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Electronic Supplementary Information

Diketopyrrolopyrrole-based fluorescence probes for the imaging of lysosomal Zn^{2+} and identification of prostate cancer in human tissue

Chenchen Du,[‡a] Shibo Fu,[‡b] Xiaohua Wang,[a] Adam C. Sedgwick,[c] Wei Zhen,[a] Minjie Li,[a] Xinqiang Li,[d] Juan Zhou,[*b] Zhong Wang,[*b] Hongyu Wang,[*a] and Jonathan L. Sessler[*a,d]

[a] Department of Chemistry, College of Science, and Center for Supramolecular Chemistry & Catalysis, Shanghai University, 99 Shangda Road, Shanghai, 200444, P. R. China. E-mail: wanghy@shu.edu.cn

[b] Department of Urology, Shanghai Ninth People's Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, 200011, P. R. China. E-mail: zhoujuan0002@163.com and zhongwang2000@sina.com

[c] Department of Chemistry, The University of Texas at Austin, 105 East 24th Street, Stop A5300, Austin, Texas 78712-1224, United States. E-mail: sessler@cm.utexas.edu

[d] Pathology Department, First Affiliated Hospital of Zhengzhou University, 1 Jianshe East Road, Zhengzhou, Henan Province, P. R. China, 450052

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1. Materials and Instruments

All solvent and reagents used in the syntheses were purchased from commercial sources and were used without further purification. The starting material, 3,6-diphenyl DPP (**1**), was prepared based on our earlier reported synthetic procedure.[1] MeO-DPEN was synthesized accordingly to the procedure described by Cho, et al.[2]

^{1}H NMR and ^{13}C NMR spectra were recorded on a Brucker Avance III 500 MHz NMR spectrometer. Mass spectroscopic data were obtained at the Shanghai Institute of Organic Chemistry (SIOC) using either an HP5973 mass spectrometer or a GCMS-QP2010 SE (SHIMADZU) instrument. UV-Vis spectra were recorded on a Shimadzu UV-2501PC UV-Vis spectrophotometer. Fluorescence measurements were carried out using a Perkin-Elmer LS-55 spectrofluorophotometer using a xenon lamp as the light source. An excitation wavelength of 500 nm was used. All UV-Vis and photoluminescent (PL) spectra were collected at room temperature. A Mettler Toledo Seven Compact S220 PH meter was used for all pH measurements. Isothermal titration calorimetry (ITC) measurements were performed using a MicroCal VP-ITC.

Absorption and Fluorescence Spectra

UV-Vis and fluorescence experiments were carried out in four different solvent systems: Dichloromethane (DCM), EtOH, DMSO, and HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer (50 mM HEPES, 100 mM KCl, 10 mM EGTA (1,2-bis[2-[bis(carboxymethyl)amino]ethoxy]ethane), pH 7.2, 1% DMSO). To produce stock solutions for each probe, a solvent was chosen and used to produce a final concentration of 1.0 × 10^{-4} mol/L. This stock solution was then further diluted to 1 μM for the UV-Vis and fluorescence spectroscopic measurements. All recorded UV-Vis absorption and fluorescence spectra were determined at a concentration of 1 μM (3 mL volume) in a 1 cm standard quartz cell. For the spectroscopic measurements involving each probe in its Zn-free form, EGTA (10 μM) was added to remove residual metal ions in solution. For the spectroscopic studies of each probe in its Zn-bound form, Zn^{2+} (1 μM) was added in the form of the chloride anion salt to separate solutions of DCM, EtOH, and DMSO containing each probe (1 μM). Zn^{2+} (10 mM) was added to the HEPES buffer containing each probe (1 μM). For the Zn^{2+} titrations at pH 5 an acetate buffer was used.

Determination of fluorescence quantum yields

The fluorescence quantum yield (Φ_F) of each probe was determined using equation 1. Rhodamine 6G (Φ_F = 0.89 in CH_2Cl_2) was used as the standard.[3]

$$\Phi_{unk} = \Phi_{std} \frac{I_{unk}}{A_{unk}} \cdot \frac{A_{std}}{I_{std}} \cdot \frac{\eta_{unk}}{\eta_{std}}$$

Equation 1

where Φ = fluorescence quantum yield; I = integrated area under the corrected emission spectrum; A = absorbance at the excitation wavelength; η = refractive index of the solution (pure solvents were assumed). The subscripts 'unk' and 'std' refer to unknown and standard, respectively.

Determination of apparent dissociation constants

A series of HEPES buffer solutions (50 mM HEPES, 100 mM KCl, 10 mM EGTA, 1% DMSO) containing various amounts of $ZnSO_4$ (0.5~ 9.8 mM) were prepared. The free Zn^{2+} ion concentration ($[Zn^{2+}]_{free}$) was calculated, with the values for $K_{Zn\text{-}EGTA}^{app}$, $[EGTA]_{total}$, $[Zn^{2+}]_{total}$ derived either from the experiment or taken from the literature.[4,5]

For HEPES buffer (pH 7.2), $[Zn^{2+}]_{free}$ was calculated as follow:

$[Zn^{2+}]_{total}$ (mM)	0.50	1.00	2.00	3.00	4.00	4.99	5.99	6.98	7.98	8.97	9.47	9.8
$[Zn^{2+}]_{free}$ (nM)	0.14	0.29	0.65	1.1	1.8	2.6	3.9	6.1	10	23	47	127

For acetate buffer (pH 5), $[Zn^{2+}]_{free}$ was calculated as follow:

$[Zn^{2+}]_{total}$ (μM)	0.2	0.5	1.0	2.0	3.0	4.0	6.0	10.0	15.0	40.0	70.0	200.0
$[Zn^{2+}]_{free}$ (nM)	0.13	0.33	0.65	1.3	1.96	2.6	3.9	6.5	9.8	26.2	46	133

To determine the apparent dissociation constants (K_d) for the probe/Zn^{2+} complex, the fluorescence titration curves were obtained, and the dissociation constants were calculated by fitting to the Benesi-Hildebrand equation (Equation 2). The binding stoichiometry and detection limit were obtained from Hill plots (Equation 3).

$$\frac{F - F_{min}}{F_{max} - F_{min}} = \frac{[Zn^{2+}]_{free}}{K_d + [Zn^{2+}]_{free}}$$

Equation 2 - Benesi-Hildebrand equation

$$\log\left(\frac{F - F_{min}}{F_{max} - F}\right) = n \log[Zn^{2+}]_{free} + \log K_a$$

Equation 3 - Hill equation

where F = fluorescence intensity, F_{max} = maximum fluorescence intensity of the probe in HEPES buffer with Zn^{2+} F_{min} = fluorescence intensity of the probe in HEPES buffer without Zn^{2+}. K_d, K_a, and n are the dissociation constant, association constant, and the Hill coefficient, respectively.

Job Plots

A series of solutions were prepared containing different mole fractions of the probe in question and Zn^{2+} in HEPES buffer (50 mM HEPES, 100 mM KCl, pH 7.2, 1% DMSO) with the total concentration of the probe and Zn^{2+} being maintained at 1 μM.

Isothermal Titration Calorimetry (ITC) Titrations

ITC titrations were carried out in absolute ethanol at 25 °C. The concentration of each probe and Zn^{2+} were kept at 0.05 mM and 0.5 mM, respectively. Titration data were fitted to determine the stoichiometry (n), the equilibrium association constant (K_a), the apparent association enthalpy (ΔH), and the entropy (ΔS).

Zn^{2+} Selectivity

Stock solutions containing various metal ions in H_2O were prepared using NaCl, $CaCl_2$, $MgCl_2 \cdot 6H_2O$, $Mn(OAc)_2$, $FeSO_4$, $CoCl_2 \cdot 6H_2O$, $NiCl_2 \cdot 6H_2O$, $CuCl_2$, $CdCl_2$, and $ZnSO_4 \cdot 7H_2O$. The selectivity of each probe (1 μM) was obtained in HEPES buffer (50 mM HEPES, 100 mM KCl, 10 mM EGTA, pH 7.2, 1% DMSO). The final stock concentrations containing each metal cation were 10 mM for Na^+, Ca^{2+}, Mg^{2+}, or 5 mM for Mn^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Cd^{2+}.

After each measurement, Zn^{2+} (10 or 6 mM) was added to the relevant solutions, and the fluorescence of the samples containing the potentially competing cation was measured.

Effect of pH

A series of HEPES buffer (50 mM HEPES, 100 mM KCl, 1% DMSO) of different pH (3.5 ~ 9) were prepared. The pH was adjusted by adding either KOH or HCl as needed. The concentration of Zn^{2+} was kept at or close to 0 μM by adding 10 mM EGTA. The effect of pH on the fluorescence intensity of each probe (1 μM) with and without Zn^{2+} was then determined.

Cell Culture Studies

Human cervical carcinoma cell line (Hela), human normal prostate epithelia (RWPE1), and prostate cancer cell lines (DU145, PC3) were all purchased from American Type Culture Collection (ATCC, USA). Hela, DU145, and PC3 cells were cultured in high-glucose DMEM (HyClone, USA) supplemented with 10% FBS (HyClone, USA) and 1% penicillin/streptomycin. RWPE1 was cultured in serum-free PEpiCM (ScienCell, USA) with 1% prostatic epithelial cell growth supplement containing various growth factors, hormones, and proteins (PEpiCGS, ScienCell) and 1% penicillin/streptomycin. The cells were cultured at 37 °C under an atmosphere of 5% CO_2.

Cell Viability Assays

Hela cells were plated in 96-well plates at 2000 cells per well in 100 μL DMEM complete medium and cultured for 24 hours. Hela cells were further incubated with the probe in question (0, 5, 10 μM) for another 24 hours. Cell proliferation was analyzed using a Cell Counting Kit-8 assay (CCK-8; Dojindo, Japan) in accord with the manufacturer's instructions. Briefly, 10 μL of CCK-8 solution was added to each well and cells were cultured at 37 °C for 2 hours. The absorbance at 450 nm was measured with a Synergy H1 Multi-Mode Microplate Reader (Bio Tek, USA).

RWPE1, PC3, and DU145 cells were plated in 96-well plates at 2000 cells per well in 100 μL complete PEpiCM medium and cultured for 24 hours. The cells were further incubated with the probes in question (0, 0.25, 0.5, 1, 5 μM) for another 24 hours. Cell proliferation was analyzed using the CCK-8 assay as per the above.

Fluorescent Imaging

Hela cells were plated in 6 well plates at 2×10^5 per well and cultured for 24 hours. Then the cells were incubated with the probe under study (10 μM) for 24 hours, followed by further incubation with zinc pyrithione (50 μM) for 10 minutes or *N*,*N*,*N*′,*N*′-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN) (50 μM) for 10 minutes. Cells were then imaged under a 400× magnification using a fluorescence microscope (Nikon, Japan).

For co-localization analyses, after incubation with the specified probe for 24 hours, lysosomes were stained by 50 nM LysoTracker™ Red DND-99 (Invitrogen, USA) for 2.5 hours. Cell nuclei were stained with the blue-fluorescent indicator 4,6-diamidino-2-phenylindole (DAPI). Hela cells were observed under a laser scanning confocal microscope (Nikon A1, Japan) with 1000× magnification. Co-localization was measured with Image J and quantified using Pearson's correlation value.

Flow Cytometry

Zn abundance in RWPE1, PC3, and DU145 cells was quantified by flow cytometry. Cells (2×10^5/well) were plated in 6-well tissue culture plates. After 24 hours, cells were treated with $ZnSO_4$ (15 μM) for 24 h at 37°C. Cells were then washed once with PBS. Fresh medium (2 mL) containing **LysoDPP-C4** (1 μM) was added to the culture medium. After a 2.5 hours incubation, the cells were detached using 0.25% Trypsin-EDTA (Thermo Fisher, CA). Cells were then washed and fixed in 2% formaldehyde. The cells were analyzed by flow cytometry using a DxFLEX instrument (Beckman, USA). The data were analyzed using the Flowjo software.

Inductively Coupled Plasma-mass Spectrometry (ICP-MS)

RWPE-1, DU145, and PC3 cells were grown to 70%-80% confluence in 100 mm diameter petri dish. 24 h prior to harvest, the medium was replenished with complete medium with/without 15 μM $ZnSO_4$. Cells were detached with 3 mL 0.25% trypsin-EDTA (Thermo Fisher, CA), and isolated by centrifugation. Cells were washed by suspending the cell pellet in 5 mL of chelexed 20 mM Tris buffer followed by centrifugation. The resulting cell pellet was suspended in 1 mL of double-distilled H_2O and cells were lysed by sonication. Prostate cell lysates (200 μL) were digested in concentrated HNO_3 (500 μL) overnight at 37°C. Purified water was then added to give a total volume of 14 mL. The Zn^{2+} concentration was analyzed by inductively coupled plasma-mass spectrometry (ICP-MS) using the inherent strontium signal as an internal control.

The zinc concentration of the complete DMEM media and PepiCM media were also measured.

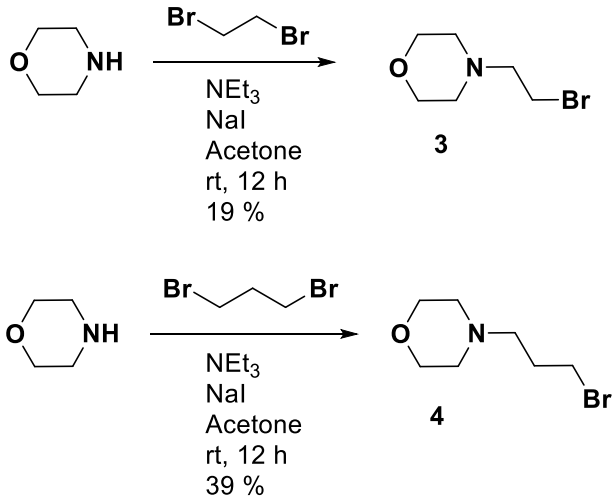
Whole-body Animal Imaging and Analysis

Male BALB/c nude mice (Shanghai Slac Laboratory Animals Co., Ltd) were used in this study. All animal experiments were performed in accord with institutional guidelines and were specifically approved by the Subcommittee on Research Animal Care at the Shanghai Ninth People's Hospital. A whole-body fluorescent imaging system (IVIS Lumina III, PerkinElmer) equipped with a 488 nm excitation and 520-nm emission filter was used in this study. Fluorescence was detected at 15 min after tail-vein injection of normal saline-diluted **LysoDPP-C4** (500 μg per mouse). All of the fluorescence imaging was conducted under the same 1 s exposure time to allow for comparative analyses. Both grayscale white-light and epi-fluorescent images were captured, merged, and analyzed using the Living Image software. Regions of interest overlying the prostate or muscle (as a control) were selected and the analysis area kept constant. The fluorescent intensity was recorded as average efficiency. Signal intensities were measured by region-of-interest (ROI) analysis using the Living Imaging software. Fluorescence intensity was represented using false multicolor scale ranging from blue (least intense) to red (most intense). Since the prostate, bladder, seminal vesicles, and rectum are adjacent within the pelvic cavity, to determine the exact origin of the observed fluorescent signal, the mice were euthanized, dissected, and the viscera then observed under a fluorescence stereomicroscope.

Human Prostate Cancer Tissues

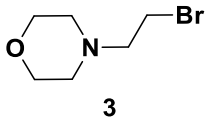
The study protocol was approved by the Medical Ethics Committee of the Shanghai Ninth People's Hospital, and the patient's informed consent was acquired. Prostate cancer tissues were acquired from a 65-year-old male patient, who had undergone holmium laser prostate enucleation at the Urology Department of the Shanghai Ninth People's Hospital two weeks prior to our analysis. Pathological examination confirmed a Gleason score of 3+3 for the cancer tissue under study. Samples ($10 \times 9 \times 3$ mm^3) were obtained from a nodule in the residual peripheral zone during a subsequent radical prostatectomy. The tissue was embedded in Tissue-Tek Optimal Cutting Temperature Compound (Sakura Finetek, Japan) and snap-frozen in liquid nitrogen. Ten-micrometer consecutive sections were prepared and numbered for different purposes. The first section was immediately cultured in PBS containing dilute **LysoDPP-C4** (1 μmol/L) under standard conditions (37°C, 5% CO_2, 100% humidity) for 2 hours, then rinsed and fixed for fluoroscopy. The remain consecutive sections were used for haematoxylin and eosin (H&E) staining.

2. Synthesis of Compounds



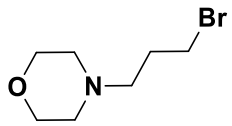
Scheme S1 – Synthesis of morpholine derivatives **3** and **4**.

4-(2-Bromoethyl)morpholine (3)



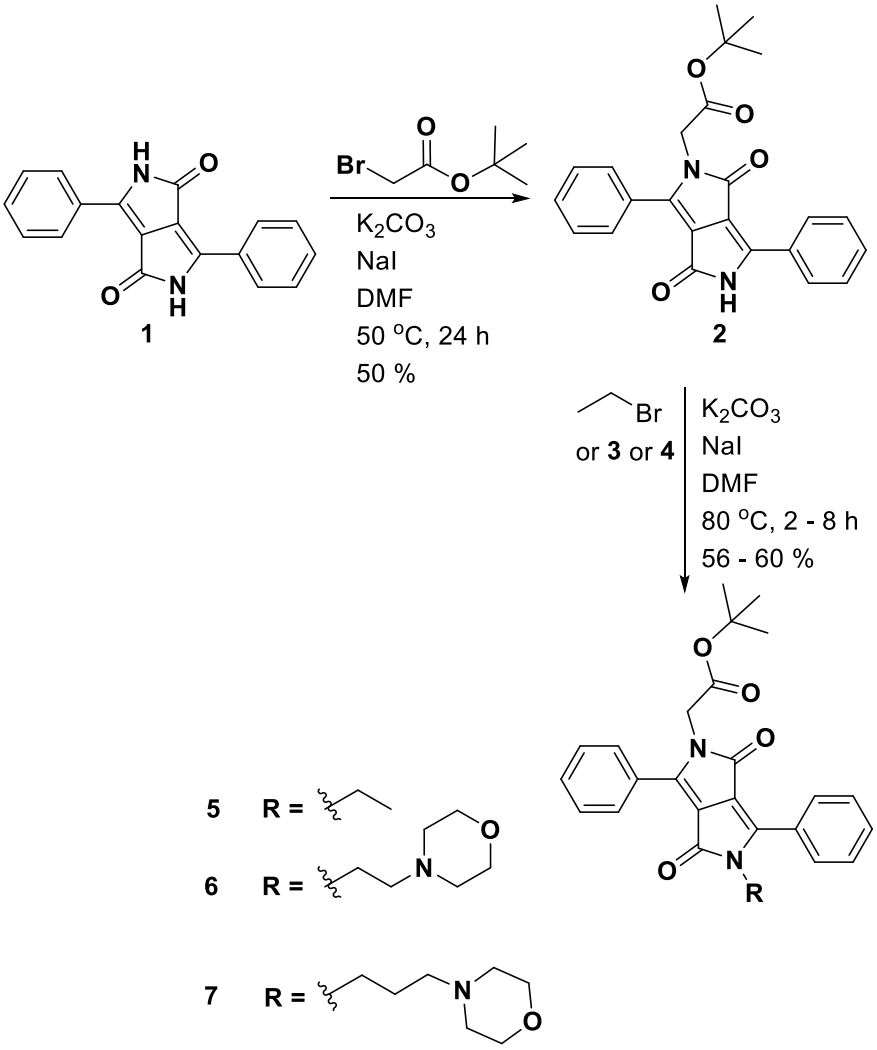
A mixture of 1,2-dibromoethane (2 g, 11 mmol), morpholine (0.93 g, 11 mmol), triethylamine (3.34 g, 33 mmol) and catalytic amounts of NaI in 30 mL acetone was stirred for 12 h at room temperature. Then the resulting mixture was extracted with ethyl acetate, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography over silica gel using a mixture of ethyl acetate/petroleum ether (v/v = 1/1) as the eluent to give the product as a colorless oil (0.4 g, 19%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.66 (t, J = 4.65 Hz, 4H), 3.38 (t, J = 7.2 Hz, 2H), 2.73 (t, J = 7.2 Hz, 2H), 2.46 (t, J = 4.55 Hz, 4H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 66.8, 60.2, 53.3, 28.6.

4-(3-Bromopropyl)morpholine (4)



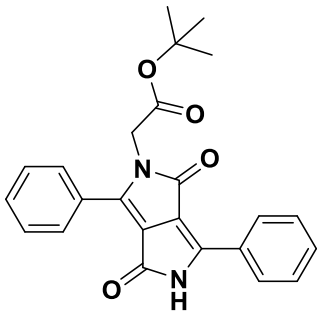
4

A mixture of 1,3-dibromopropane (2 g, 9.9 mmol), morpholine (0.86 g, 9.9 mmol), triethylamine (3.0 g, 29.7 mmol) and catalytic amounts of NaI in 20 mL acetone was stirred for 12 h at room temperature. Then the resulting mixture was filtrated, and the filtrate was concentrated under reduced pressure. The crude product obtained in this way was purified by column chromatography over silica gel using a mixture of ethyl acetate/petroleum ether (v/v = 2/1) as the eluent to give the product as white solid (0.8 g, 39%).^{1}H NMR (500 MHz, $CDCl_3$): δ 3.67 (t, J = 4.65, 4H), 3.44 (t, J = 6.6, 2H), 2.40-2.46 (m, 6H), 1.99 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 66.9, 56.8, 53.7, 31.6, 29.6.



Scheme S2 – Synthesis of morpholine functionalized DPP- fluorophores.

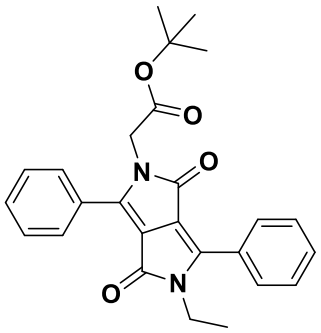
***tert*-Butyl -2-(1,4-dioxo-3,6-diphenyl-4,5-dihydropyrrolo[3,4-c]pyrrol-2(1H)-yl)acetate (2)**



2

A mixture of compound **1** (1 g, 3.47 mmol), *t*-butyl bromoacetate (0.81 g, 4.16 mmol), K_2CO_3 (0.95 g, 8.67 mmol) and catalytic amounts of NaI in 50 mL DMF was stirred for 24 h at 50 ^oC. The resulting mixture was extracted with dichloromethane, dried with anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude product obtained in this way was purified by column chromatography over silica gel using a mixture of ethyl acetate/petroleum ether (v/v = 1/2) as the eluent to give the product as a light red solid (0.35 g, 25%). ^1H NMR (500 MHz, $CDCl_3$): δ 10.41 (s, 1H), 8.38 (m, 2H), 7.82 (m, 2H), 7.47-7.55 (m, 6H), 4.49 (s, 2H), 1.39 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.6, 163.9, 162.1, 147.4, 145.9, 132.1, 131.3, 129.1, 128.9, 128.8, 128.2, 127.9, 127.6, 111.7, 108.8, 82.7, 44.44, 27.9.

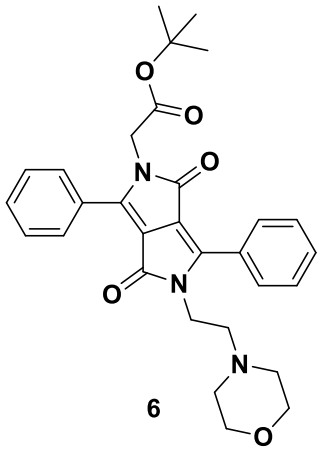
***tert*-Butyl-2-(5-ethyl-1,4-dioxo-3,6-diphenyl-4,5-dihydropyrrolo[3,4-c]pyrrol-2(1H)-yl)acetate (5)**



5

A mixture of compound **2** (0.1 g, 0.25 mmol), bromoethane (0.11 g, 1 mmol), K_2CO_3 (0.1 g, 0.72 mmol), and catalytic amounts of NaI in 20 mL DMF was stirred for 2 h at 80 °C. The resulting mixture was extracted with dichloromethane, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude product obtained in this way was purified by column chromatography over silica gel using a mixture of dichloromethane/petroleum ether (v/v = 1/1) as the eluent to give the product as an orange solid (0.065 g, 60%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.77-7.83 (m, 4H), 7.48-7.55 (m, 6H), 4.43 (s, 2H), δ 3.82 (q, J = 7.1 Hz, 2H), 1.37 (s, 9H), 1.25 (t, J = 7.1 Hz, 3H),. ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.6, 162.7, 162.1, 149.1, 148.3, 131.3, 129.9, 128.9, 128.9, 128.8, 128.9, 128.6, 127.9, 110.5, 108.9, 82.6, 44.0, 36.9, 27.8, 14.9.

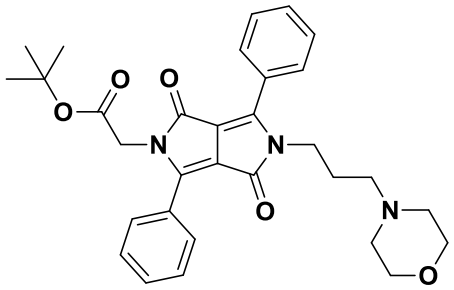
***tert*-Butyl-2-(5-(2-morpholinoethyl)-1,4-dioxo-3,6-diphenyl-4,5-dihydropyrrolo[3,4-c]pyrrol-2(1H)-yl)acetate (6)**



6

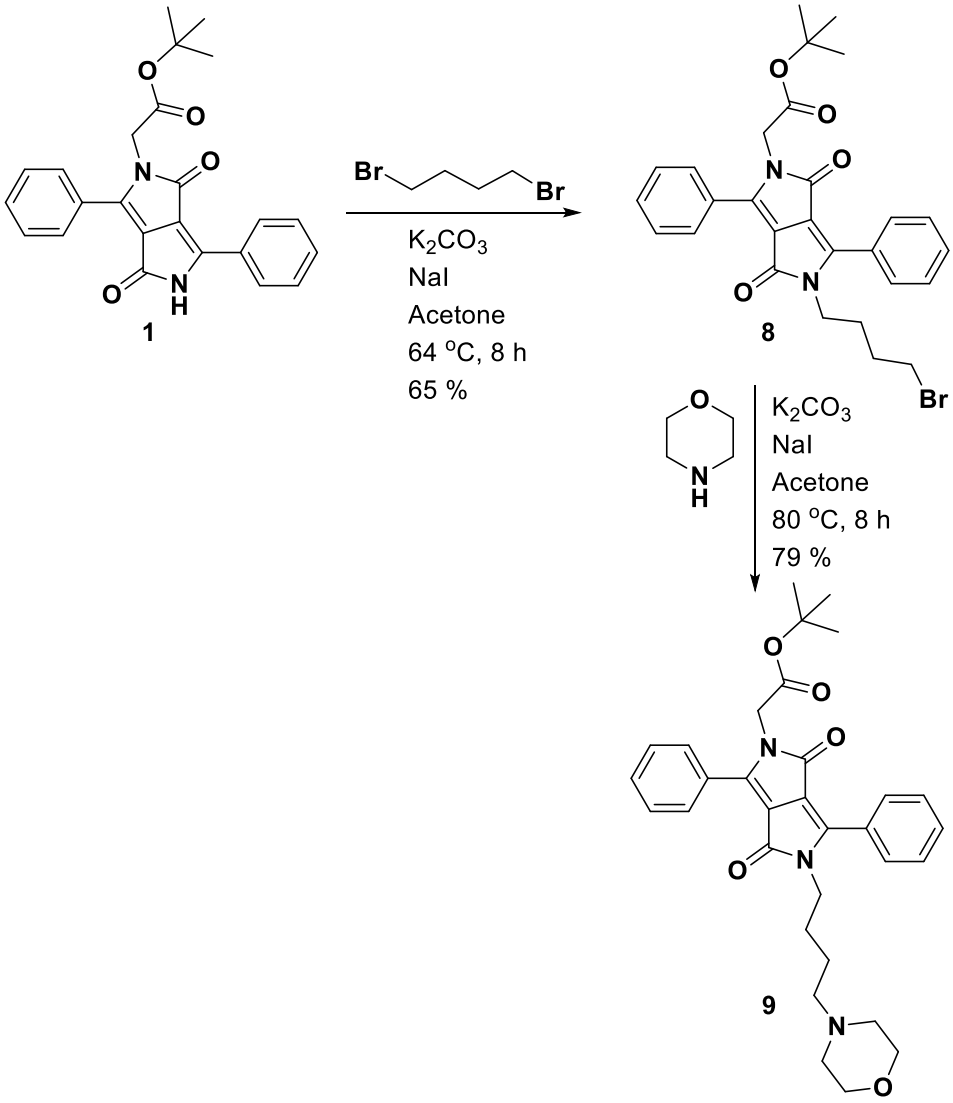
A mixture of compound **2** (0.12 g, 0.3 mmol), 4-(2-bromoethyl)morpholine **3** (0.13 g, 0.67 mmol), K_2CO_3 (0.1 g, 0.72 mmol), and catalytic amounts of NaI in 20 mL DMF was stirred for 8 h at 90 ^oC. The resulting mixture was extracted with dichloromethane, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography over silica gel using a mixture of ethyl acetate/petroleum ether (v/v = 1/1) as the eluent to give the product as an orange solid (0.09 g, 58%).^{1}H NMR (500 MHz, $CDCl_3$): δ 7.78 (m, 2H), 7.72 (m, 2H), 7.43-7.47 (m, 6H), 4.37 (s, 2H), δ 3.87 (t, J = 6.5 Hz, 4H), 3.50 (br, 4H), 2.47 (t, J = 6.5 Hz, 2H), 2.28 (br, 4H), 1.32 (s, 9H). ^{13}C NMR (125MHz, $CDCl_3$): δ 167.6, 162.9, 162.1, 149.2, 147.8, 131.2, 131.1, 128.9, 128.8, 128.8, 128.6, 128.2, 127.9, 110.1, 109.2, 82.5, 66.8, 57.1, 53.6, 43.9, 38.9, 27.8.

***tert*-Butyl-2-(5-(3-morpholinopropyl)-1,4-dioxo-3,6-diphenyl-4,5-dihydropyrrolo[3,4-c]pyrrol-2(1H)-yl)acetate (7)**



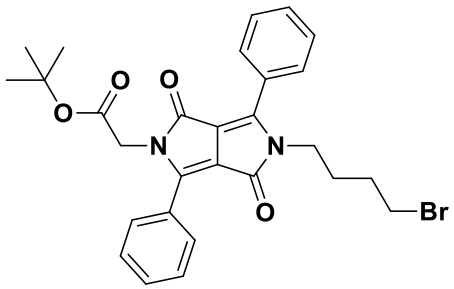
7

A mixture of compound **2** (0.15 g, 0.37 mmol), 4-(3-bromopropyl)morpholine **4** (0.23 g, 1.12 mmol), K_2CO_3 (0.15 g, 1.12 mmol) and catalytic amounts of NaI in 20 mL acetone was refluxed for 8 h. The resulting mixture was filtered, the filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography over silica gel using a mixture of dichloromethane/methanol (v/v = 100/1) as the eluent to give the product as an orange solid (0.11 g, 56%).^{1}H NMR (500 MHz, $CDCl_3$): δ 7.77-7.82 (m, 4H), 7.53 (m, 6H), 4.42 (s, 2H), δ 3.88 (t, J = 7.4 Hz, 2H), 3.61 (br, 4H), 2.31 (br, 6H), 1.78 (m, 2H), 1.38 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.6, 162.9, 162.1, 148.9, 147.8, 131.3, 131.2, 129.0, 128.9, 128.8, 128.6, 128.1, 127.9, 110.3, 109.2, 82.6, 66.8, 55.8, 53.5, 44.0, 40.1, 27.9, 25.9.



Scheme S3 – Synthesis of morpholine functionalized DPP- fluorophore **9**.

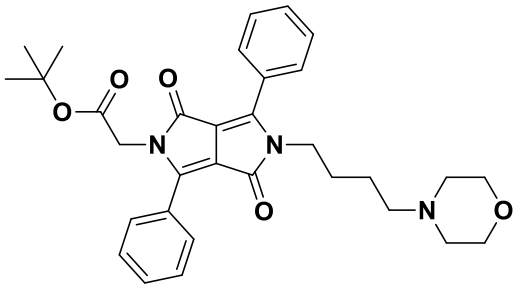
***tert*-Butyl-2-(5-(4-bromobutyl)-1,4-dioxo-3,6-diphenyl-4,5-dihydropyrrolo[3,4-c]pyrrol-2(1H)-yl)acetate (8)**



8

A mixture of compound **2** (0.08 g, 0.20 mmol), 1,4-dibromobutane (0.13 g, 0.6 mmol), K_2CO_3 (0.08 g, 6 mmol), and catalytic amounts of NaI in 20 mL acetone was refluxed for 8 h. The resulting mixture was filtered, and the filtrate was concentrated under reduced pressure. The crude product obtained in this way was purified by column chromatography over silica gel using a mixture of petroleum ether/ethyl acetate (v/v = 2/1) as the eluent to give the product as an orange solid (0.07 g, 65%).^{1}H NMR (500 MHz, $CDCl_3$): δ 7.76-7.81 (m, 4H), 7.49-7.56 (m, 6H), 4.42 (s, 2H), δ 3.81 (t, J = 7.25 Hz, 2H), 3.33 (t, J = 6.3 Hz, 2H), 1.73-1.84(m, 4H), 1.37 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.6, 162.7, 162.1, 148.7, 148.1, 131.3, 131.3, 129.1, 129.0, 128.7, 128.6, 127.9, 127.8, 110.1, 109.2, 82.6, 44.0, 40.8, 32.8, 29.7, 27.9, 27.9.

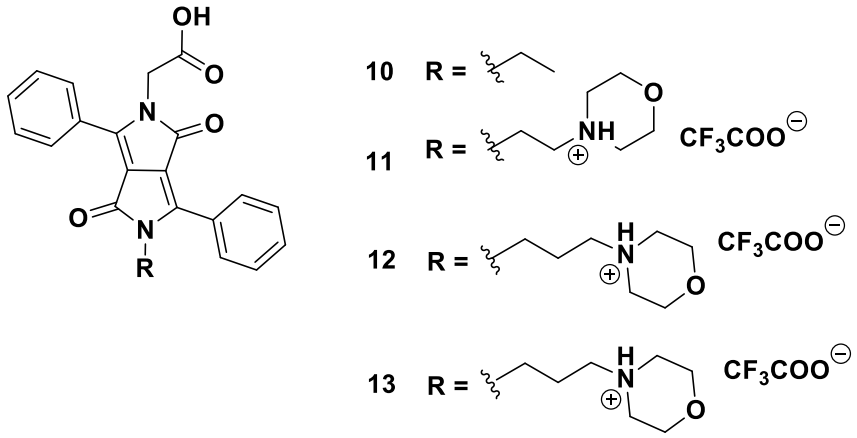
***tert*-Butyl-2-(5-(4-morpholinobutyl)-1,4-dioxo-3,6-diphenyl-4,5-dihydropyrrolo[3,4-c]pyrrol-2(1H)-yl)acetate (9)**



9

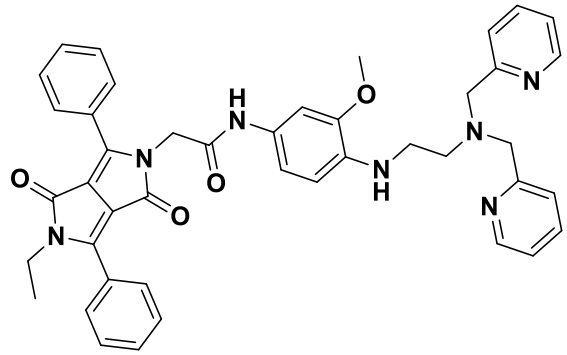
A mixture of compound **8** (0.1 g, 0.19 mmol), morpholine (0.05 g, 0.56 mmol), K_2CO_3 (0.15 g, 1.12 mmol), and catalytic amounts of NaI in 20 mL acetone was refluxed for 8 h. Then the resulting mixture was filtered, and the filtrate was concentrated under reduced pressure. The crude product obtained in this way was purified by column chromatography over silica gel using a mixture of dichloromethane/methanol (v/v = 100/1) as the eluent to give the product as an orange solid (0.08 g, 79%).^{1}H NMR (500 MHz, $CDCl_3$): δ 7.76-7.81 (m, 4H), 7.50-7.54 (m, 6H), 4.42 (s, 2H), δ 3.80 (t, J = 7.5 Hz, 2H), 3.66 (br, 4H), 2.35 (br, 4H), 2.25 (t, J = 7.35 Hz, 2H), 1.62 (m, 2H), 1.43 (m, 2H), 1.37 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.6, 162.7, 162.1, 148.9, 147.8, 131.2, 131.2, 128.9, 128.9, 128.7, 128.6, 128.1, 127.9, 110.3, 109.1, 82.6, 66.9, 65.8, 58.1, 53.6, 44.0, 41.6, 27.8, 27.3, 23.6, 15.3.

General procedures for the synthesis of compounds 10-13



A representative procedure is as follows: A mixture of corresponding precursor (0.3 mmol) in 5 mL TFA was stirred for 4 h at room temperature. The resulting mixture was then concentrated under reduced pressure. The resulting orange solid was used in the next reaction without further purification.

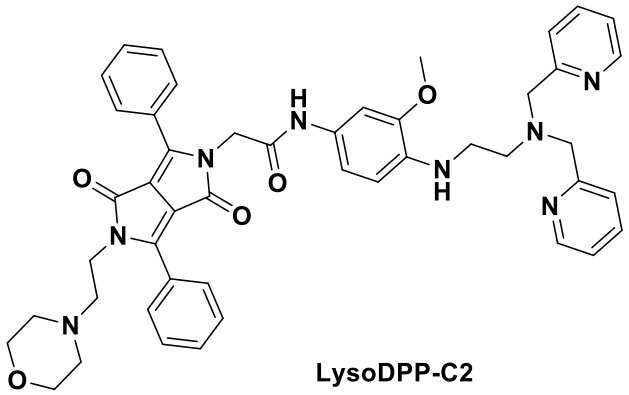
***N*-(4-((2-(Bis(pyridin-2-ylmethyl)amino)ethyl)amino)-3-methoxyphenyl)-2-(5-ethyl-1,4-dioxo-3,6-diphenyl-4,5-dihydropyrrolo[3,4-c]pyrrol-2(1H)-yl)acetamide (DPP-C2)**



DPP-C2

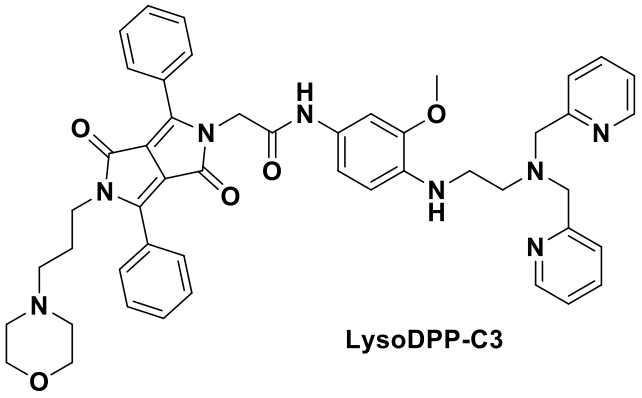
A mixture of compound **10** (30 mg, 0.08 mmol), *O*-(benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium tetrafluoroborate (TBTU) (25.7 mg, 0.08 mmol), *N*,*N*-diisopropylethylamine (DIPEA) (31 mg, 0.24 mmol) in 4 mL DMF was stirred for 30 min at room temperature. To this mixture, amino-functionalized MeO-DPEN (44 mg, 0.12 mmol) was added and stirred for 8 h at room temperature. The resulting mixture was extracted with dichloromethane, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude product obtained in this way was purified by column chromatography over silica gel using a mixture of dichloromethane/methanol (v/v = 100/1) as the eluent to give the product as a yellow solid (40 mg, 69%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.61 (s, 1H), 8.52 (m, 2H), 8.05 (m, 2H), 7.83(m, 2H), 7.51- 7.65 (m, 10H), 7.23 (d, J = 1.70 Hz, 1H), 7.15 (m, 2H), 6.75 (dd, J_1 = 1.65 Hz, J_2 = 8.40 Hz, 1H), 6.38 (d, J = 8.40 Hz, 1H), 4.45 (s, 2H), δ 3.82-3.92 (m, 9H), 3.18 (t, J = 5,9 Hz, 2H), 2.9 (t, J = 5.80 Hz, 2H), 1.28 (t, J = 7.05 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.8,163.4, 162.6,159.0, 150.3, 148.9, 147.9, 146.8, 136.5, 135.6, 131.7, 131.6, 129.4, 129.1, 129.1, 128.8, 127.8, 127.3, 127.2, 123.1, 122.2, 113.3, 110.4, 109.7, 108.5, 103.9, 60.3, 55.7, 52.9, 47.6, 41.3, 37.1, 14.9. ESI-MS calcd for [$C_{43}H_{41}N_7O_4$], 719.32; found, 719.2 [M^+].

***N*-(4-((2-(Bis(pyridin-2-ylmethyl)amino)ethyl)amino)-3-methoxyphenyl)-2-(5-(2-morpholinoethyl)-1,4-dioxo-3,6-diphenyl-4,5-dihydropyrrolo[3,4-c]pyrrol-2(1H)-yl)acetamide (LysoDPP-C2)**



A mixture of compound **11** (70 mg, 0.15 mmol), TBTU (49 mg, 0.15 mmol), and DIPEA (80 mg, 0.6 mmol) in 5 mL DMF was stirred for 30 min at room temperature. To this mixture, amino-functionalized MeO-DPEN (84 mg, 0.23 mmol) was added and stirred for 8 h at room temperature. The resulting mixture was extracted with dichloromethane, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude product obtained in this way was purified by column chromatography over silica gel using a mixture of dichloromethane/methanol (v/v = 25/1) as the eluent to give the product as a yellow solid (0.08 g, 66%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.67 (s, 1H), 8.48 (d, J = 4.4, 2H), 7.99 (m, 2H), 7.80 (m, 2H), 7.59(m, 2H), 7.46- 7.51 (m, 8H), 7.20 (d, J = 2.05 Hz, 1H), 7.10 (m, 2H), 6.69 (dd, J_1 = 2.05 Hz, J_2 = 8.45 Hz, 1H), 6.32 (d, J = 8.45 Hz, 1H), 4.39 (s, 2H), δ 3.88 (t, J = 6.3 Hz, 2H), 3.84 (s, 4H), 3.81 (s, 3H), 3.51 (br, 4H), 3.12 (t, J = 6 Hz, 2H), 2.84 (t, J = 5.95 Hz, 2H), 2.48 (t, J = 6.3 Hz, 2H), 2.28 (br, 4H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.7,163.3, 162.8, 159.3, 150.3, 148.9, 148.1, 146.8, 136.5, 135.7, 131.7, 131.4, 129.3, 129.1, 128.9, 128.8, 128.1, 127.3, 127.3, 122.9, 122.1, 113.2, 110.1, 109.5, 108.9, 103.8, 66.8, 60.3, 57.2, 55.6, 53.7, 52.9, 47.3, 41.3, 39.1. ESI-MS calcd for [$C_{47}H_{48}N_8O_5$], 804.37; found, 805.4 [$M^+ + H^+$], 827.4 [$M^+ + Na^+$].

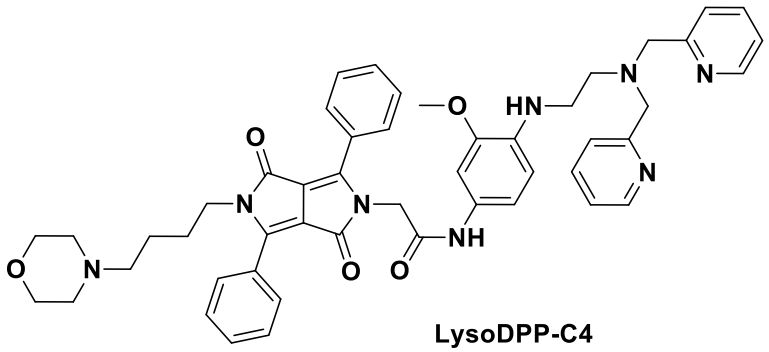
***N*-(4-((2-(Bis(pyridin-2-ylmethyl)amino)ethyl)amino)-3-methoxyphenyl)-2-(5-(3-morpholinopropyl)-1,4-dioxo-3,6-diphenyl-4,5-dihydropyrrolo[3,4-c]pyrrol-2(1H)-yl)acetamide (LysoDPP-C3)**



LysoDPP-C3

A mixture of compound **12** (0.16 g, 0.34 mmol), TBTU (0.11 g, 0.34 mmol), and DIPEA (0.13 g, 1.02 mmol) in 5 mL DMF was stirred for 30 min at room temperature. To this mixture, amino-functionalized MeO-DPEN (0.18 g, 0.51 mmol) was added and stirred for 8 h at room temperature. The resulting mixture was extracted with dichloromethane, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude product obtained in this way was purified by column chromatography over silica gel using a mixture of dichloromethane/methanol (v/v = 25/1) as the eluent. Recrystallization from ethyl ether gave the product as a yellow solid (0.13 g, 47%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.52 (m, 2H), 8.45 (s, 1H), 8.06 (m, 2H), 7.82 (m, 2H), 7.51- 7.65 (m, 10H), 7.23 (d, J = 2.00 Hz, 1H), 7.15 (m, 2H), 6.74 (dd, J_1 = 1.80 Hz, J_2 = 8.35 Hz, 1H), 6.39 (d, J = 8.40 Hz, 1H), 4.44 (s, 2H), δ 3.88-3.92 (br, 9H), 3.62 (br, 4H), 3.17 (t, J = 5.9 Hz, 2H), 2.88 (t, J = 5.90 Hz, 2H), 2.32 (br, 6H), 1.80 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.7, 163.4, 162.7, 159.4, 150.2, 148.9, 148.0, 146.8, 136.4, 135.9, 131.8, 131.6, 129.4, 129.2, 129.1, 128.8, 127.9, 127.2, 127.1, 122.9, 122.1, 113.4, 110.3, 109.5, 108.7, 103.9, 66.8, 60.4, 55.7, 55.7, 53.4, 52.9, 47.7, 41.3, 40.3, 25.9. ESI-MS calcd for [$C_{48}H_{50}N_8O_5$], 818.39; found, 818.2 [M^+].

***N*-(4-((2-(Bis(pyridin-2-ylmethyl)amino)ethyl)amino)-3-methoxyphenyl)-2-(5-(4-morpholinobutyl)-1,4-dioxo-3,6-diphenyl-4,5-dihydropyrrolo[3,4-c]pyrrol-2(1H)-yl)acetamide (LysoDPP-C4)**

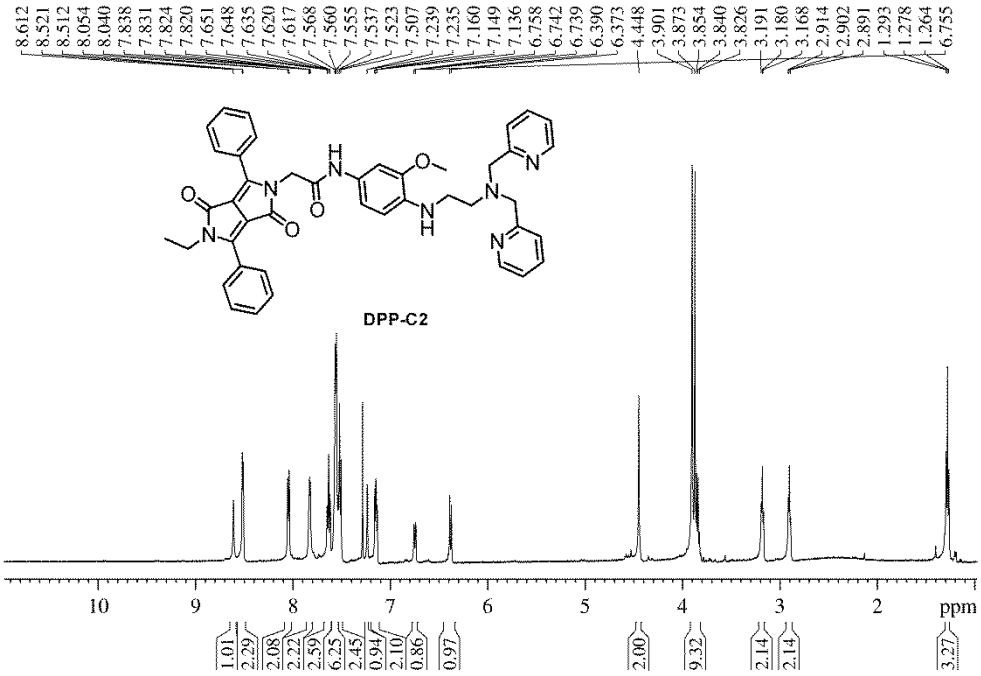


LysoDPP-C4

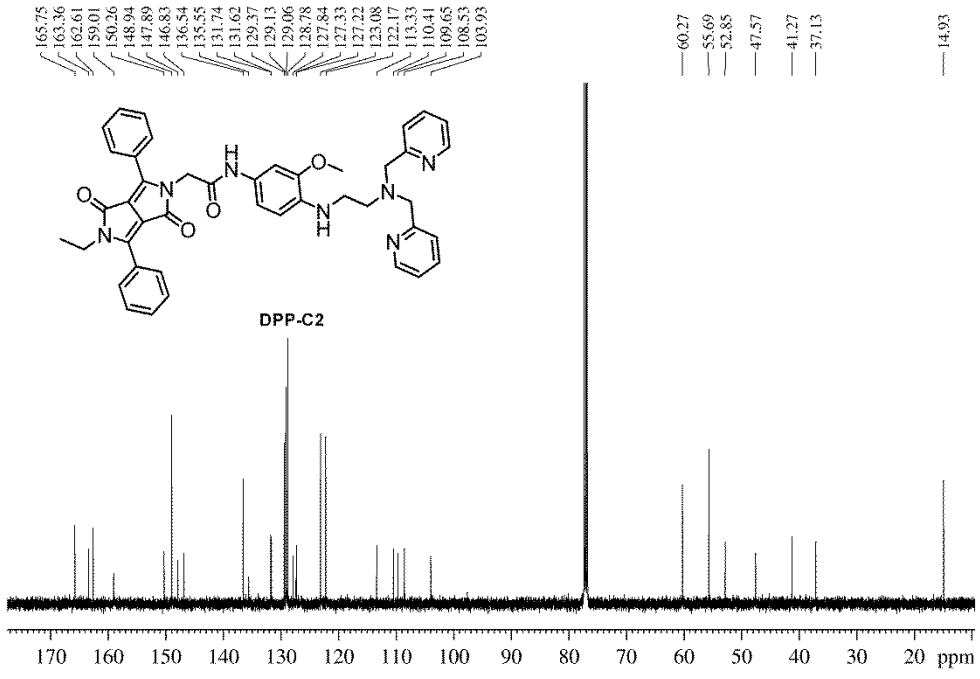
A mixture of compound **13** (0.10 g, 0.21 mmol), TBTU (0.07 g, 0.21 mmol), and DIPEA (0.08 g, 0.63 mmol) in 5 mL DMF was stirred for 30 min at room temperature. To this mixture, amino-functionalized MeO-DPEN (0.12 g, 0.32 mmol) was added and stirred for 8 h at room temperature. The resulting mixture was extracted with dichloromethane, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography over silica gel using a mixture of dichloromethane/methanol (v/v = 20/1) as the eluent. Recrystallization from ethyl ether gave the product as a yellow solid (0.10 g, 57%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.54 (s, 1H), 8.51 (m, 2H), 8.04 (m, 2H), 7.80 (m, 2H), 7.62 (m, 2H), 7.50- 7.58 (m, 8H), 7.23 (d, J = 2.20 Hz, 1H), 7.14 (m, 2H), 6.73 (dd, J_1 = 2.20 Hz, J_2 = 8.45 Hz, 1H), 6.38 (d, J = 8.45 Hz, 1H), 4.42 (s, 2H), 3.87 (br, 7H), δ 3.82 (t, J = 7.5 Hz, 2H), 3.68 (br, 4H), 3.16 (t, J = 6.00 Hz, 2H), 2.87 (t, J = 6.00 Hz, 2H), 2.37 (br, 4H), 2.27 (t, J = 7.35 Hz, 2H), 1.63 (m, 2H), 1.45 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.7,163.3, 162.6, 159.4, 149.9, 148.9, 148.1, 146.8, 136.4, 135.8, 131.8, 131.6, 129.4, 129.1, 129.1, 128.7, 127.9, 127.2, 127.1, 122.9, 122.1, 113.3, 110.2, 109.5, 108.7, 103.9, 66.8, 60.4, 58.1, 55.7, 53.6, 52.9, 47.5, 41.7, 41.3, 27.3, 23.6. ESI-MS calcd for [$C_{49}H_{52}N_8O_5$], 832.41; found, 832.3 [M^+].

3. NMR and MS Data for Compounds of Interest

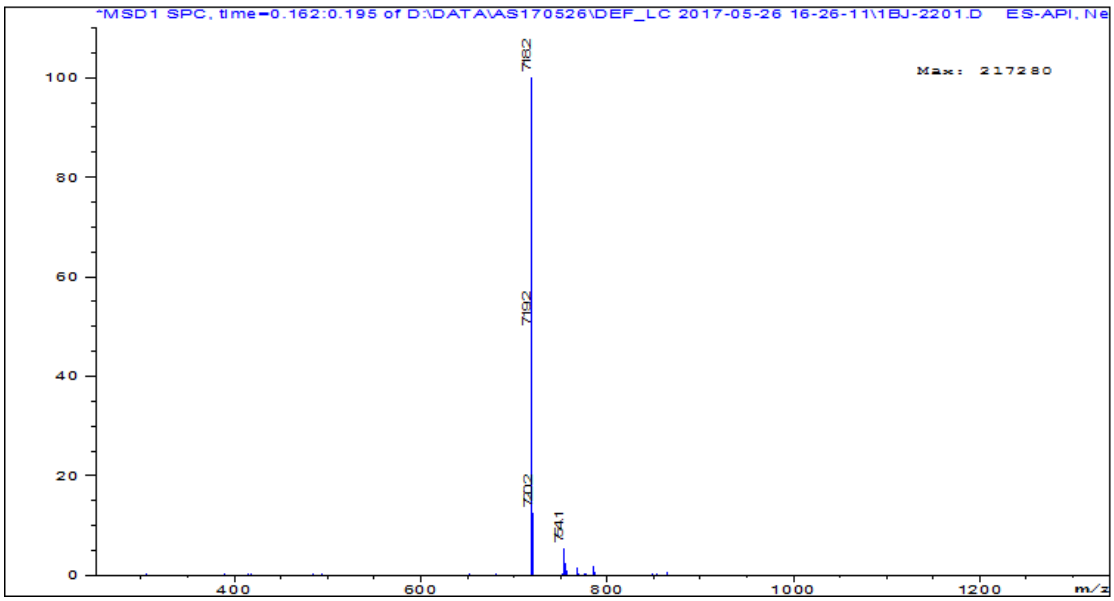
^{1}H NMR spectrum of **DPP-C2** in $CDCl_3$



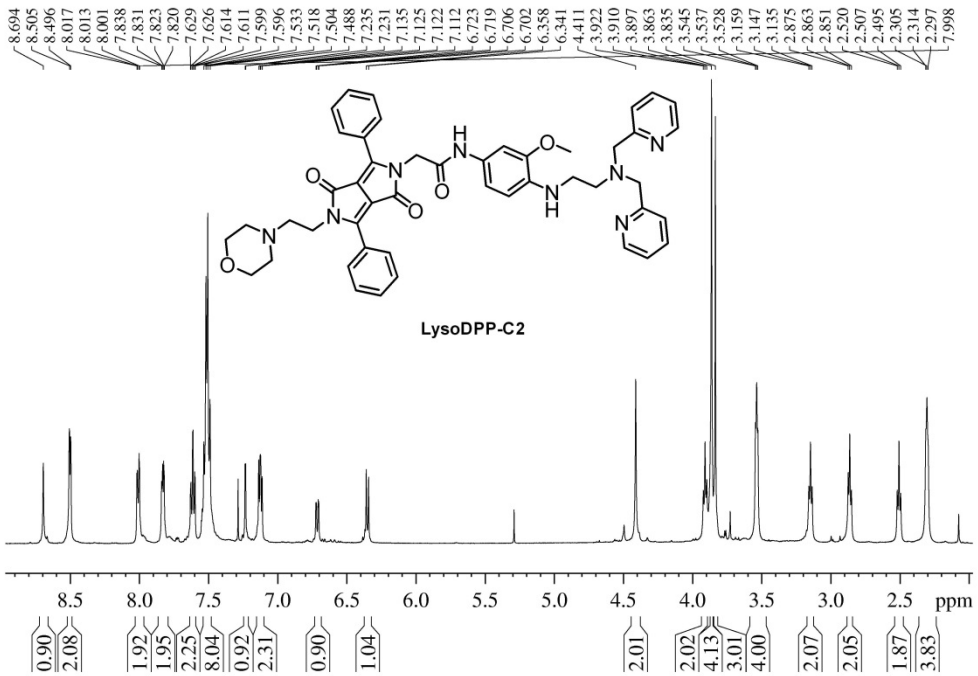
^{13}C NMR spectrum of **DPP-C2** in $CDCl_3$



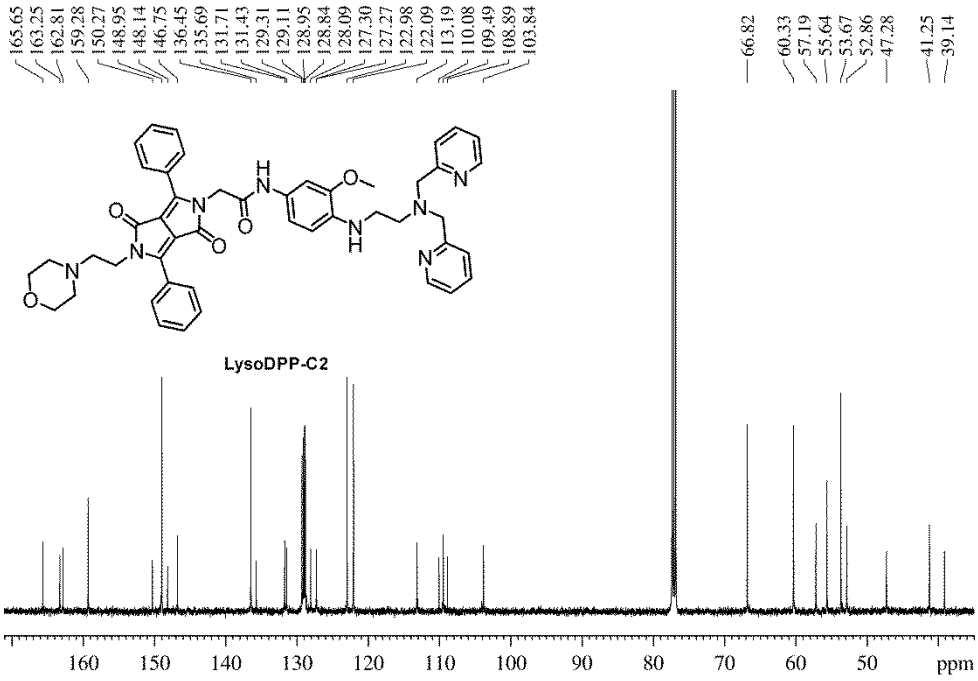
Mass Spectrum of **DPP-C2**



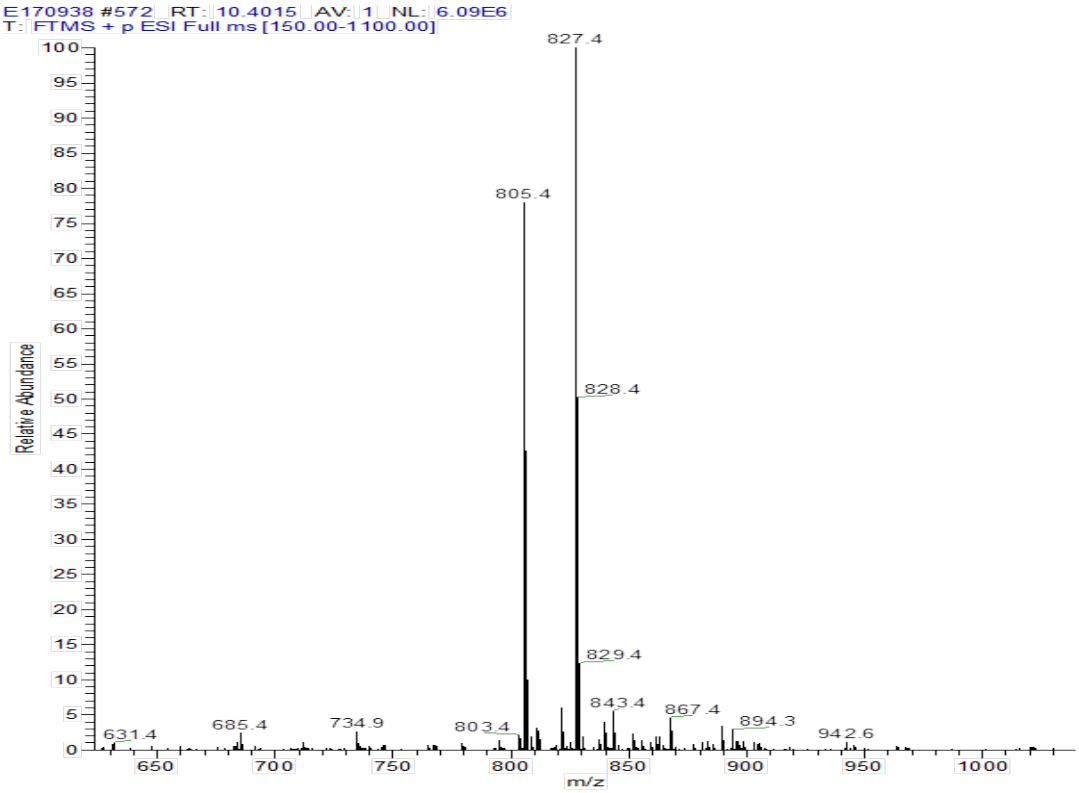
^{1}H NMR spectrum of **LysoDPP-C2** in $CDCl_3$



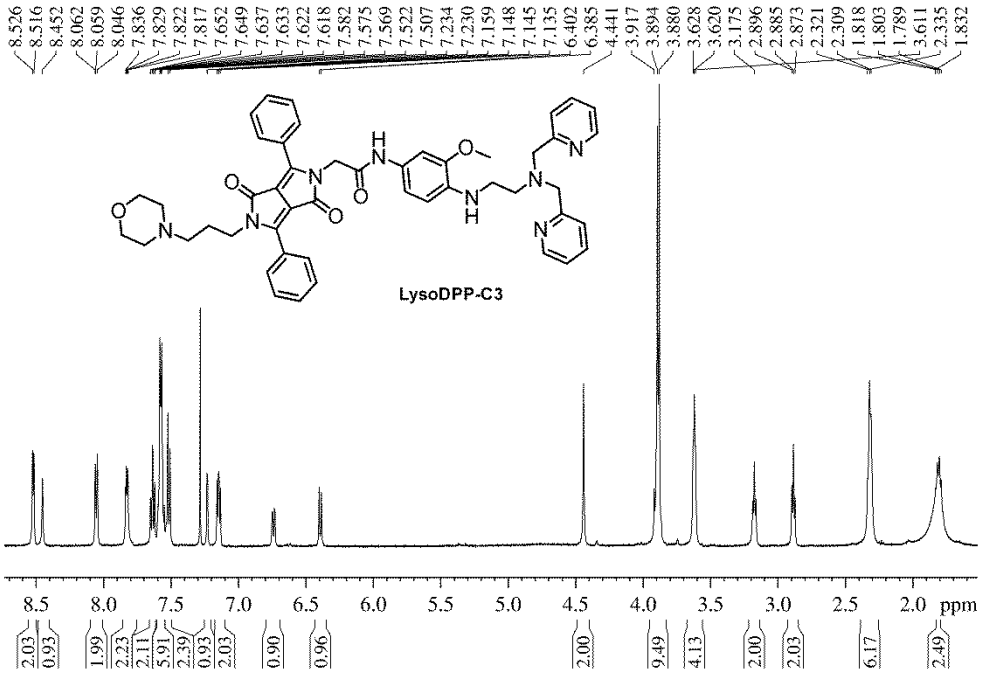
^{13}C NMR spectrum of **LysoDPP-C2** in $CDCl_3$



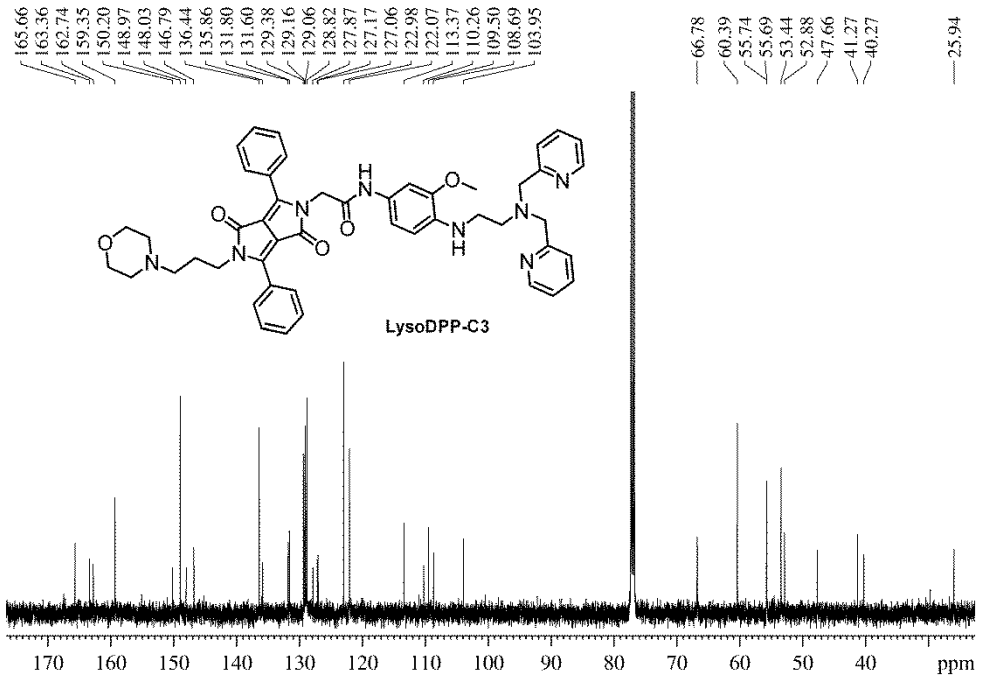
Mass spectrum of **LysoDPP-C2**



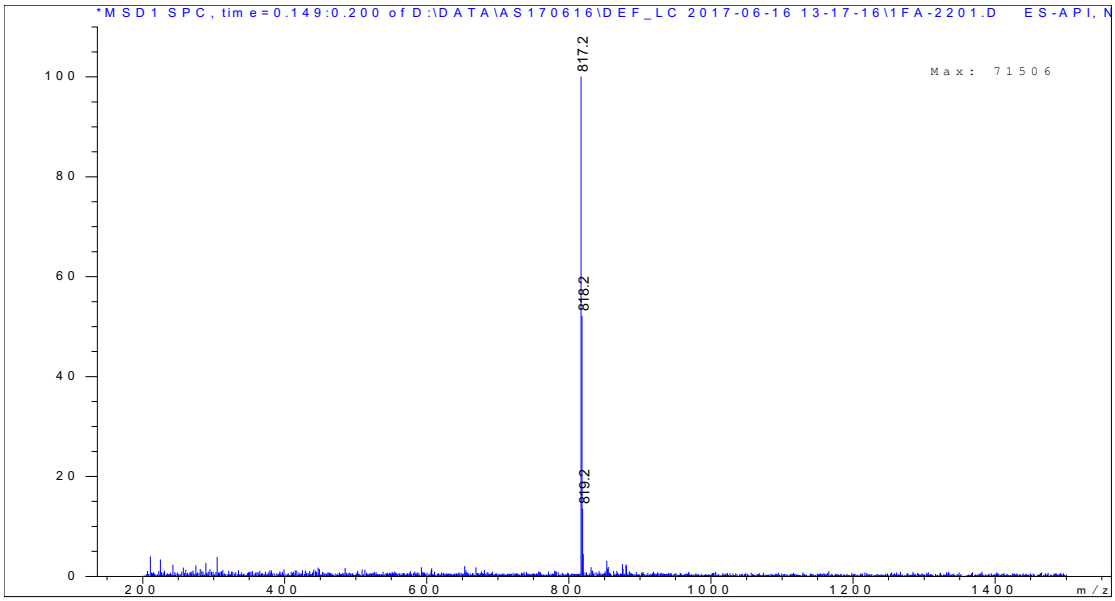
^{1}H NMR spectrum of **LysoDPP-C3** in $CDCl_3$



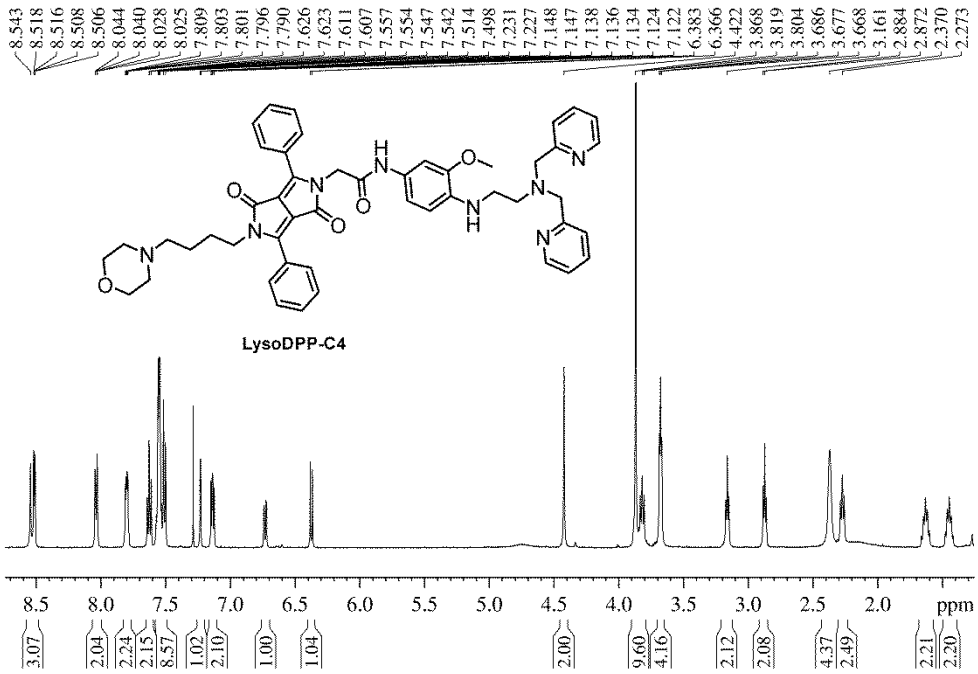
^{13}C NMR spectrum of **LysoDPP-C3** in $CDCl_3$



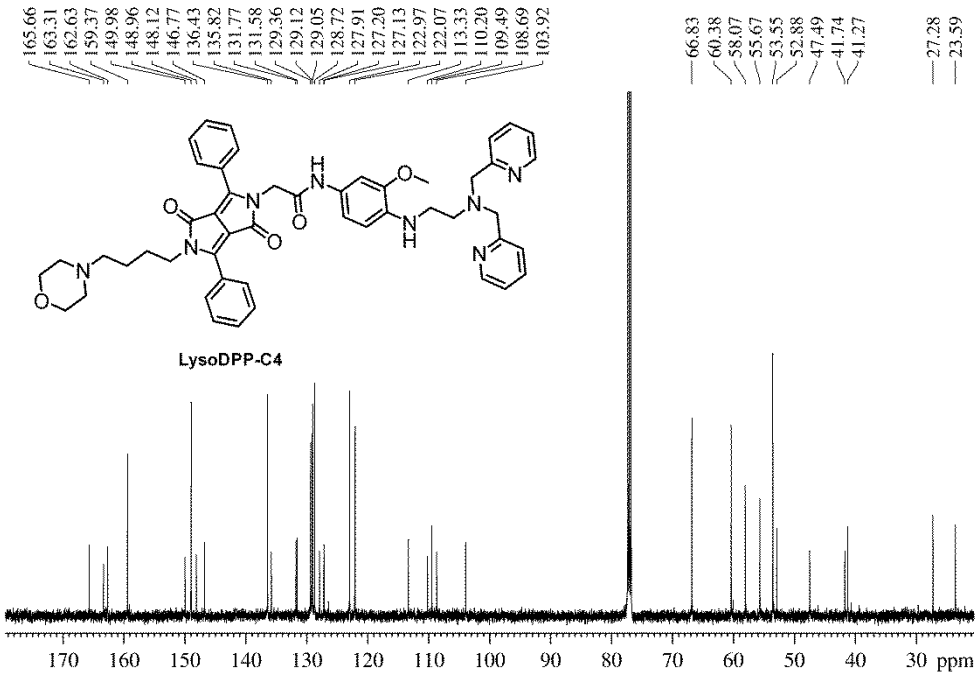
Mass spectrum of **LysoDPP-C3**



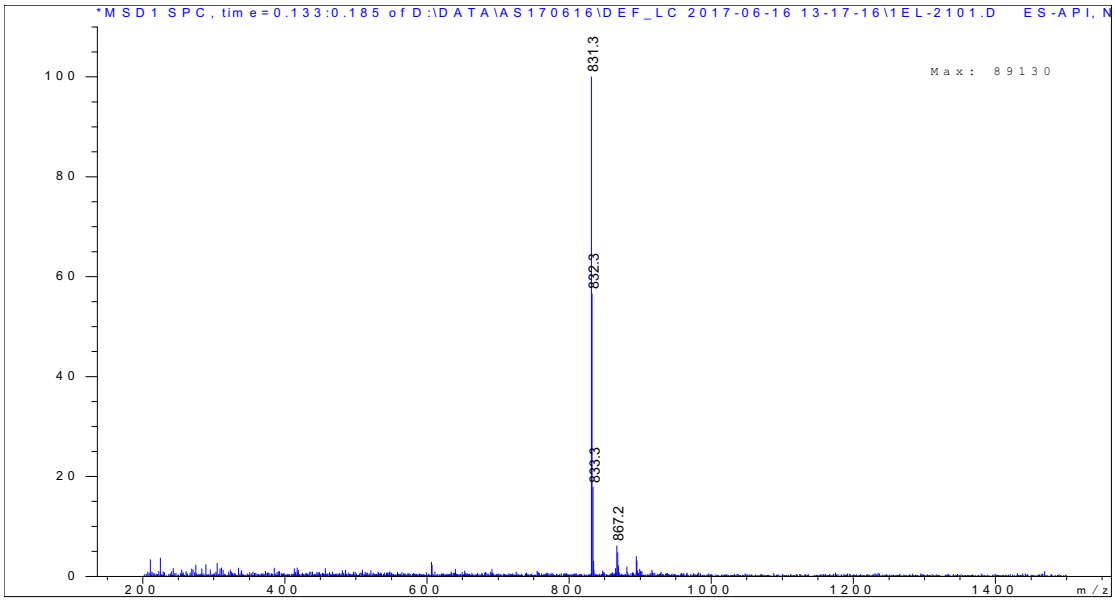
^{1}H NMR spectrum of **LysoDPP-C4** in $CDCl_3$



^{13}C NMR spectrum of **LysoDPP-C4** in $CDCl_3$



Mass spectrum of **LysoDPP-C4**



4. Photophysical Properties of the Probes of this Study in Different Solvent Systems

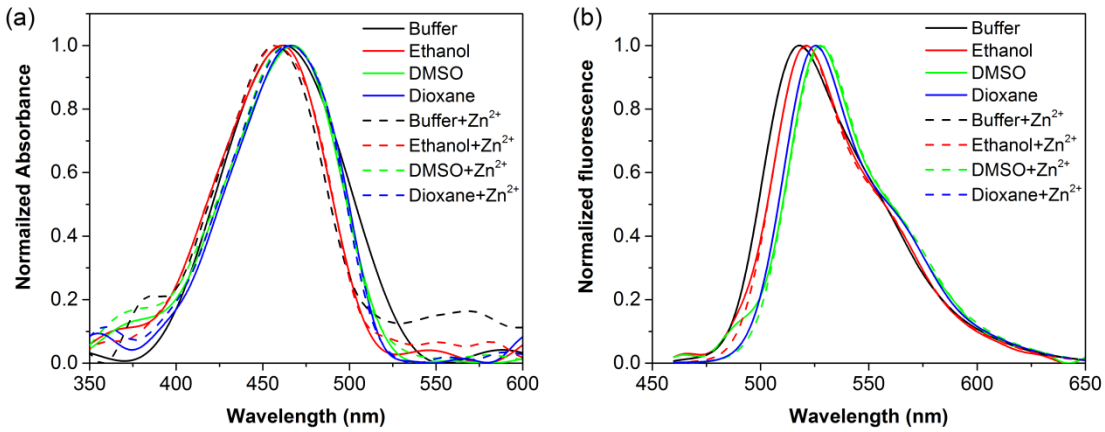


Fig. S1 - Normalized absorption (a) and emission (b) spectra of 1 µM **DPP-C2** with/without Zn^{2+} in 1,4-dioxane, DMSO, ethanol, and aqueous buffer (50 mM HEPES, 100 mM KCl, 10 mM EGTA, 1% DMSO, pH 7.2). λex = 430 nm.

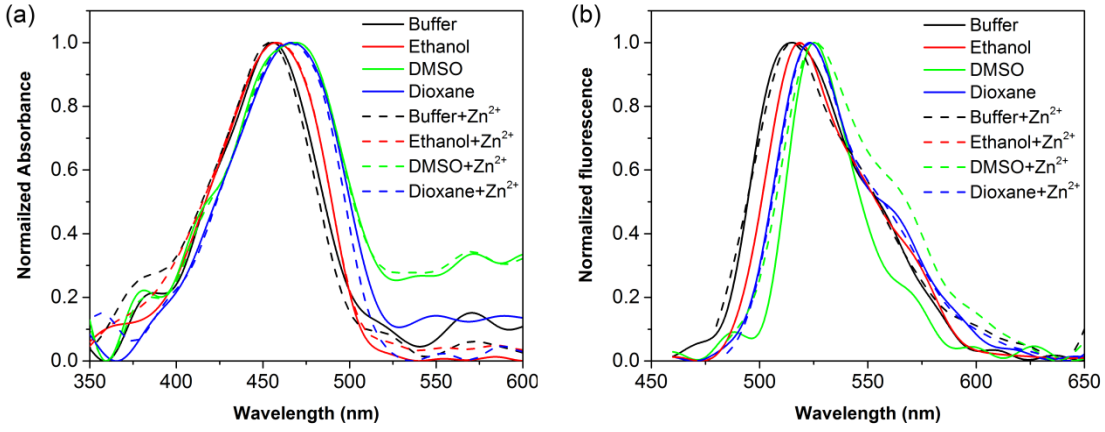


Fig. S2 - Normalized absorption (a) and emission (b) spectra of 1 µM **LysoDPP-C2** with/without Zn^{2+} in 1,4-dioxane, DMSO, ethanol, and aqueous buffer (50 mM HEPES, 100 mM KCl, 10 mM EGTA, 1% DMSO, pH 7.2). λex = 430 nm.

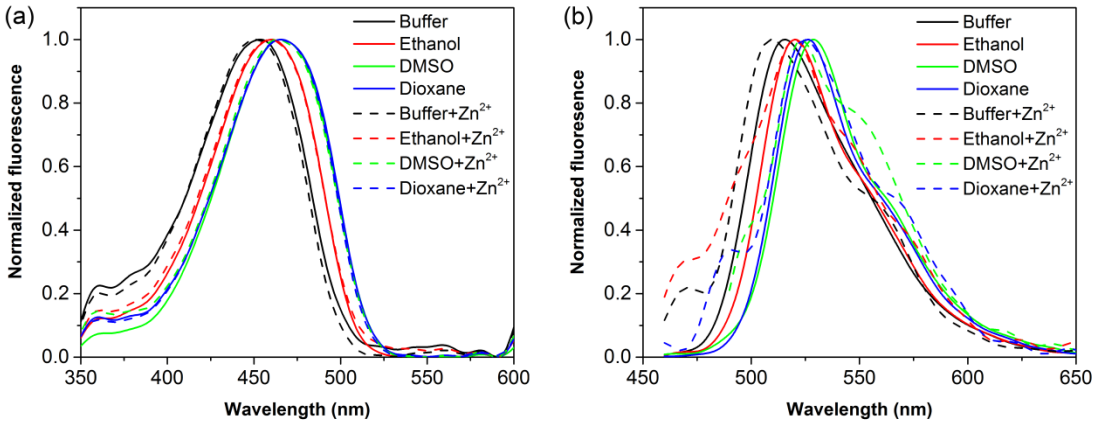


Fig. S3 - Normalized absorption (a) and emission (b) spectra of 1 µM **LysoDPP-C3** with/without Zn^{2+} in 1,4-dioxane, DMSO, ethanol, and aqueous buffer (50 mM HEPES, 100 mM KCl, 10 mM EGTA, 1% DMSO, pH 7.2). λex = 430 nm.

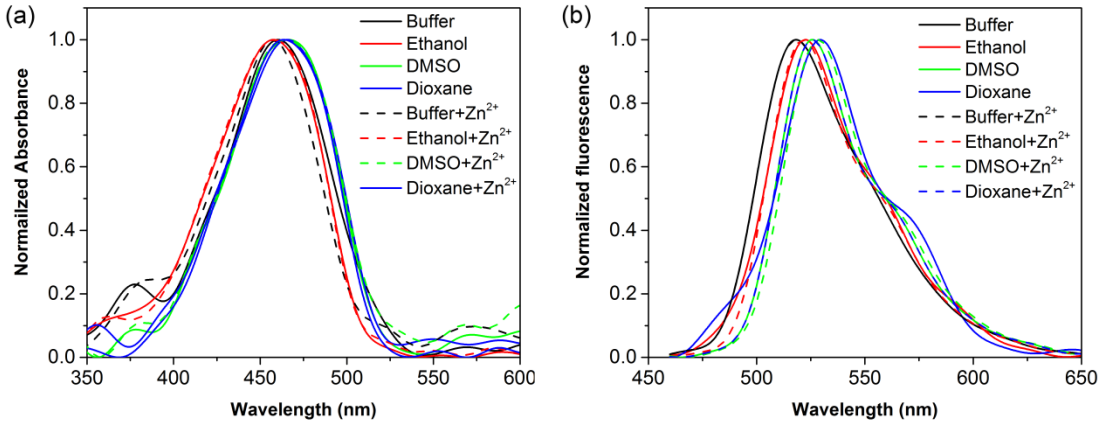


Fig. S4 - Normalized absorption (a) and emission (b) spectra of 1 µM **LysoDPP-C4** with/without Zn^{2+} in 1,4-dioxane, DMSO, ethanol, and aqueous buffer (50 mM HEPES, 100 mM KCl, 10 mM EGTA, 1% DMSO, pH 7.2). λex = 430 nm.

Table S1. Photophysical properties of the probes of this study with/without Zn^{2+} in solvents of different polarity.

Compound	Solvent [a]	λ^{abs}_{max} (nm) [b]	λ^{fl}_{max} (nm) [c]	Φ_F [d]	Stokes shift (nm)
DPP-C2	1,4-dioxane (0.164)	466	526	0.0039	60
	DMSO (0.444)	467	528	0.0237	61
	ethanol (0.654)	461	521	0.0215	60
	aqueous buffer (1.00) [e]	462	518	0.0027	56
DPP-C2+Zn^{2+}	1,4-dioxane (0.164)	465	526	0.6303	61
	DMSO (0.444)	464	529	0.4848	65
	ethanol (0.654)	458	521	0.642	63
	aqueous buffer (1.00)	457	518	0.3988	61
LysoDPP-C2	1,4-dioxane (0.164)	467	523	0.003	56
	DMSO (0.444)	469	526	0.001	57
	ethanol (0.654)	458	519	0.0023	61
	Buffer (1.00)	456	515	0.0027	59
LysoDPP-C2+Zn^{2+}	1,4-dioxane (0.164)	465	523	0.1061	58
	DMSO (0.444)	468	526	0.0202	58
	ethanol (0.654)	458	519	0.0337	61
	aqueous buffer (1.00)	454	515	0.0482	61
LysoDPP-C3	1,4-dioxane (0.164)	465	524	0.0082	59
	DMSO (0.444)	465	523	0.0039	58
	ethanol (0.654)	460	520	0.0053	60
	aqueous buffer (1.00)	454	509	0.0064	55
LysoDPP-C3+Zn^{2+}	1,4-dioxane (0.164)	465	526	0.3778	61
	DMSO (0.444)	463	528	0.1110	65
	ethanol (0.654)	459	521	0.2087	62
	aqueous buffer (1.00)	451	515	0.2606	64
LysoDPP-C4	1,4-dioxane (0.164)	465	526	0.0144	61
	DMSO (0.444)	465	529	0.0167	64
	ethanol (0.654)	460	520	0.0309	60
	aqueous buffer (1.00)	453	512	0.0077	59

LysoDPP-C4+Zn^{2+}	1,4-dioxane (0.164)	465	527	0.5034	62
	DMSO (0.444)	464	529	0.2819	65
	ethanol (0.654)	459	522	0.3836	63
	aqueous buffer (1.00)	453	517	0.3283	64

[a]The numbers in the parenthesis are a normalized empirical parameter of solvent polarity. [b] The maximum absorption wavelength in solution. [c]The maximum emission wavelength in solution. The excitation wavelength was 430 nm. [d]Fluorescent quantum yields were determined in reference to rhodamine 6G (Φ_F = 0.89, in CH_2Cl_2). The aqueous buffer solutions consisted of 50 mM HEPES, 100 mM KCl, 10 mM EGTA, 1% DMSO, pH 7.2. The solvent polarity used for the buffer solution is that for water.

Table S2. Spectroscopic properties of the probes prepared in this study and their zinc complexes as measured in aqueous HEPES buffer.[a]

Compound	λ^{abs}_{max} [b] (nm)	λ^{fl}_{max} [c] (nm)	Φ_F [d]	Φ_F [e]	*FEF* [f]	*FEF* [g]	K_a [h] ($\times 10^8$ M^{-1})	K_d [i] (nM)
DPP-C2	462	518	0.0027	0.0567	-	-	-	-
DPP-C2+Zn^{2+}	457	518	0.3988	0.3985	83	87	4.76	2.83
LysoDPP-C2	456	515	0.0027	0.0238	-	-	-	-
LysoDPP-C2+Zn^{2+}	454	515	0.0482	0.1522	29	89	2.31	1.54
LysoDPP-C3	454	509	0.0064	0.0848	-	-	-	-
LysoDPP-C3+Zn^{2+}	451	515	0.2606	0.3114	55	84	1.46	2.57
LysoDPP-C4	453	512	0.0077	0.0915	-	-	-	-
LysoDPP-C4+Zn^{2+}	453	517	0.3283	0.3722	75	84	2.94	1.91

[a]Measurements were made in HEPES buffer (50 mM HEPES, 100 mM KCl, 10 mM EGTA, 1% DMSO, pH = 7.2) with or without 10 mM Zn^{2+}. [b]The maximum absorption wavelength in HEPES buffer. [c]The maximum emission wavelength in HEPES buffer. The excitation wavelength was 430 nm. Fluorescent quantum yields were determined in buffer at pH = 7.2[d] and pH = 5 [e], with reference to rhodamine 6G (Φ_F = 0.89, in CH_2Cl_2). Fluorescence enhancement factor measured by $(F - F_{min})/F_{min}$ in buffer at pH = 7.2 [f] and pH = 5 [g]. [h]Association constants measured by ITC in ethanol. [i]Dissociation constants for Zn^{2+} in nM measured by fluorescence titration, with the titration curves fitted to the Benesi-Hildebrand equation.

5. Photostability Studies

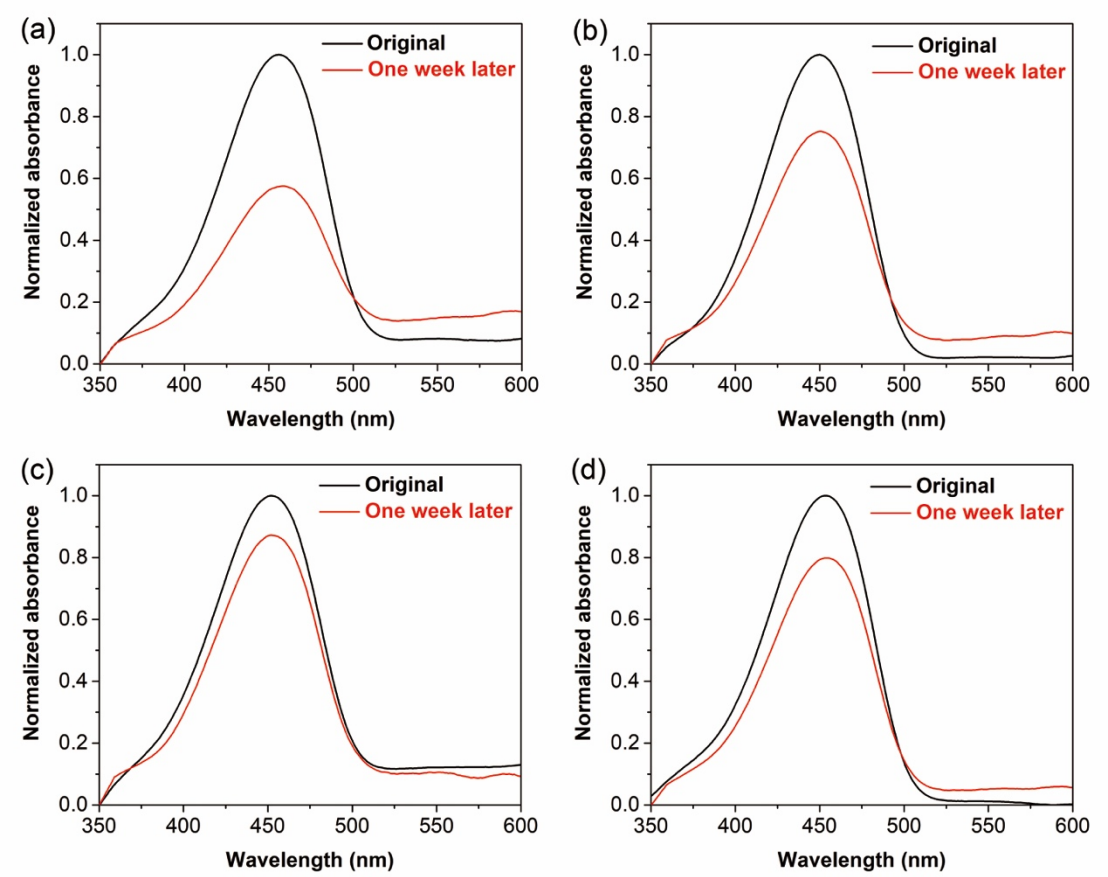


Fig. S5 - UV-vis absorption spectra of 2 μM probe/Zn^{2+} complexes recorded in aqueous HEPES buffer (50 mM HEPES, 100 mM KCl, 1% DMSO, pH 7.2) before and after exposure to sun light for one week. (a) **DPP-C2**/Zn^{2+}; (b) **LysoDPP-C2**/Zn^{2+}; (c) **LysoDPP-C3**/Zn^{2+}; (d) **LysoDPP-C4**/Zn^{2+}. λ_{ex} = 430 nm.

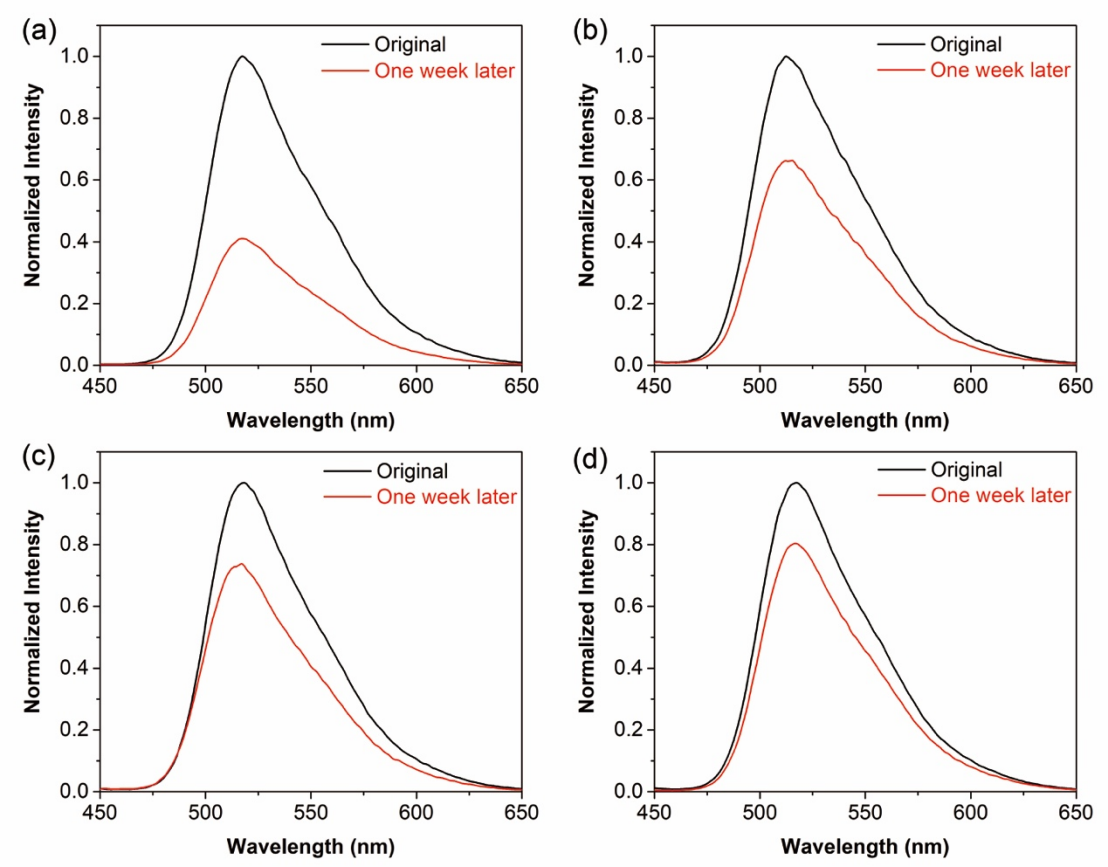


Fig. S6 - Fluorescence spectra of 2 μM probe/Zn^{2+} complex recorded in aqueous HEPES buffer (50 mM HEPES, 100 mM KCl, 1% DMSO, pH 7.2) before and after exposure to sun light for one week. (a) **DPP-C2**/Zn^{2+}; (b) **LysoDPP-C2**/Zn^{2+}; (c) **LysoDPP-C3**/Zn^{2+}; (d) **LysoDPP-C4**/Zn^{2+}. λ_{ex} = 430 nm.

6. UV-Vis and Fluorescence Spectral Analyses of Zn^{2+} Complexation

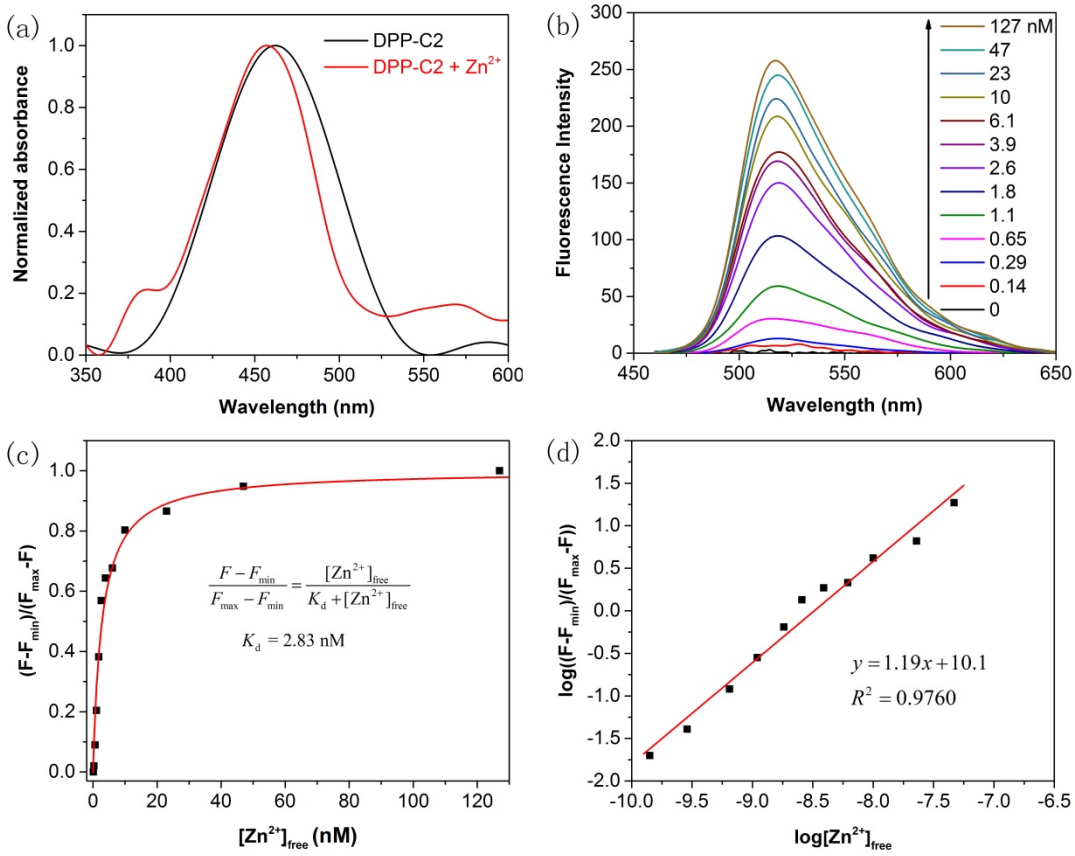


Fig. S7 - (a) UV-vis absorption (1 μM), and (b) fluorescence emission spectra of **DPP-C2** recorded in aqueous HEPES buffer (50 mM HEPES, 100 mM KCl, 10 mM EGTA, 1% DMSO, pH = 7.2) in the presence of free Zn^{2+} (in the form of its sulfate salt, 0~127 nM), (c) fluorescence titration curves, and (d) Hill's plot corresponding to the complexation of **DPP-C2** with free Zn^{2+} (0~127 nM). λ_{ex} = 430 nm.

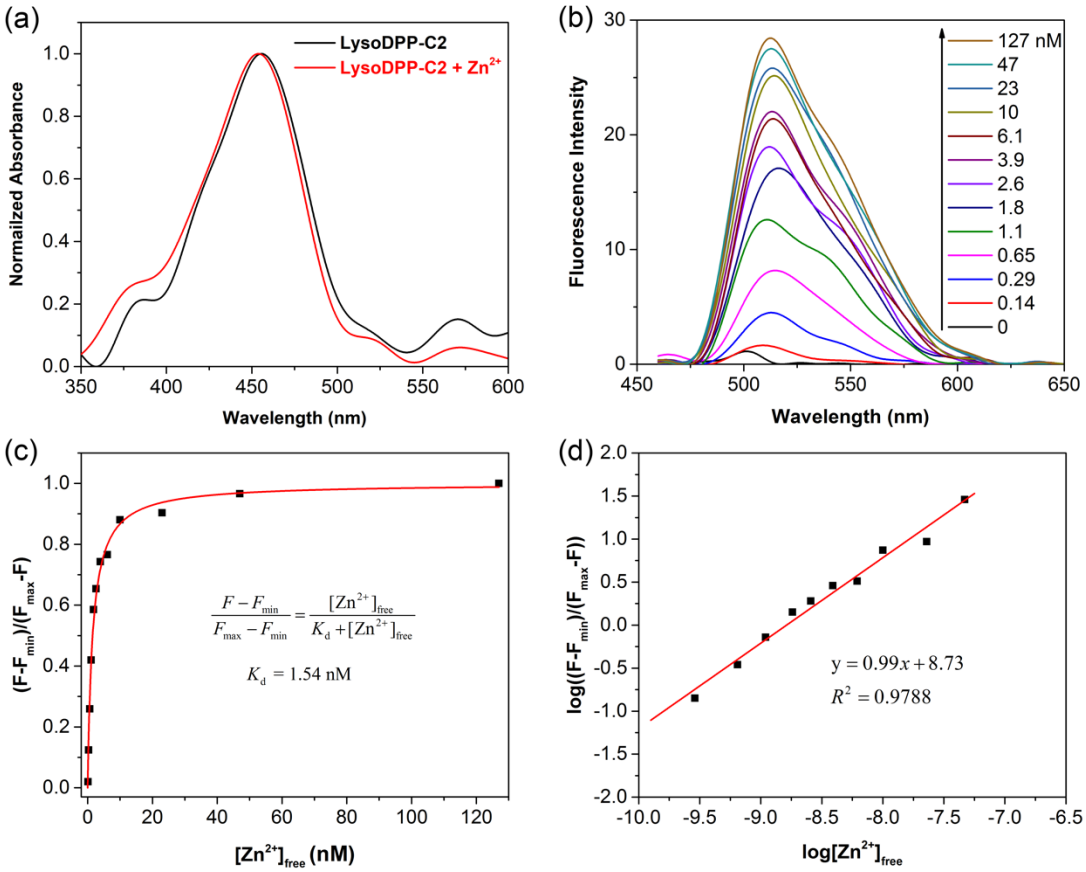


Fig. S8 - (a) UV-vis absorption (1 μM), and (b) fluorescence emission spectra of **LysoDPP-C2** recorded in aqueous HEPES buffer (50 mM HEPES, 100 mM KCl, 10 mM EGTA, 1% DMSO, pH = 7.2) in the presence of free Zn^{2+} (in the form of its sulfate salt, 0~127 nM), (c) fluorescence titration curves, and (d) Hill's plot corresponding to the complexation of **LysoDPP-C2** with free Zn^{2+} (0~127 nM). λ_{ex} = 430 nm.

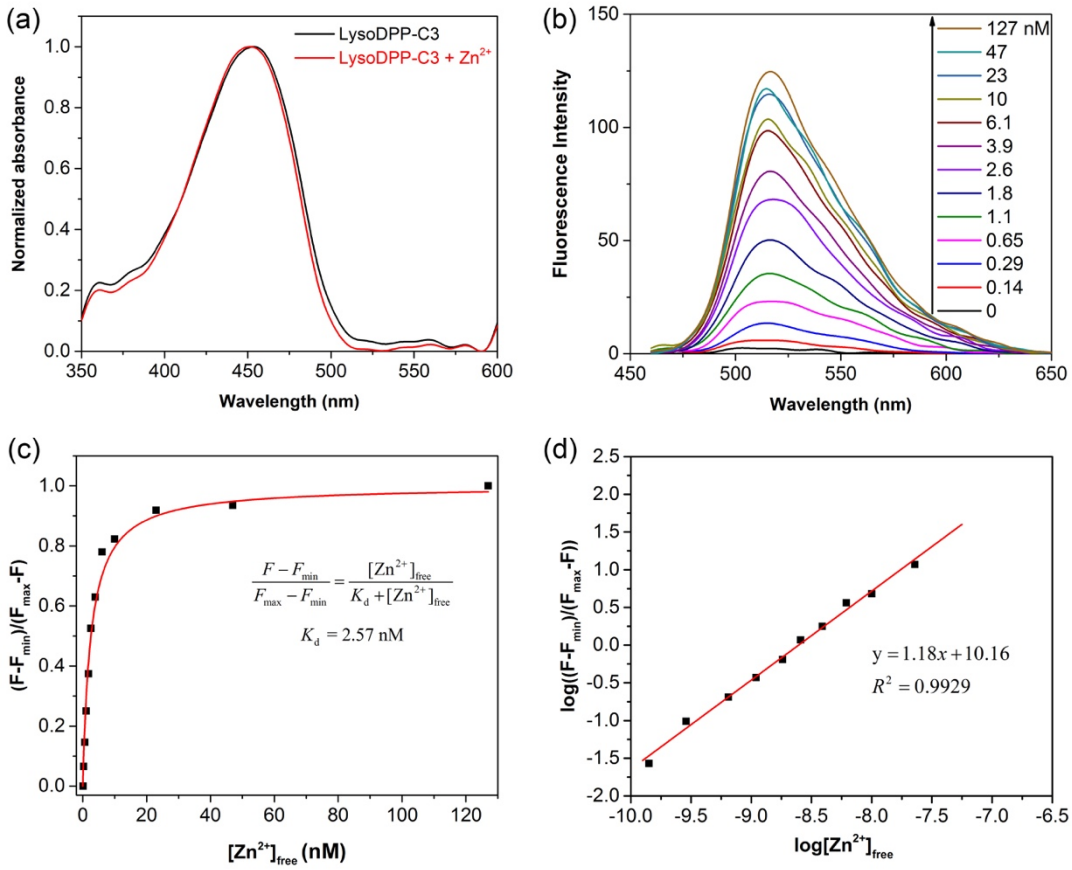


Fig. S9 - (a) UV-vis absorption (1 μM), and (b) fluorescence emission spectra of **LysoDPP-C3** recorded in aqueous HEPES buffer (50 mM HEPES, 100 mM KCl, 10 mM EGTA, 1% DMSO, pH = 7.2) in the presence of free Zn^{2+} (in the form of its sulfate salt, 0~127 nM), (c) fluorescence titration curves, and (d) Hill's plot corresponding to the complexation of **LysoDPP-C3** with free Zn^{2+} (0~127 nM). λ_{ex} = 430 nm.

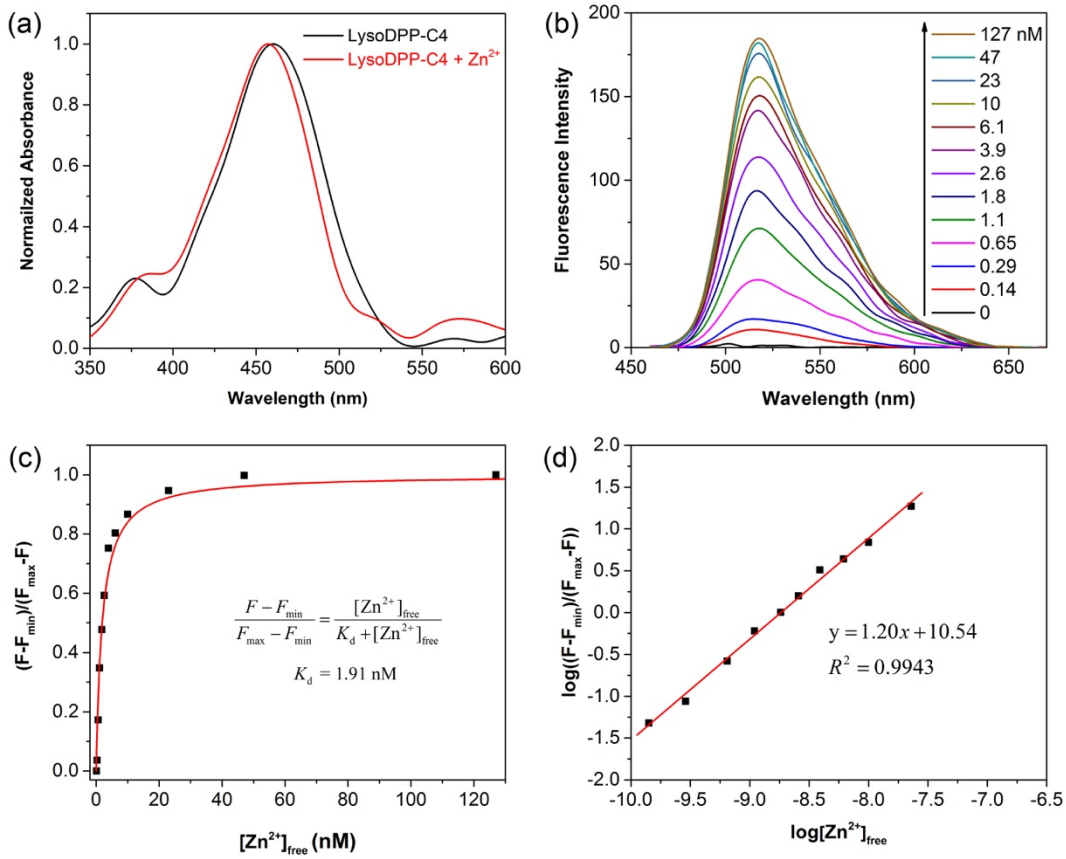


Fig. S10 - (a) UV-vis absorption (1 μM), and (b) fluorescence emission spectra of **LysoDPP-C4** recorded in aqueous HEPES buffer (50 mM HEPES, 100 mM KCl, 10 mM EGTA, 1% DMSO, pH = 7.2) in the presence of free Zn^{2+} (in the form of its sulfate salt, 0~127 nM), (c) fluorescence titration curves, and (d) Hill's plot corresponding to the complexation of **LysoDPP-C4** with free Zn^{2+} (0~127 nM). λ_{ex} = 430 nm.

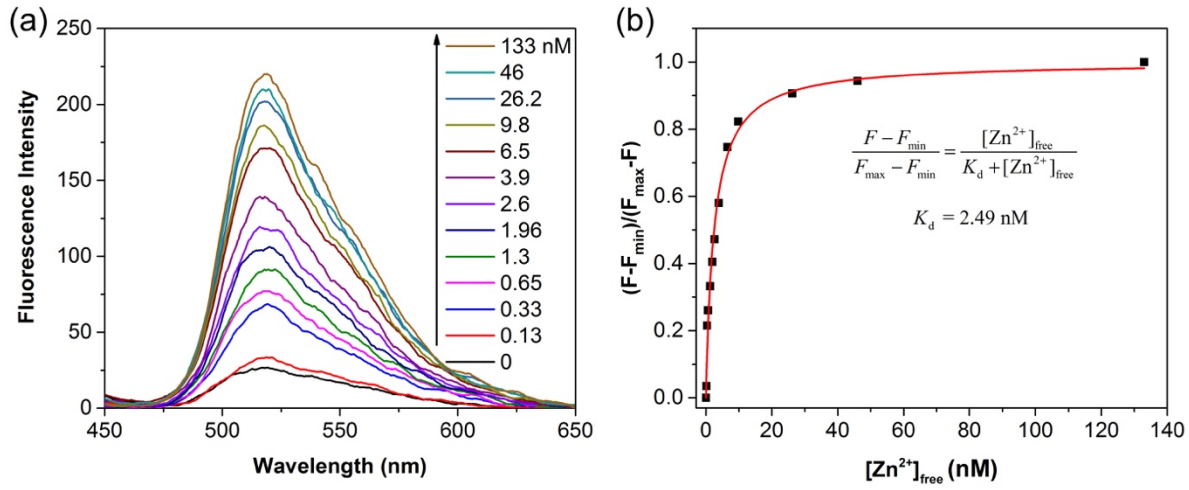


Fig. S11 - (a) Fluorescence emission spectra of **DPP-C2 (**1 μM**)** recorded in acetate buffer (100 mM sodium acetate, 100 mM KCl, 10 mM EGTA, 1% DMSO, pH = 5) in the presence of free Zn^{2+} (in the form of its sulfate salt, 0~133 nM), (b) fluorescence titration curves were fitted to the Benesi-Hildebrand equation in accord with a 1:1 binding model.

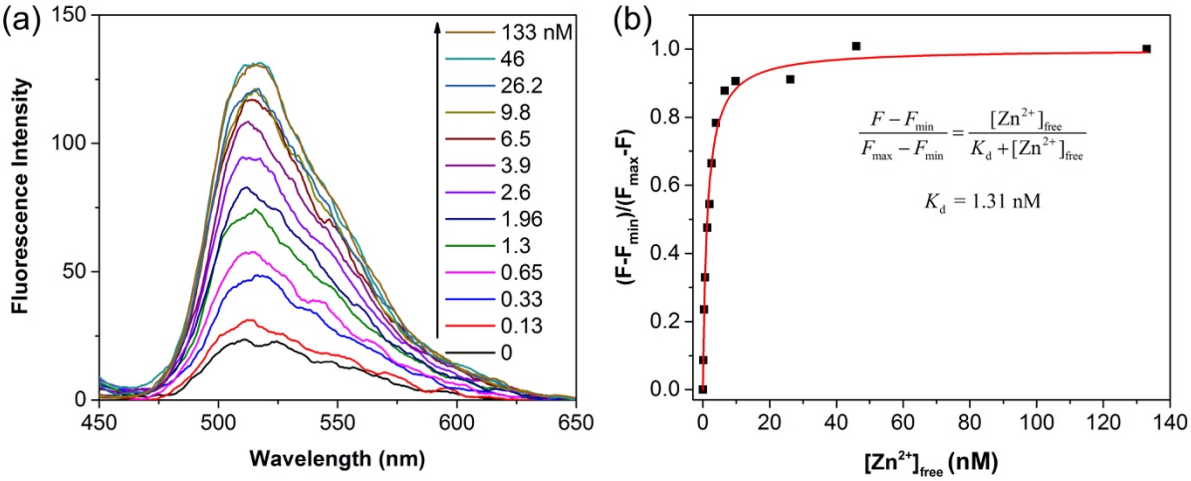


Fig. S12 - (a) Fluorescence emission spectra of **LysoDPP-C2 (**1 μM**)** recorded in acetate buffer (100 mM sodium acetate, 100 mM KCl, 10 mM EGTA, 1% DMSO, pH = 5) in the presence of free Zn^{2+} (in the form of its sulfate salt, 0~133 nM), (b) fluorescence titration curves were fitted to the Benesi-Hildebrand equation in accord with a 1:1 binding model.

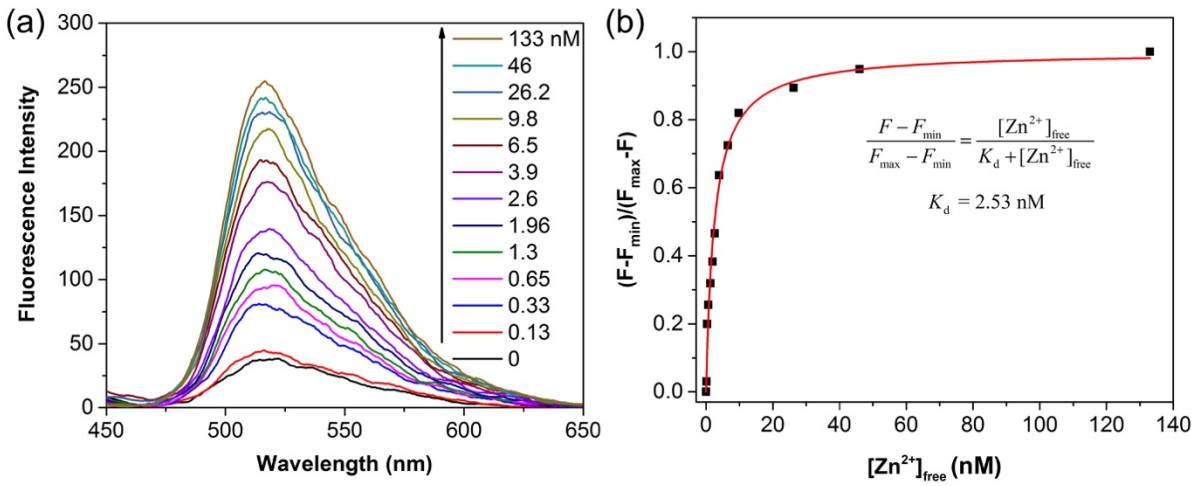


Fig. S13 - (a) Fluorescence emission spectra of **LysoDPP-C4 (**1 μM**)** recorded in acetate buffer (100 mM sodium acetate, 100 mM KCl, 10 mM EGTA, 1% DMSO, pH = 5) in the presence of free Zn^{2+} (in the form of its sulfate salt, 0~133 nM), (b) fluorescence titration curves were fitted to the Benesi-Hildebrand equation according to a 1:1 binding model.

7. Job Plots

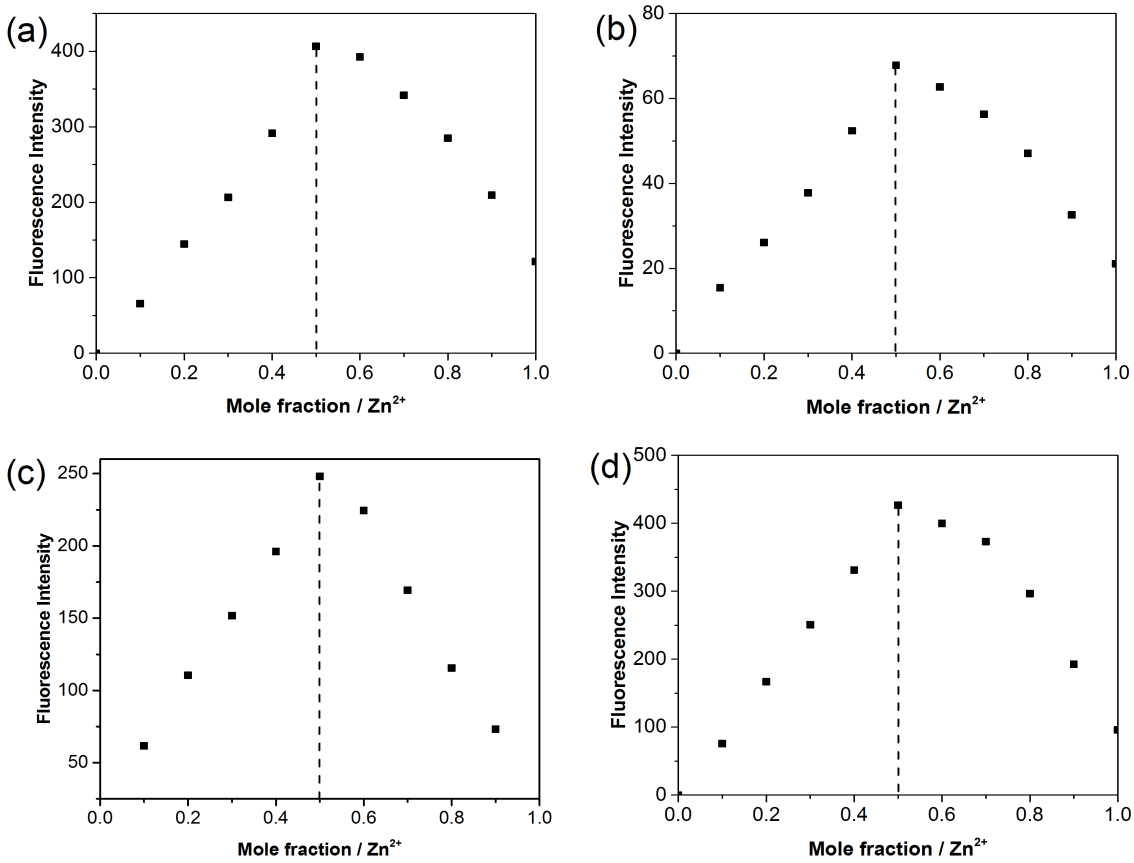


Fig. S14 - Job plots corresponding to the interaction of Zn^{2+} with the probes (the total concentration is 1 μM) of this study in aqueous HEPES buffer (50 mM HEPES, 100 mM KCl, 1% DMSO, pH 7.2): (a) **DPP-C2**, (b) **LysoDPP-C2**, (c) **LysoDPP-C3**, and (d) **LysoDPP-C4**. λex = 430 nm/ λ_{ex} = 515 nm.

8. Isothermal Titration Calorimetry (ITC)

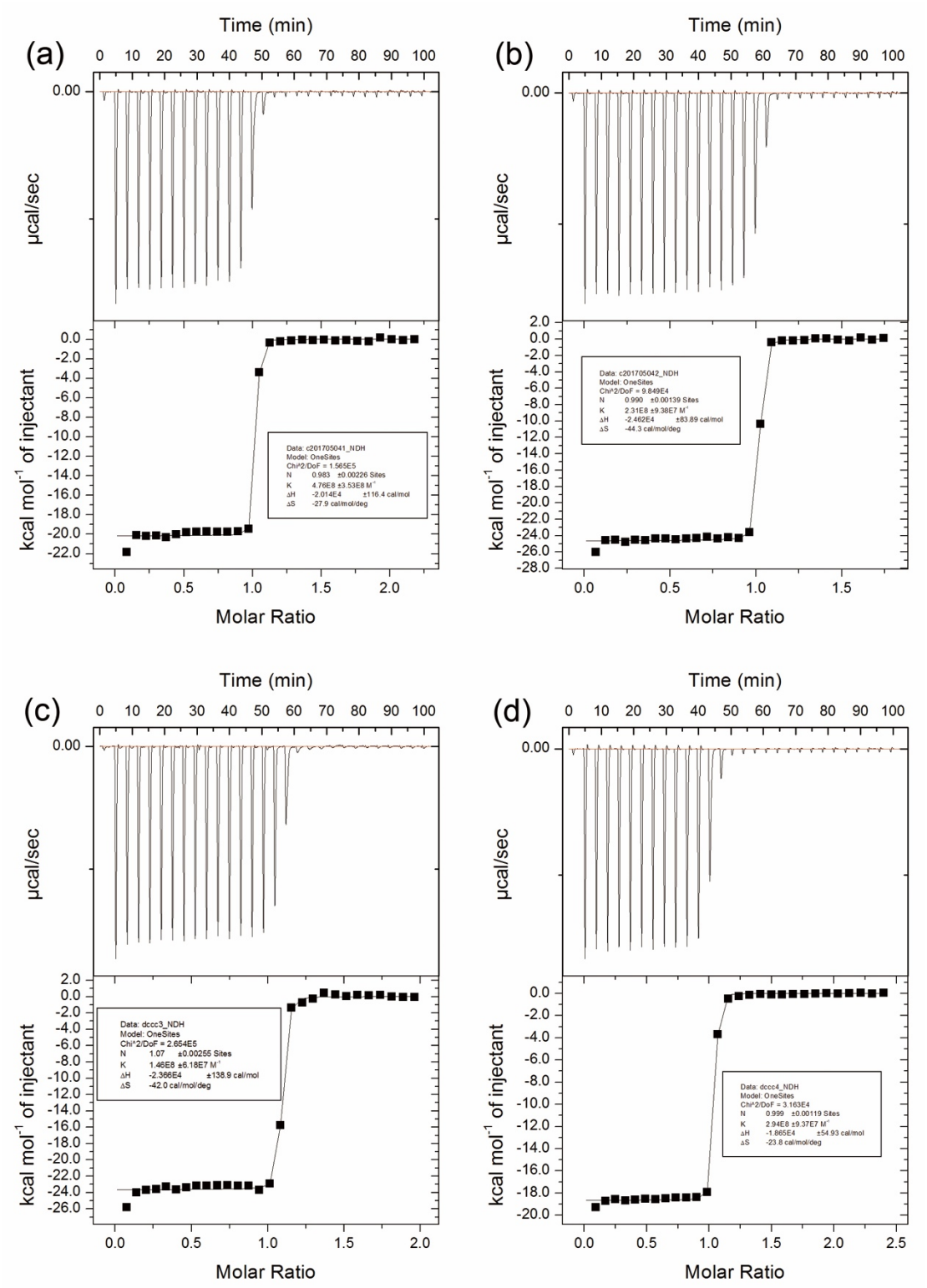


Fig. S15 - ITC traces obtained from the titration of 0.5 mM (a) **DPP-C2**, (b) **LysoDPP-C2**, (c) **LysoDPP-C3**, and (d) **LysoDPP-C4** with 0.05 mM Zn^{2+} in ethanol at 25 °C. Integrated heats of binding plotted as a function of the probe/Zn^{2+} molar ratio.

9. pH titration

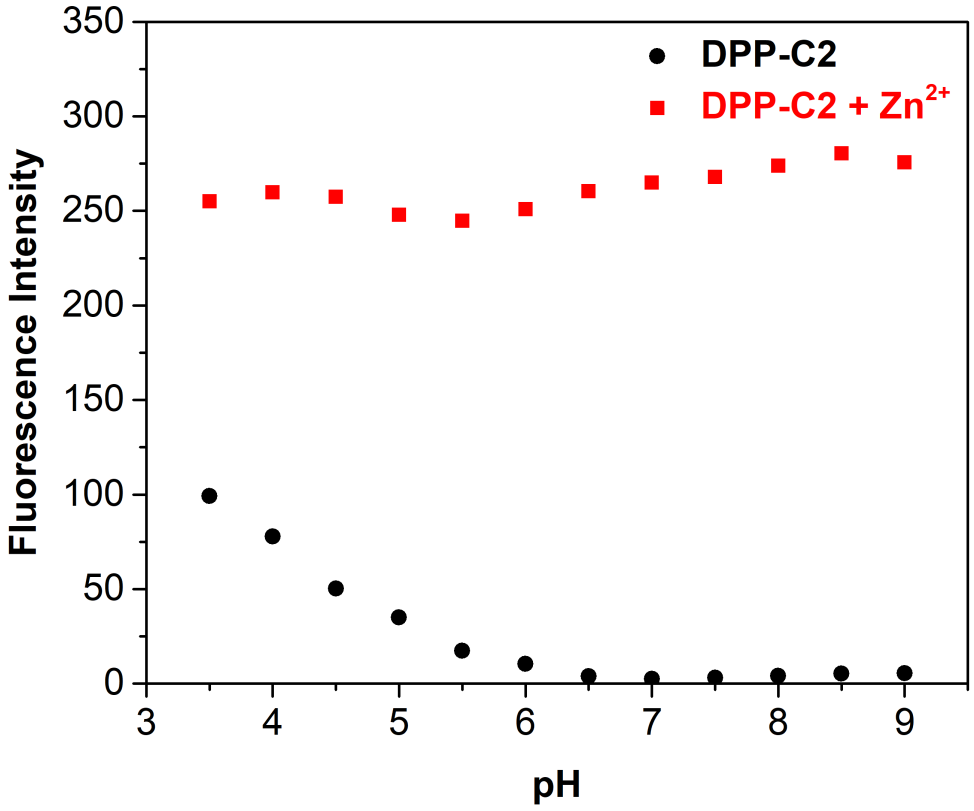


Fig. S16 – Fluorescence intensity of **DPP-C2** (1 μM) and its zinc complexes in aqueous solutions of varying pH. 0.05 M NaCl aqueous solution. λ_{ex} = 430 nm/ λ_{em} = 515 nm.

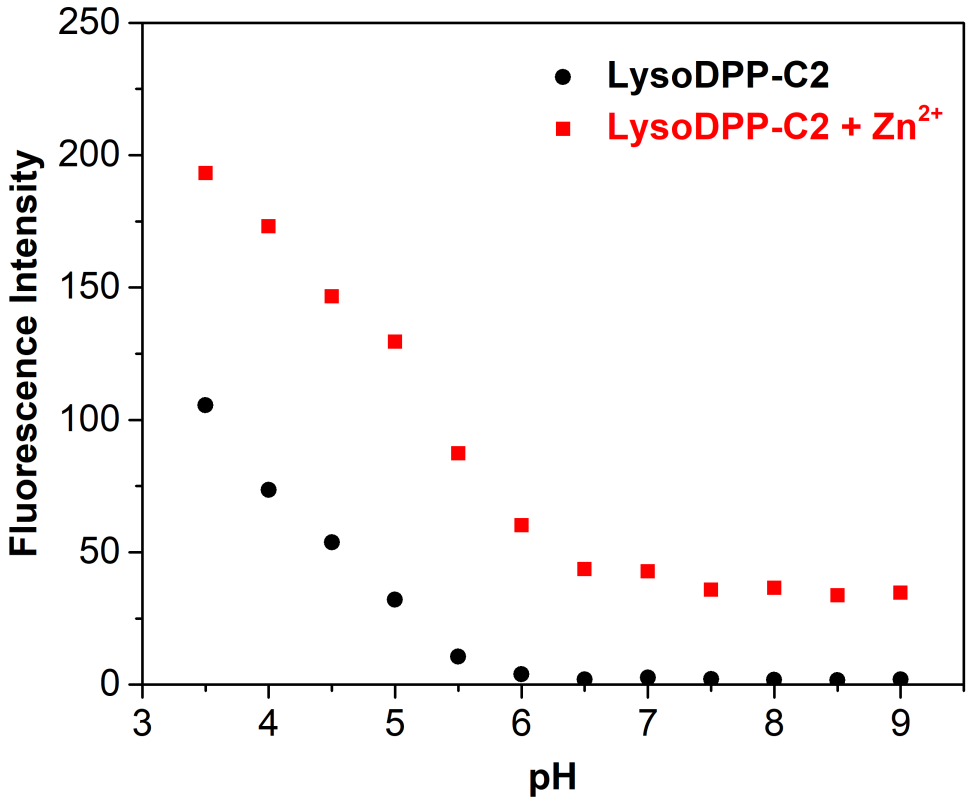


Fig. S17 - Fluorescence intensity of **LysoDPP-C2** (1 μM) and its zinc complexes in aqueous solutions of varying pH. 0.05 M NaCl aqueous solution. λ_{ex} = 430 nm/ λ_{em} = 515 nm.

10. Metal Ion Selectivity Studies

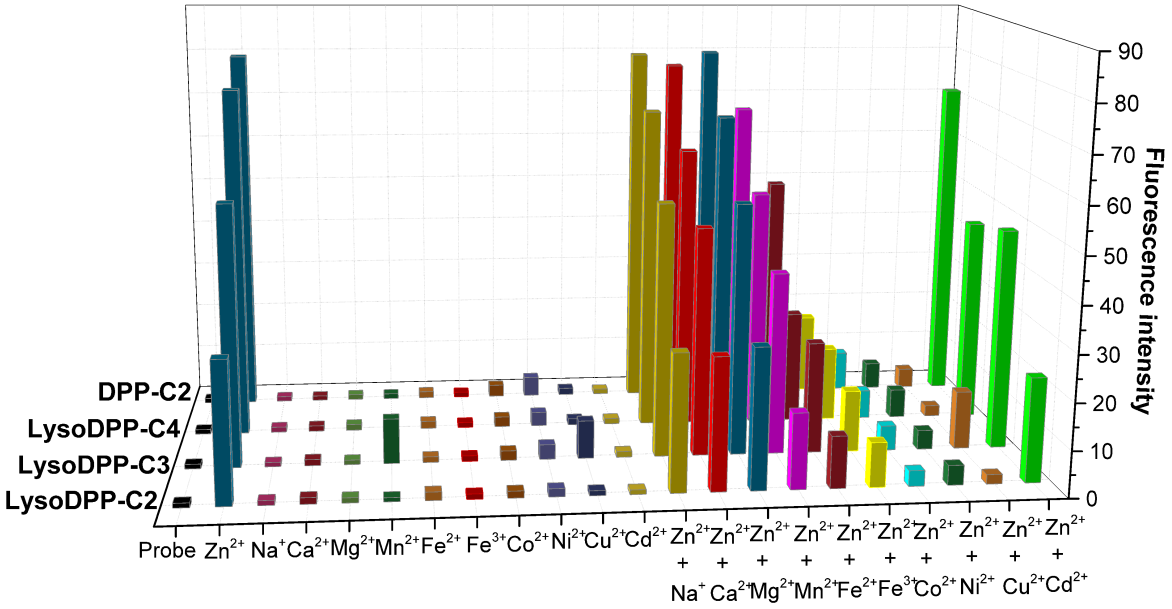


Fig. S18 - Metal ion selectivity profiles for 1 μM probes in aqueous HEPES buffer (50 mM HEPES, 100 mM KCl, 10 mM EGTA, 1% DMSO, pH 7.2). The final concentrations containing each metal cation were 10 mM for Na^{+}, Ca^{2+}, Mg^{2+}, or 5 mM for Mn^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Cd^{2+}. After each measurement, Zn^{2+} (10 or 6 mM) was added to the relevant solutions, and the fluorescence of the potentially competing samples was measured. λ_{ex} = 430 nm/ λ_{em} = 515 nm.

11. Cell Viability Studies

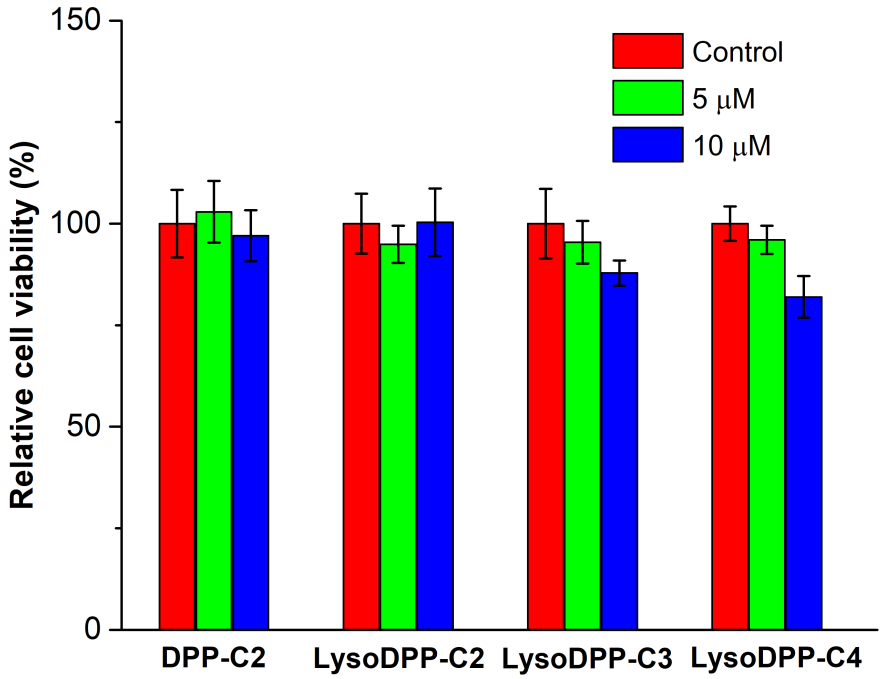


Fig. S19 - Viability of Hela cells after incubation in cell culture medium containing 5 and 10 μM of the indicated probe for 24 h as measured using the CCK-8 cell-viability assay.

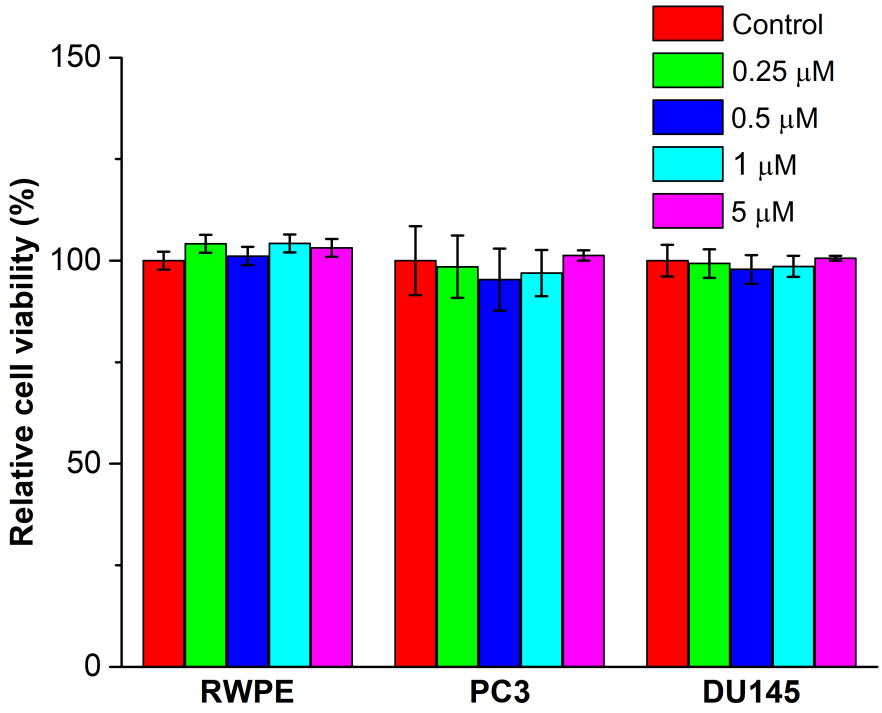


Fig. S20 - Viability of prostate cells after incubation in cell culture medium containing different concentrations of **LysoDPP-C4** for 24 h as measured by the CCK-8 cell-viability assay.

12. Flow Cytometry Analyses

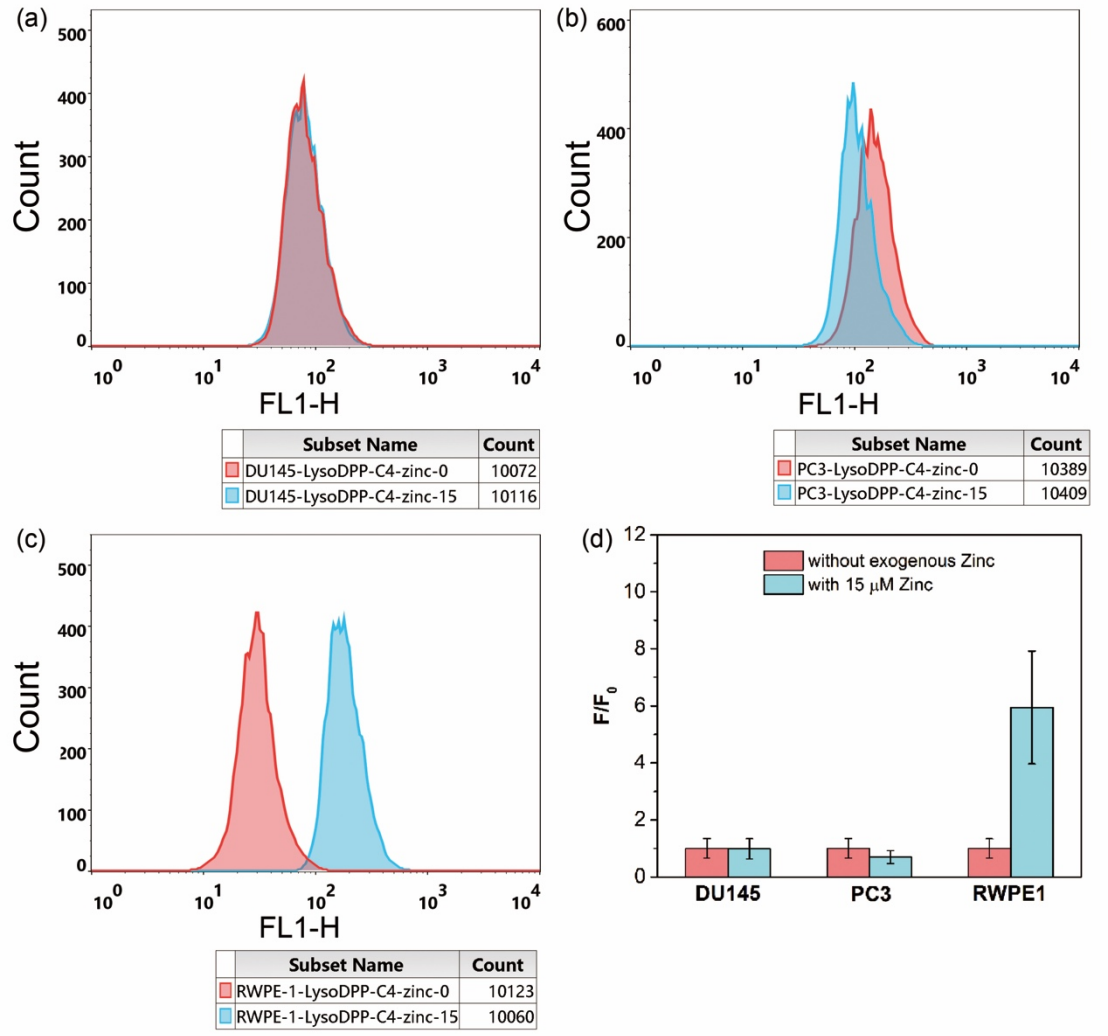


Fig. S21 - Flow cytometry analyses of (a) DU145, (b) PC3, and (c) REPE1 cells stained without or with 15 μM ZnPT as an exogenous zinc source. (d) Quantification of the change in fluorescence signal intensity of **LysoDPP-C4** in DU145, PC3, and RWPE1 cells without (red bars) or with (blue bars) 15 μM $ZnSO_4$. Only live cells were counted.

13. ICP-MS Analyses

Table S3. Zn^{2+} concentrations inferred from ICP-MS analyses of DU145, PC3, and RWPE1 cells using Sr^{2+} as an internal control.

Cells	$ZnSO_4$	Zn^{2+} (ug/L)	Sr^{2+} (ug/L)	Ratio (Zn^{2+} /Sr^{2+})
DU145	0 μM	263	61	4.31
	15 μM	283	55	5.15
PC3	0 μM	223	40	5.58
	15 μM	335	58	5.78
RWPE1	0 μM	213	47	4.53
	15 μM	785	70	11.21

14. References

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