-exchange resin column (pre-washed with 18 Mohm Deionized water) to remove other cations. The solvents were removed by Freeze drying to give **MPFCP-1** as a red viscous liquid (34mg, 89%). The ¹H-NMR spectrum of **MPFCP-1** is shown in Fig.S29. ¹H-NMR(600MHz, DMSO-d₆):δ 8.42(d, J=7.8Hz, 4H), 8.35(s, 2H), 7.86(s, 4H), 7.21(s, 2H), 7.12(s, 2H), 7.04(s, 2H), 6.94(m, 2H), 6.65(t, J=7.8Hz, 2H), 4.31(m, 8H), 4.11(s, 4H), 3.98(s, 4H), 3.54-3.48(m, 80H), 3.19(d, J=7.8Hz, 24H), 2.46(s, 6H), 2.12(d, J=10.2Hz, 3H), 1.43(d, J=7.8Hz, 6H);

MALDI-TOF spectrum is shown in Fig.S30. MALDI-TOF MS m/z Calcd for $C_{104}H_{155}BF_2N_8O_{38}$:2174.05, found:2173.2[M-H].

MPFCP-2

To a solution of 8b (40 mg, 10 μ mol) in methanol (4 mL), 0.1 M KOH aq. (12 mL) was added. The reaction mixture was stirred at room temperature for 12 h. The produce was dialyzed for three day against ultrapure water using Spectrumlabs dialysis membrane (molecular weight cutoff = 1000, Spectrum, CA), then eluted through an Dowex® 50WX8-200 ion-exchange resin column (pre-washed with 18 Mohm Deionized water) to remove other cations. The solvents were removed by Freeze drying to give **MPFCP-2** as a red viscous liquid(28mg, 71%). The 1H -NMR spectrum of **MPFCP-2** is shown in Fig.S31. 1H -NMR(600MHz, DMSO- d_6): δ 8.41(d, J=7.8Hz, 8H), 8.33(s, 4H), 7.86(s, 8H), 7.01(s, 2H), 6.88(s, 2H), 4.30(m, 12H), 4.06(s, 4H), 3.53-3.47(m, 160H), 3.18(d, J=3.0Hz, 48H), 2.45(s, 12H), 1.44(s, 12H); MALDI-TOF spectrum is shown in Fig.S32. MALDI-TOF MS m/z Calcd for $C_{184}H_{282}B_2F_4N_{14}O_{66}$:3843.93, found:3842.9[M-H].

3 Supplementary Figures

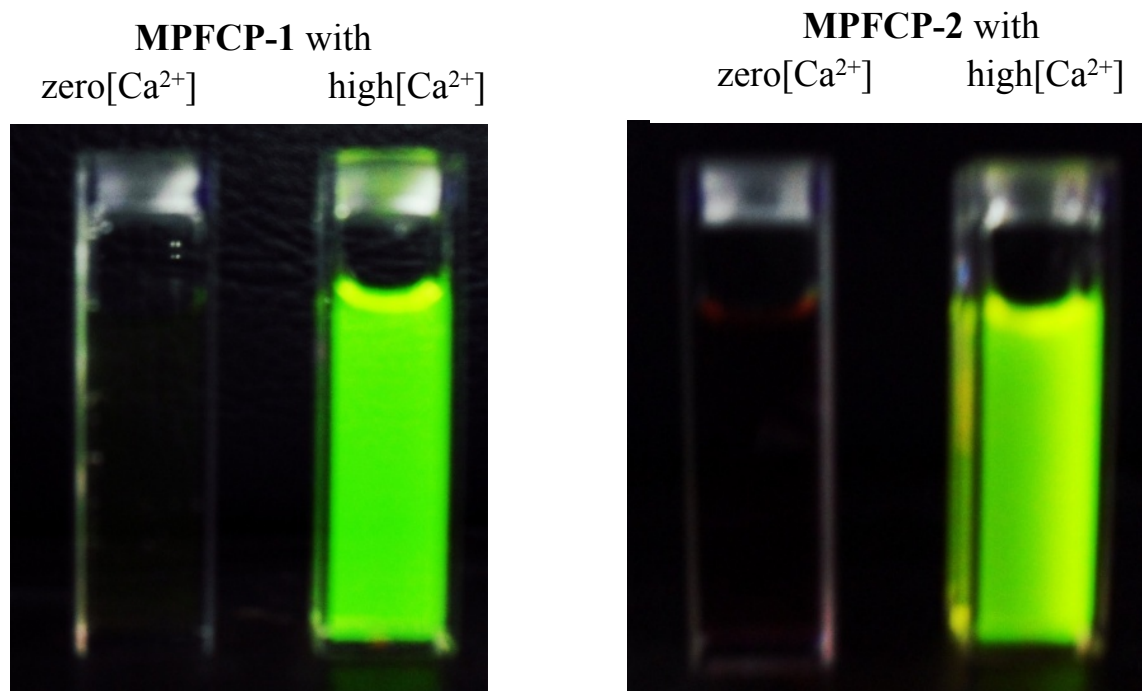


Fig.S1 MPFACP-1 and MPFACP-2 probe with Ca²⁺ detection

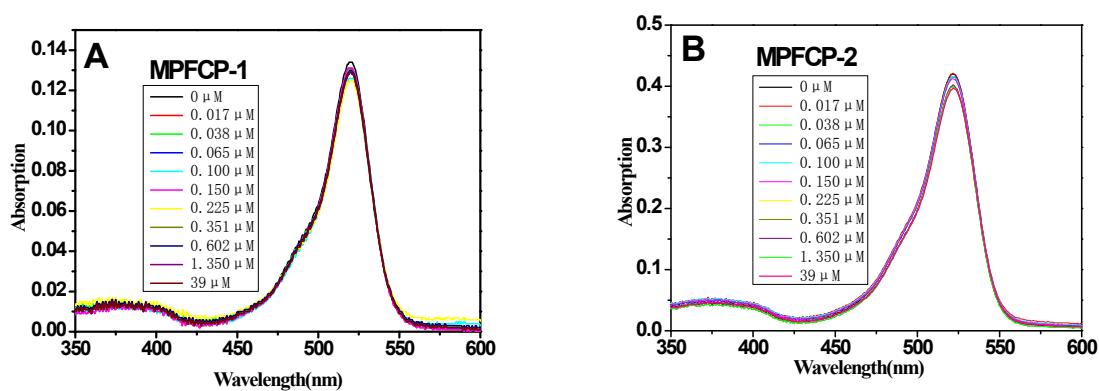


Fig.S2 Ca²⁺-UV-vis absorption spectra of MPFACP in the presence of free Ca²⁺ at various concentrations (0, 0.017, 0.038, 0.065, 0.100, 0.150, 0.225, 0.351, 0.602, 1.35, 39 μM) in 3-(N-morpholino)propanesulfonic acid (MOPS) buffer (30mM) containing KCl (100 mM) and ethyleneglycol tetraacetic acid (EGTA; 10mM) with the concentrations of probes 1 μM at pH 7.2 and 22 °C.

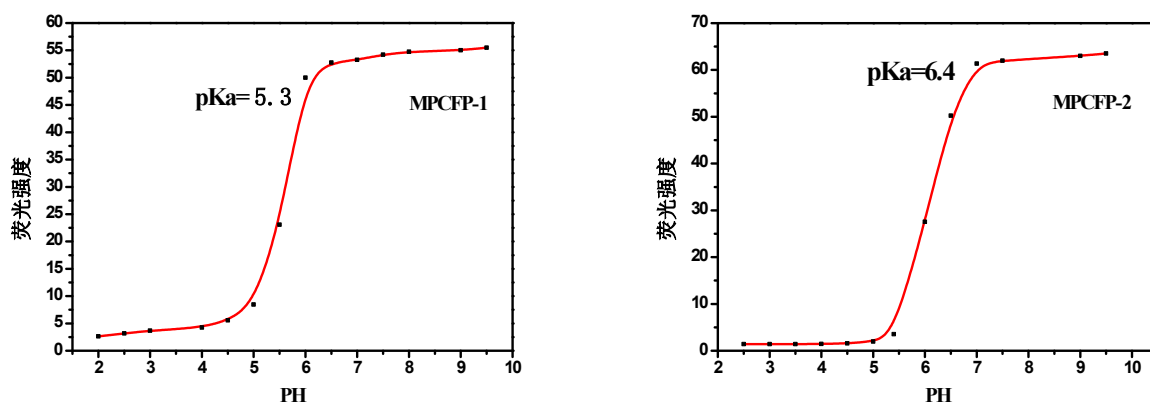


Fig.S3 pH-dependent fluorescence emission spectra of MPCFP-1 (A) and MPCFP-2 (B)

Table S1 K_d values and optical properties of MPCFP and some previously reported Ca^{2+} probes.

| Compound | λ_{max} | emission maximum | | quantum efficiency(ϕ) | | Fluorescence increase ($F_{\text{max}}/F_{\text{min}}$) | K_d (μM) |
|-----------------------|------------------------|------------------------|-------------------------|------------------------------|-------------------------|--|----------------------------|
| | (nm) | Ca^{2+} -free | Ca^{2+} -bound | Ca^{2+} -free | Ca^{2+} -bound | | |
| MPCFP-1 | 520 | 545 | 545 | 0.0018 ^a | 0.15 | 83 | 0.44 |
| | 2.6×10^4 | | | | | | |
| MPCFP-2 | 525 | 549 | 550 | 0.0013 ^a | 0.13 | 100 | 1.21 |
| | 8.0×10^4 | | | | | | |
| Fluo-4 | 491 | nd ^b | 516 | nd ^b | 0.14 | 120 | 0.35 |
| CalciumGreen-1 | 506 | 531 | 531 | nd ^b | 0.75 | 14 | 0.19 |

^a Estimated from the following calculation: $\phi_{\text{Ca}^{2+}\text{-free}} = \phi_{\text{Ca}^{2+}\text{-bound}} \times (F_{\text{min}}/F_{\text{max}})$. ^b No date.

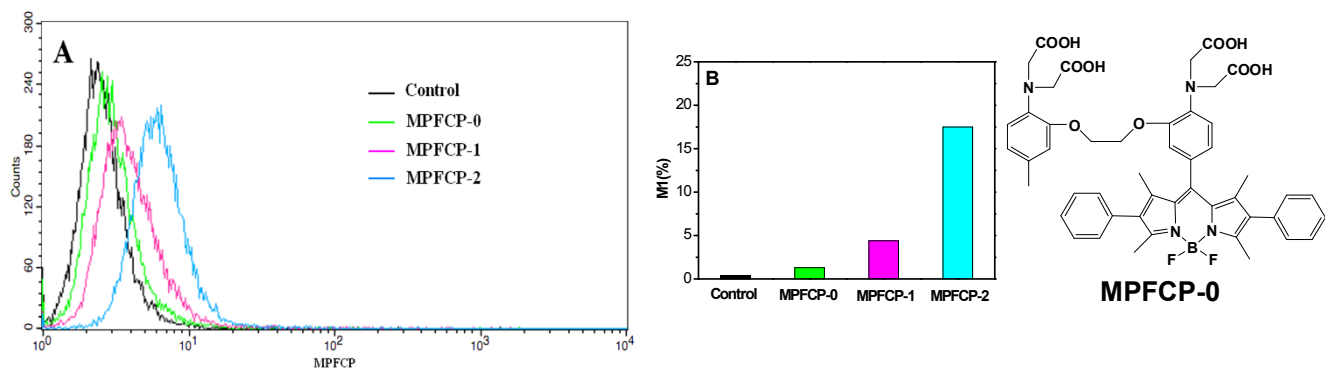


Fig.S4 Uptake of MPFCP into cell by flow cytometry. The cells were incubate with same concentration(20 μ M) of MPFCP for 2 h. The Black, green, magenta and cyan line were Control, MPFCP-0 (BODIPY alone), MPFCP-1 and MPFCP-2 respectively.

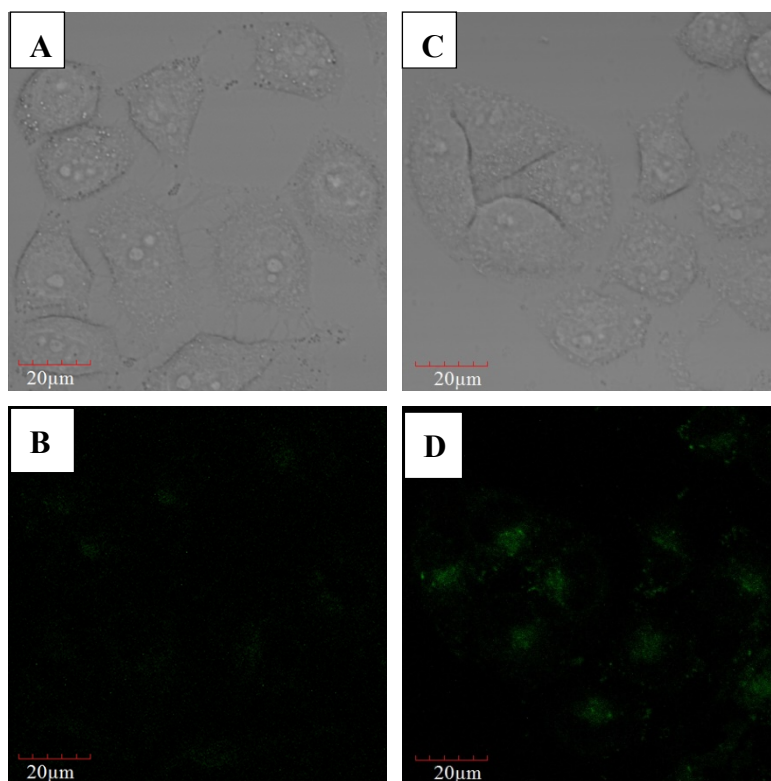


Fig.S5 The bright-field images of HeLa cells incubated by 20 μ M MPFCP-2 (A), and the confocal images of HeLa cells incubated by 20 μ M MPFCP-2 (B), the bright-field (C) and the confocal images (D) of HeLa cells incubated by 20 μ M MPFCP-2 with ATP (100 μ M).

Part B: ^1H -NMR spectrum, ^{13}C NMR spectrum and MALDI-TOF

spectrum

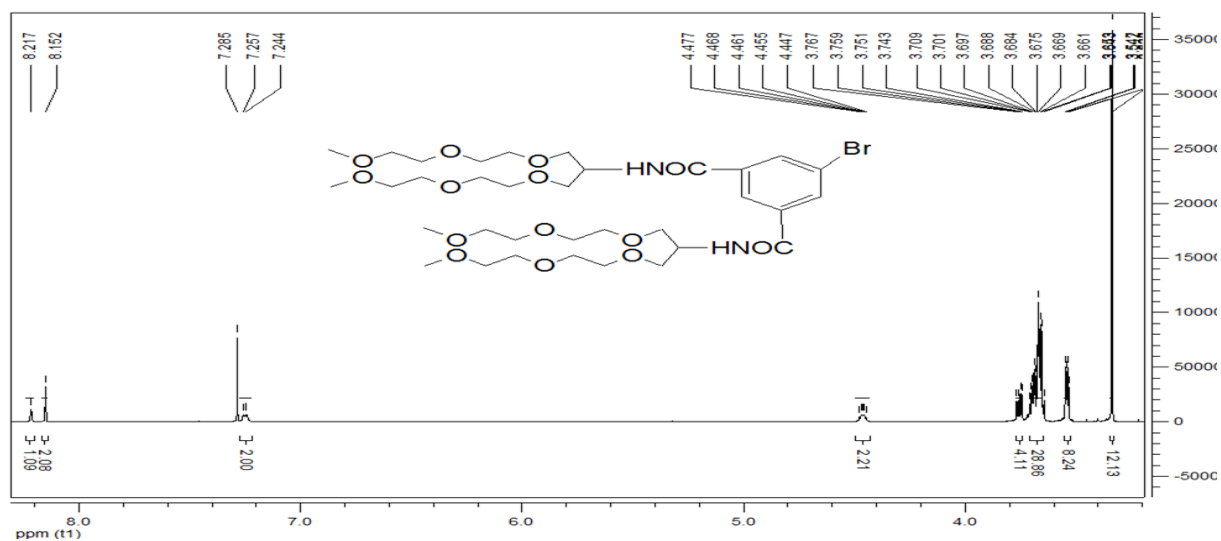


Fig.S6 ^1H -NMR spectrum(600MHz, CDCl_3 , 20 °C) of 2

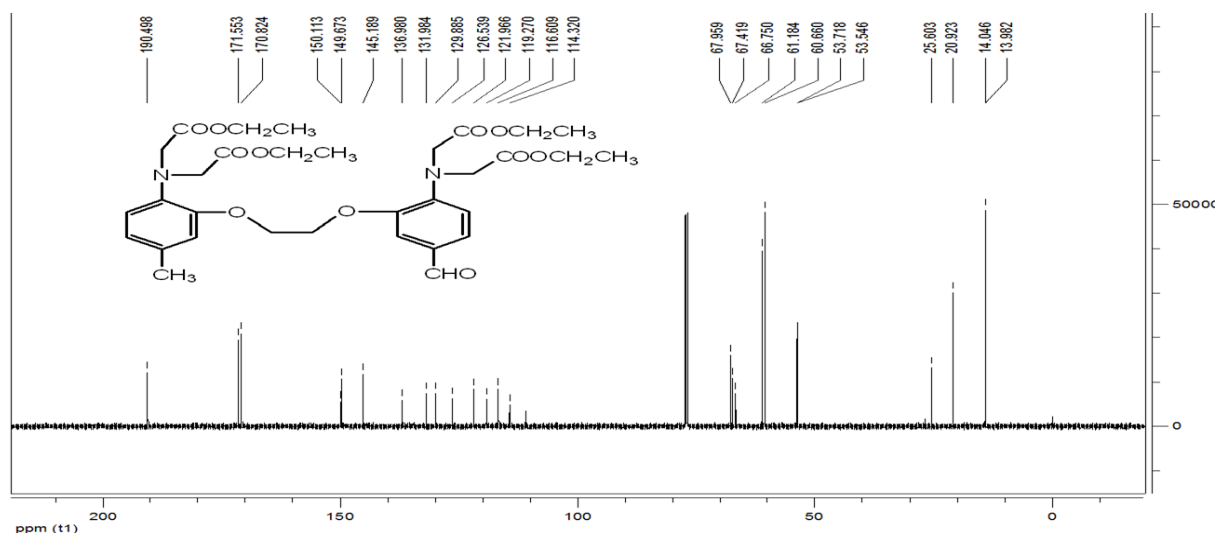


Fig.S7 ^{13}C -NMR spectrum(150MHz, CDCl_3 , 20 °C) of 5a

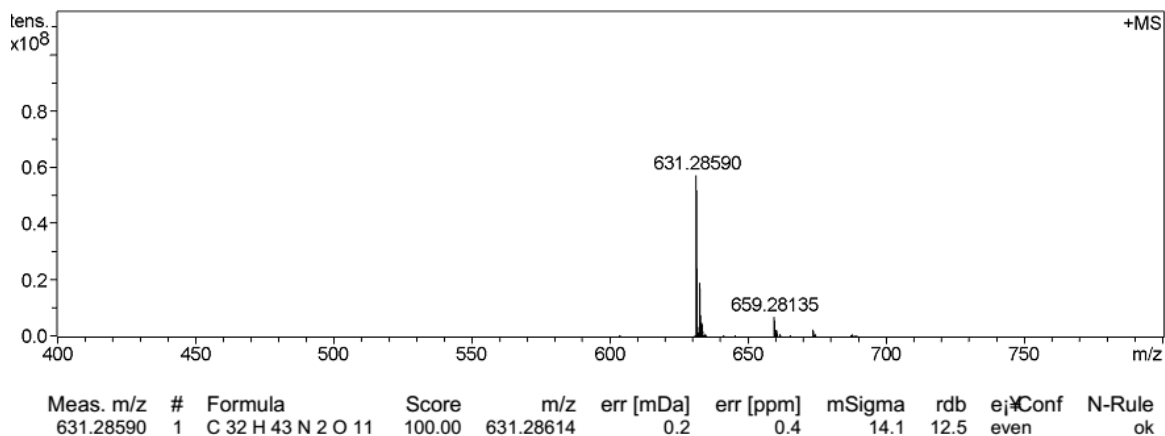


Fig.S8 Electrospray ionization mass spectrum of **5a**

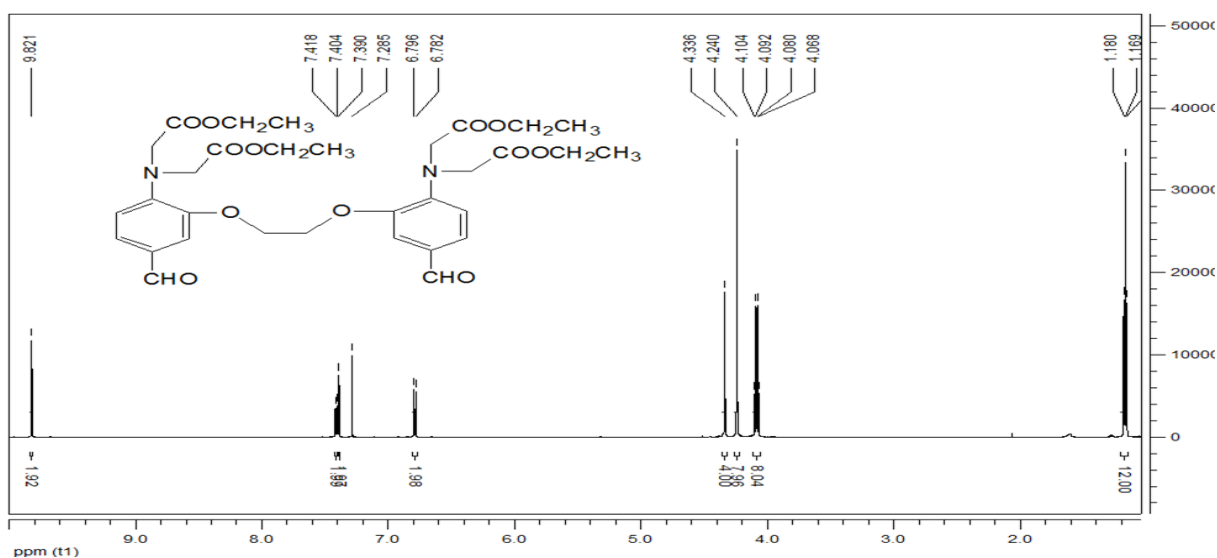


Fig.S9 ¹H-NMR spectrum(600MHz, CDCl₃, 20 °C) of **5b**

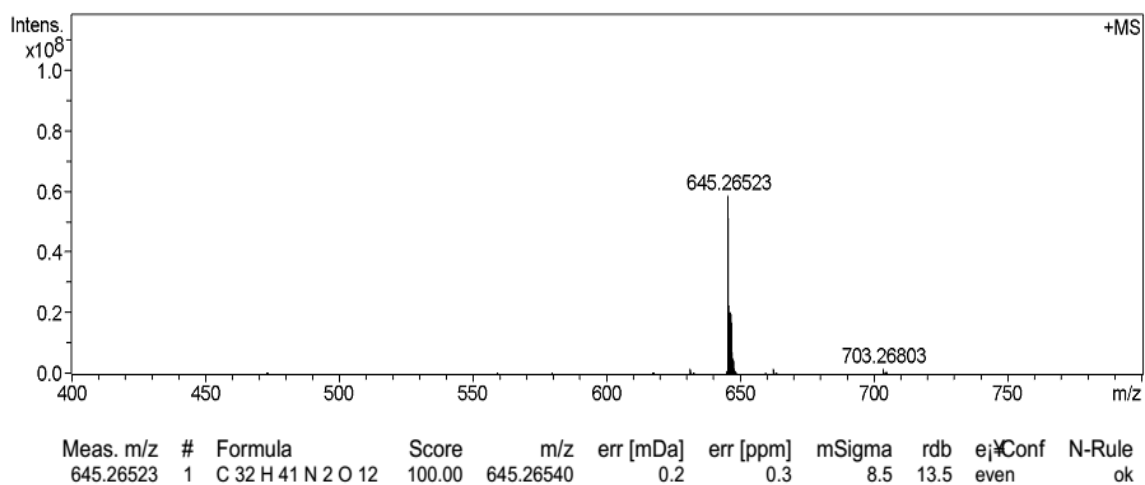
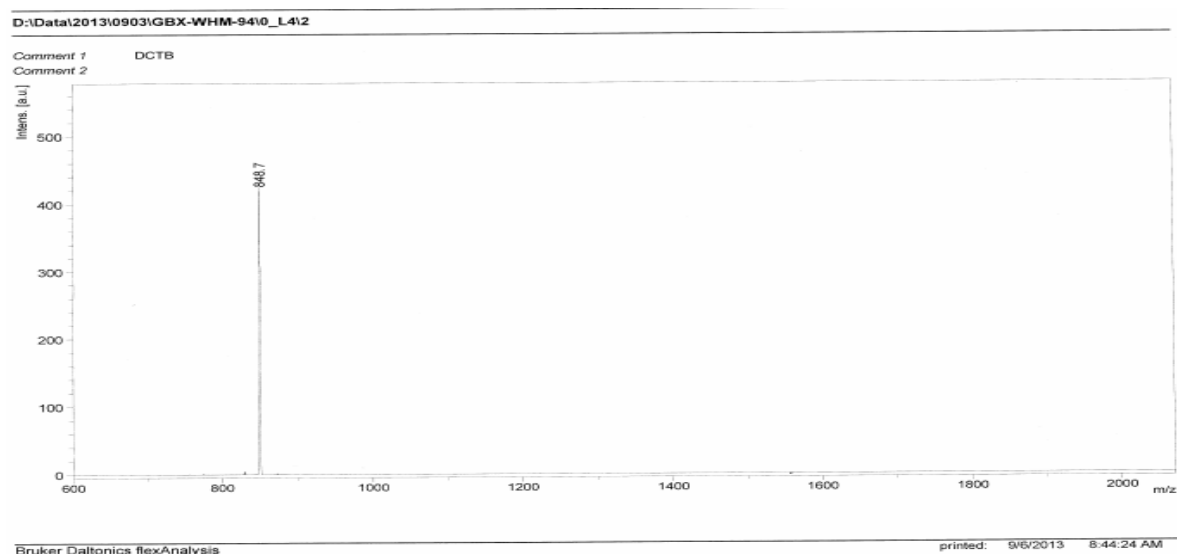
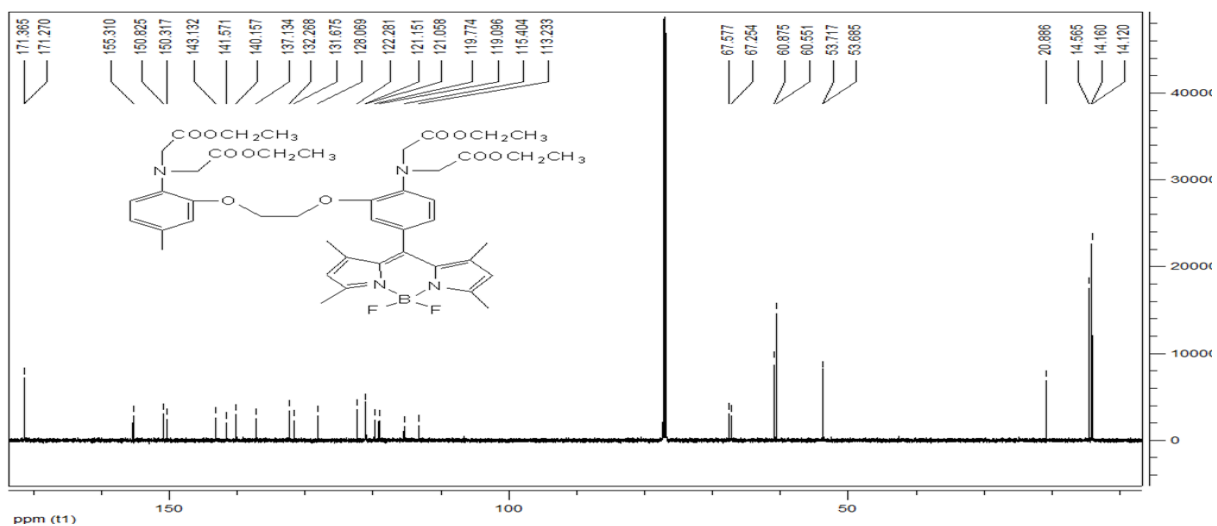
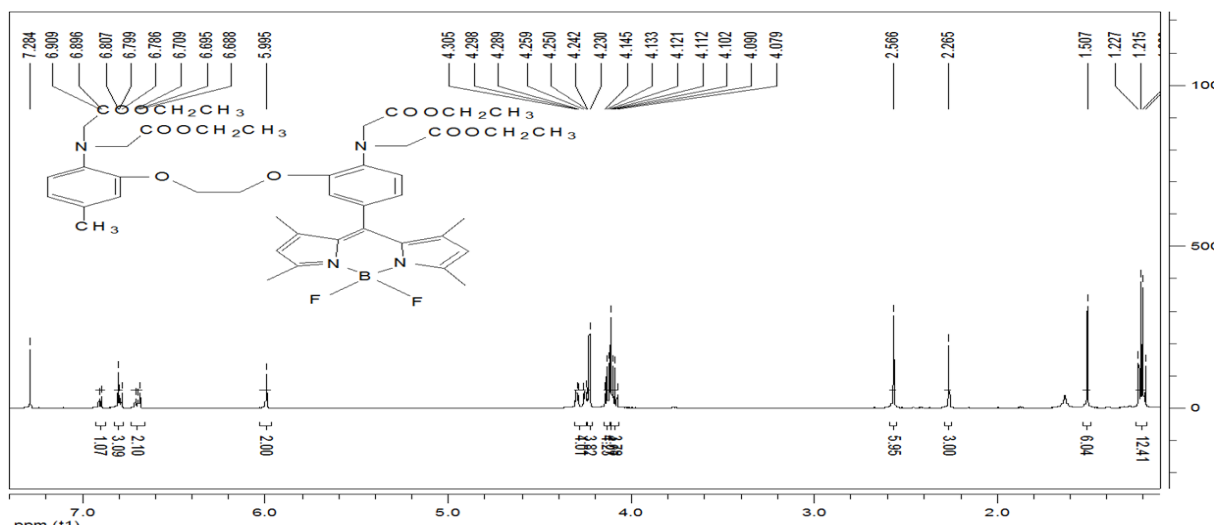


Fig.S10 Electrospray ionization mass spectrum of **5b**



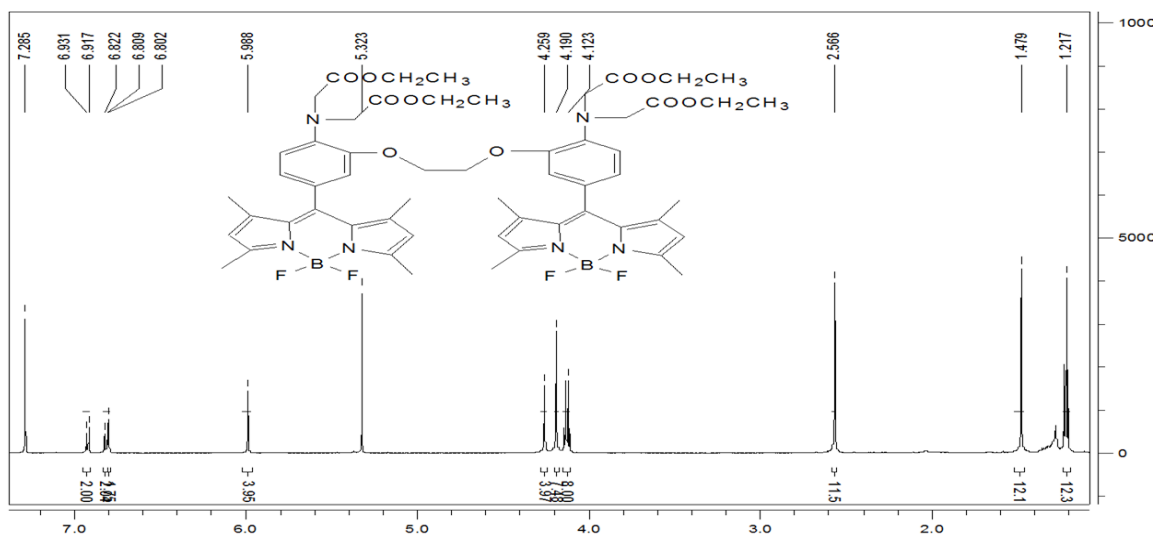


Fig.S14 $^1\text{H-NMR}$ spectrum(600MHz, CDCl_3 , 20 °C) of **6b**

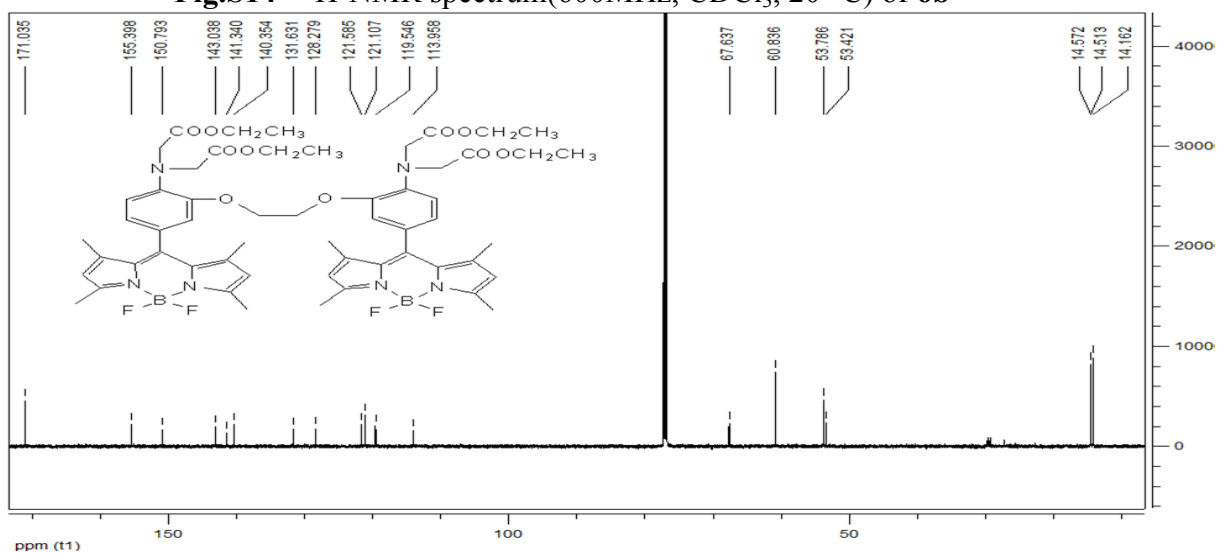


Fig.S15 $^{13}\text{C-NMR}$ spectrum(150MHz, CDCl_3 , 20 °C) of **6b**

D:\Data\2014\0221\GBX-WHM-104\0_A17\1

Comment 1 DCTB
Comment 2

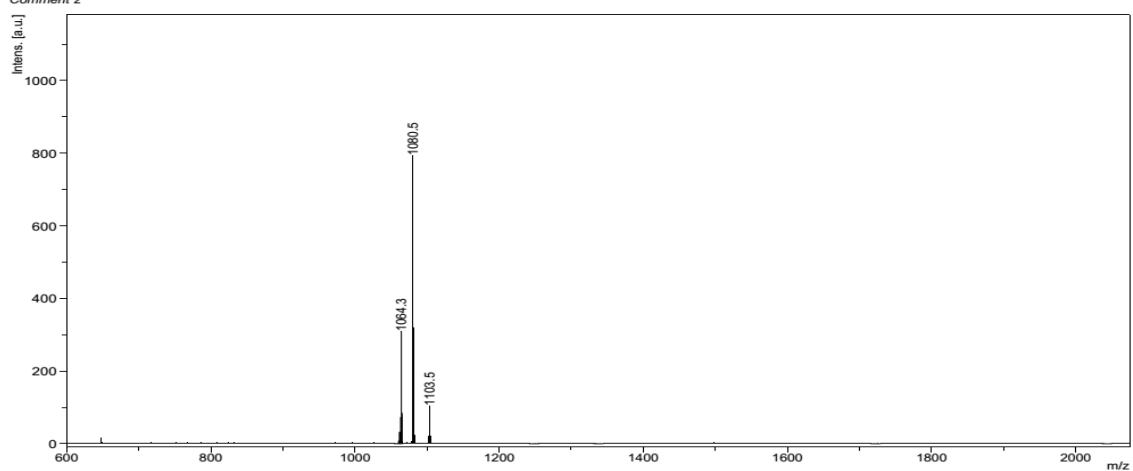


Fig.S16 MALDI-TOF spectrum of **6b**

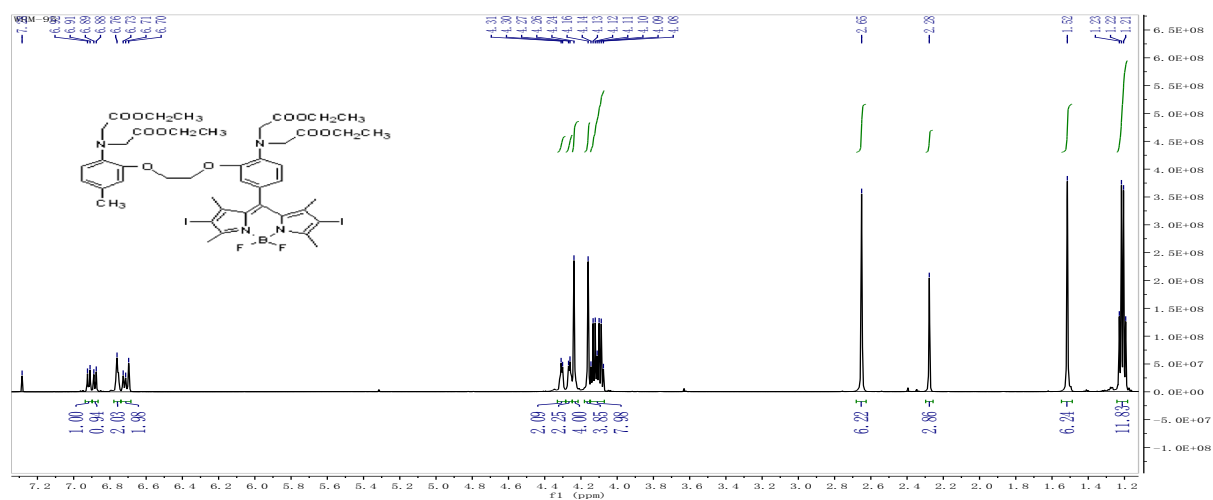


Fig.S17 ¹H-NMR spectrum(600MHz, CDCl₃, 20 °C) of 7a

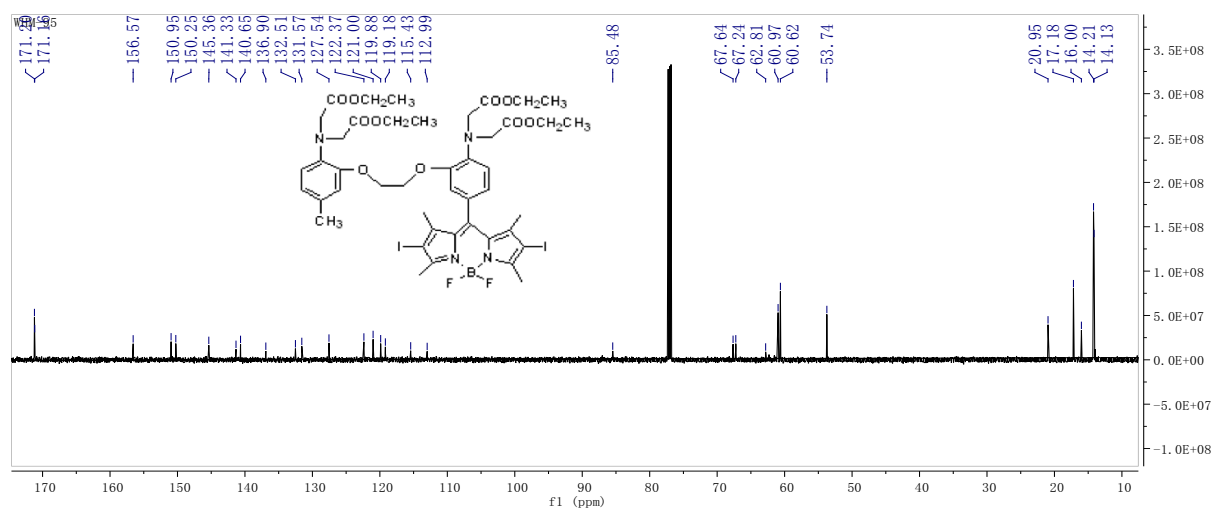


Fig.S18 ¹³C-NMR spectrum(150MHz, CDCl₃, 20 °C) of 7a

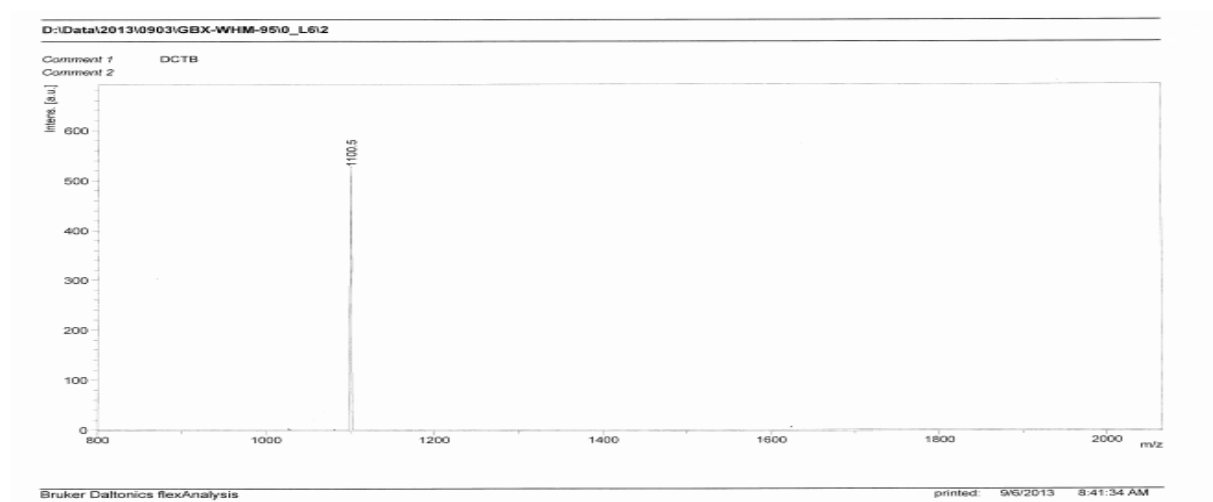


Fig.S19 MALDI-TOF spectrum of 7a

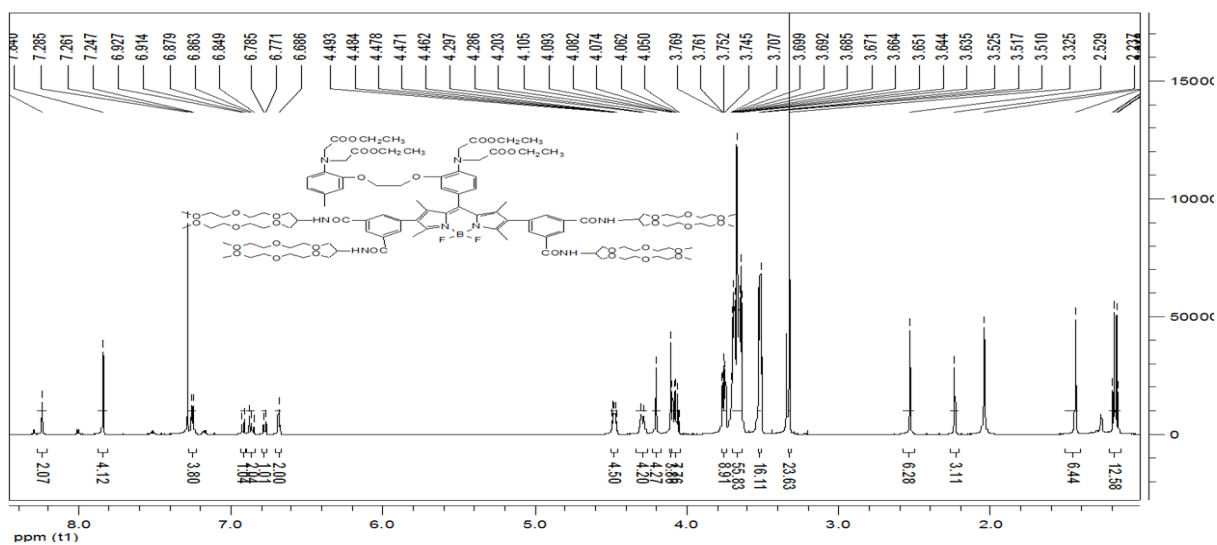


Fig.S23 $^1\text{H-NMR}$ spectrum(600MHz, CDCl_3 , 20 °C) of **8a**

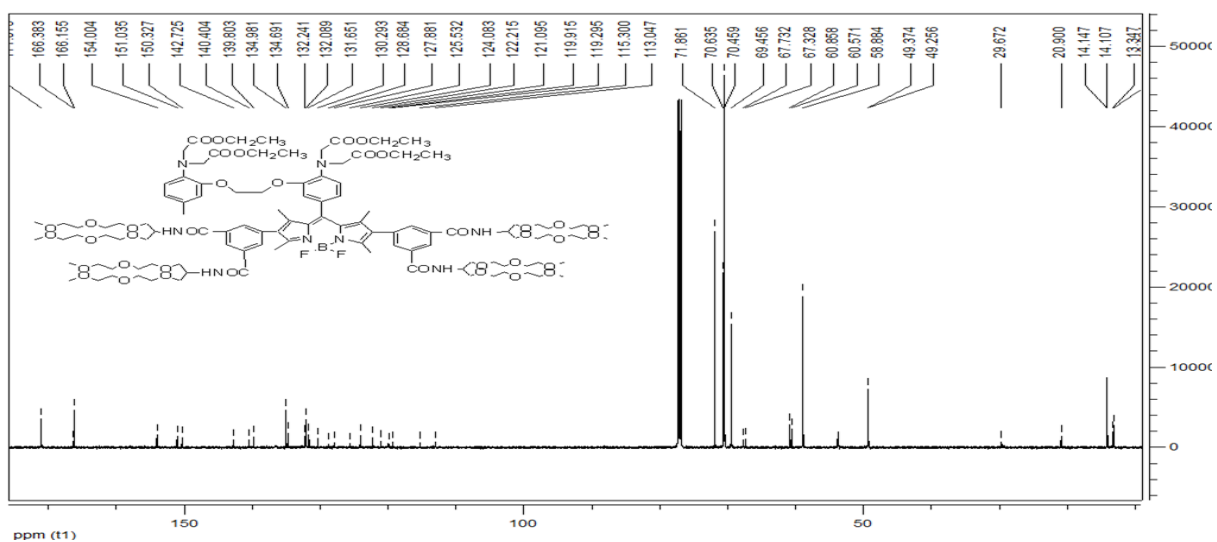


Fig.S24 $^{13}\text{C-NMR}$ spectrum(150MHz, CDCl_3 , 20 °C) of **8a**

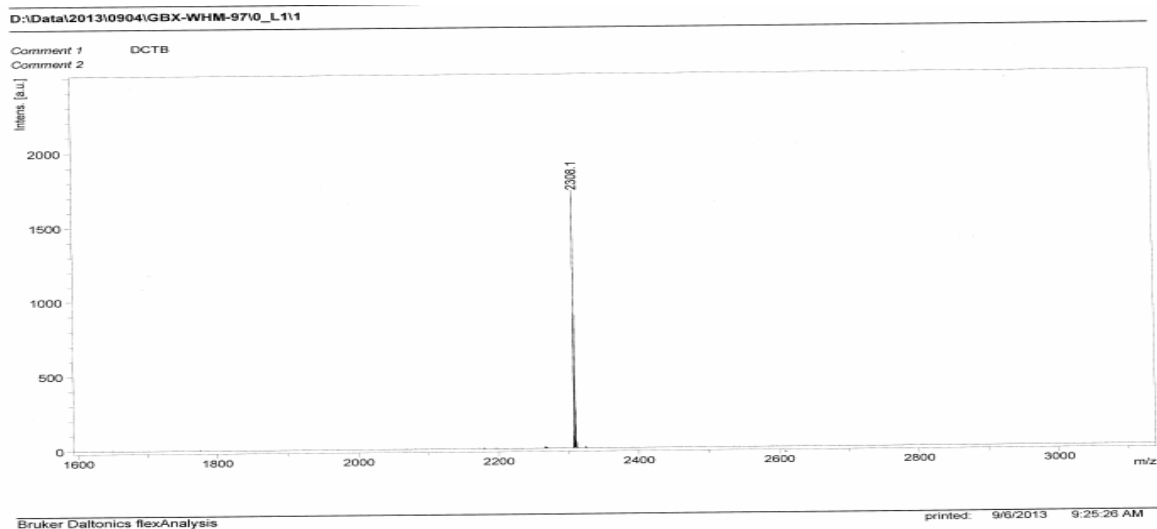
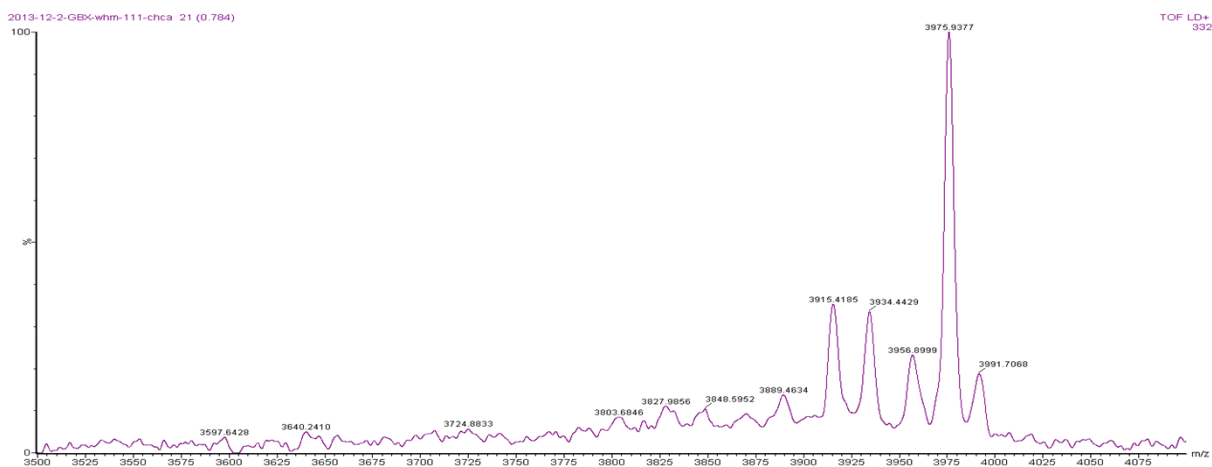
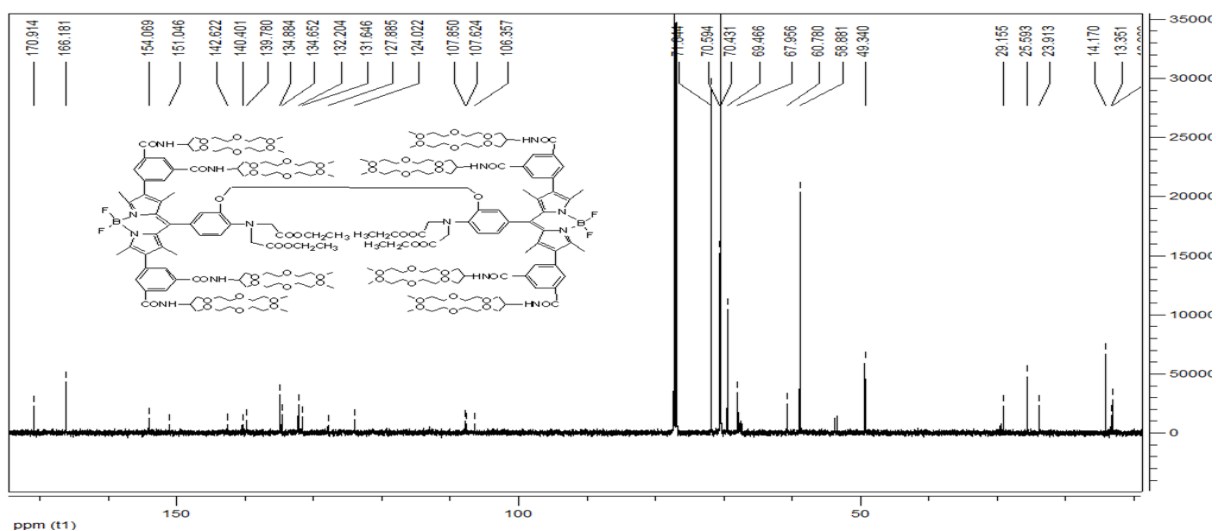
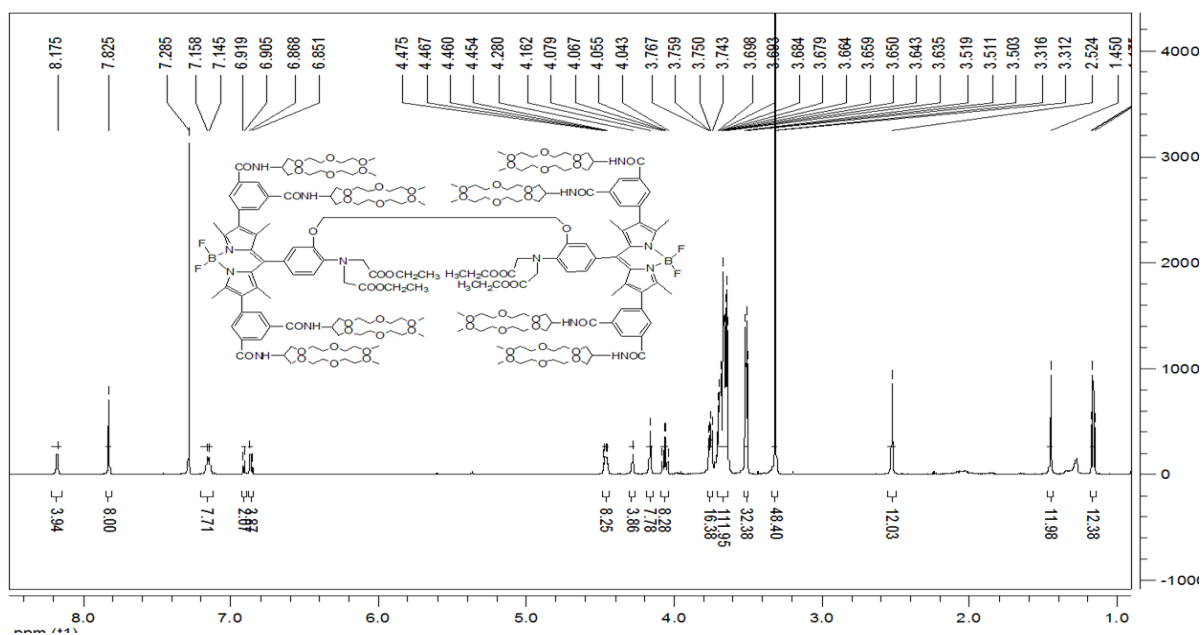


Fig.S25 MALDI-TOF spectrum of **8a**



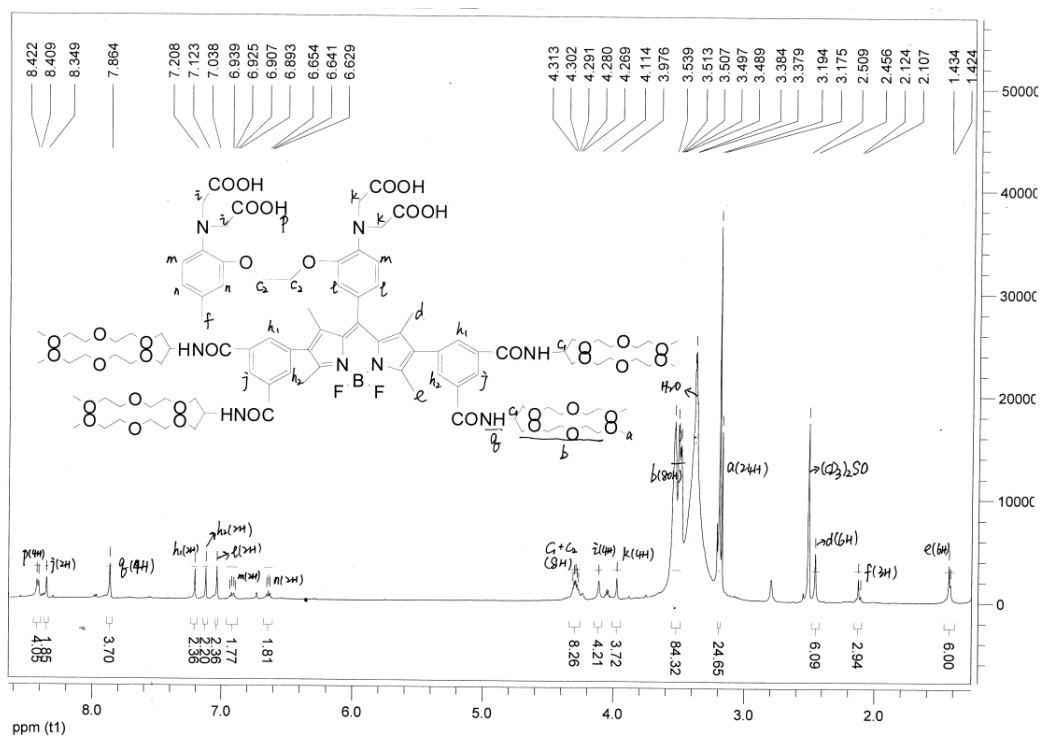


Fig.S29 $^1\text{H-NMR}$ spectrum(600MHz, DMSO- d_6 , 20 °C) of MPFCP-1

Comment 1 DCTB NEG
 Comment 2

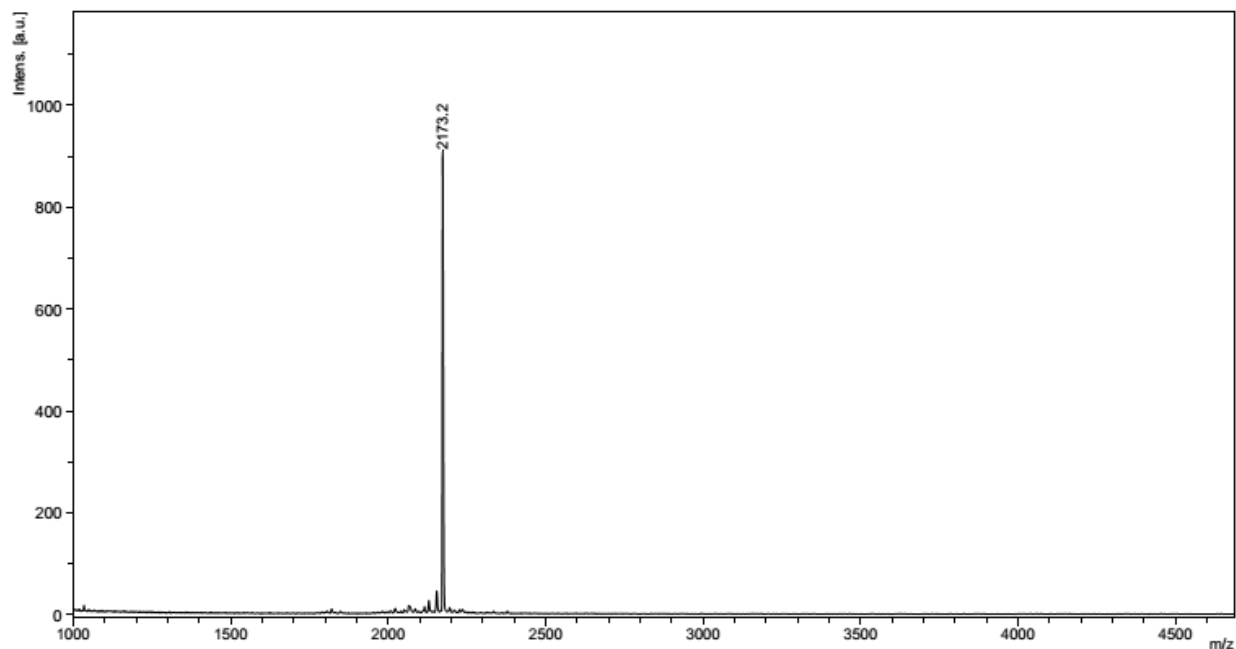


Fig.S30 MALDI-TOF spectrum of MPFCP-1

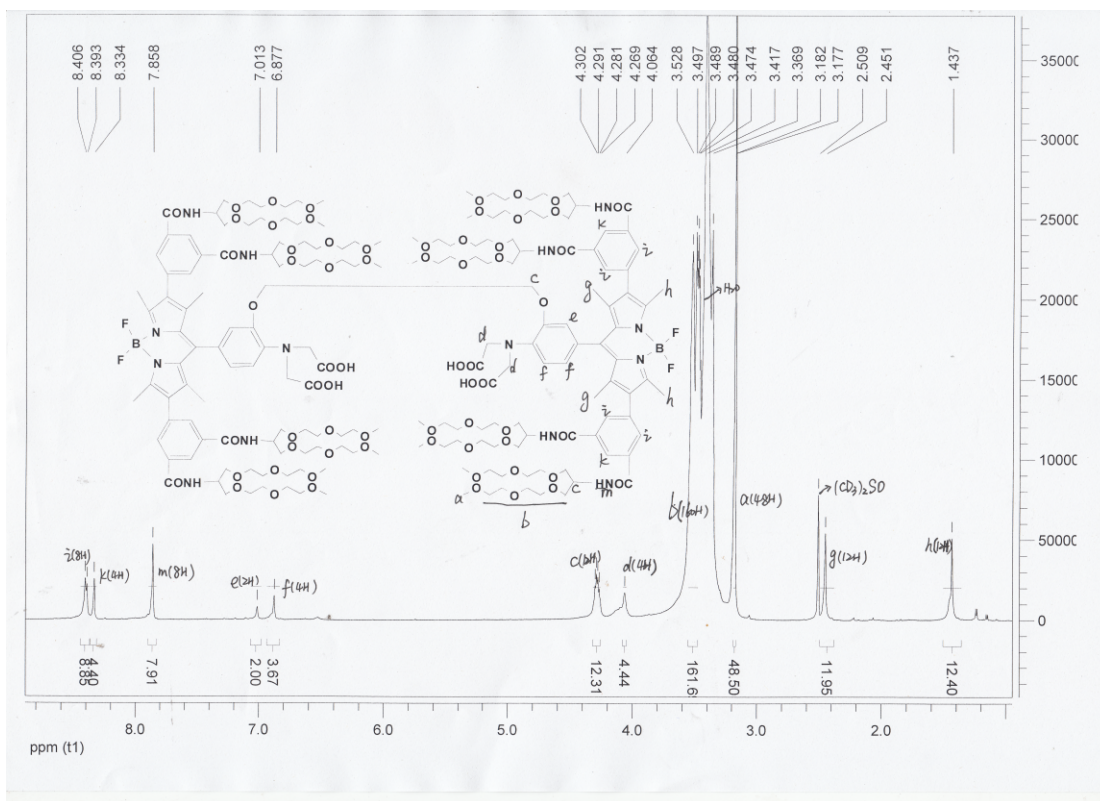


Fig.S31 ¹H-NMR spectrum(600MHz, DMSO-d₆, 20 °C) of MPFCP-2

Comment 1 DCTB NEG
 Comment 2

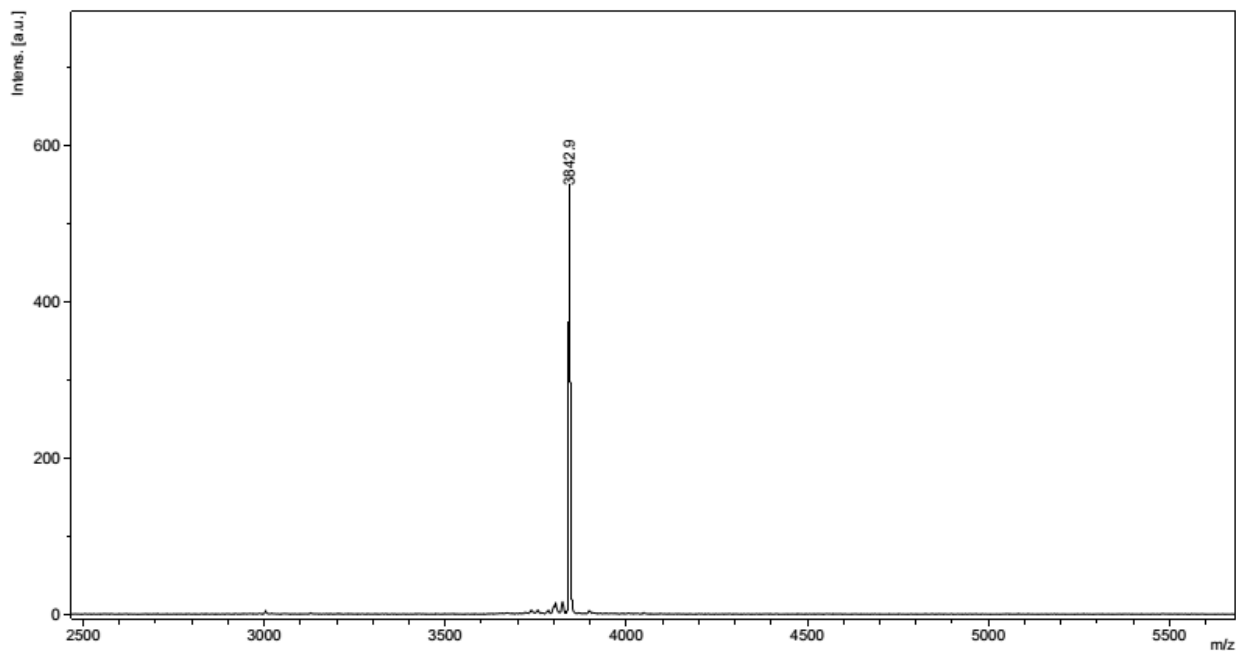


Fig.S32 MALDI-TOF spectrum of MPFCP-2