Electronic Supplementary Material (ESI) for Analyst

Supporting Information

A long wavelength hydrophobic probe for intracellular lipid droplets

TABLE OF CONTENTS

Syntheses

General procedure for the preparation of NPBDP

We prepared the novel 3,5-dichloro-BODIPY derivative ***1c*** in a few simple steps adapting literature procedures[1,2] , starting from 4-nitrobenzaldehyde that was converted to the dipyrromethane ***1a*** with an excess of pyrrole and trifluoroacetic acid (TFA) as a catalyst. Chlorination of ***1a*** with N-chlorosuccinimide (NCS), followed by oxidation of ***1b*** with 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone(DDQ) and complexation with $BF_3 \cdot Et_2O$ gave our starting material ***1c*** in a 20% overall yield for the four steps.

5-(4-Nitrophenyl)dipyrromethane (1a)[2]

Pyrrole (2 ml, 29.6 mmol) and 4-nitrobenzaldehyde (2.23 g, 14.8 mmol) were added to a dry round-bottomed flask and degassed with a stream of N_2 for 5 min. TFA (0.1 ml) was then added, and the solution was stirred under N_2 at room temperature for 1 h and then quenched with 0.1 M NaOH. Ethyl acetate (50 ml) was then added. The organic phase was washed with water (3 x 50 ml), dried over anhydrous Na_2SO_4, filtered, and dried in vacuo. The crude product was purified by silica gel column chromatography using of petroleum ether/acetone (75:25) to afford ***1a*** as a yellow powder (3.6 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19(d, 2H, J = 8.7 Hz), 8.02(br, 2H), 7.39(d, 2H, J = 8.7 Hz), 6.77 (s, 2H), 6.20 (s, 2H), 5.89(s,2H),5.60(s,1H). ESI-MS, m/z (ES) (M^-): 266.47.

1,1'-Dichloro-5-(4-nitrophenyl) dipyrromethene (1b)

A solution of ***1a*** (3.3 g, 12.3 mmol) in 100 ml dry THF was purged with N_2 and cooled to -78^{o}C. A suspension of N-chlorosuccinimide (3.5 g, 25.9 mmol) in 40 ml THF was added to the cooled solution. The reaction mixture was stirred at -78^{o}C for 1.5 h, warmed to room temperature then stirred for an additional 3 h. Water (50 ml) was added to the mixture. After extraction with CH_2Cl_2 (3 x 100 ml), the combined organic layers were dried over anhydrous Na_2SO_4, filtered, and the solution was evaporated to dryness. The residue was used for oxidation immediately without further purification. The

oxidant DDQ (2.8 g, 12.3 mmol) was added to the solution of dichlorodipyrromethane generated above in 150 ml CH_2Cl_2. The mixture was stirred at room temperature for 1 h. After evaporation of the solvent, the residue was loaded onto a silica gel flash column, and eluted using PE/CH_2Cl_2=1:1 to afford an orange powder (2.1g, 52 % for 2 steps). ^{1}H NMR (400 MHz, CDCl3) δ 8.34 (d, 2H, *J* = 8.7 Hz), 7.65 (d, 2H, *J* = 8.7Hz), 6.43 (d, 2H, *J* = 4.3 Hz), 6.30 (d, 2H, *J* = 4.3Hz), 1.58 (br, 1H); ESI-MS, m/z (ES) (M^-): 334.42; (M^+): 336.35.

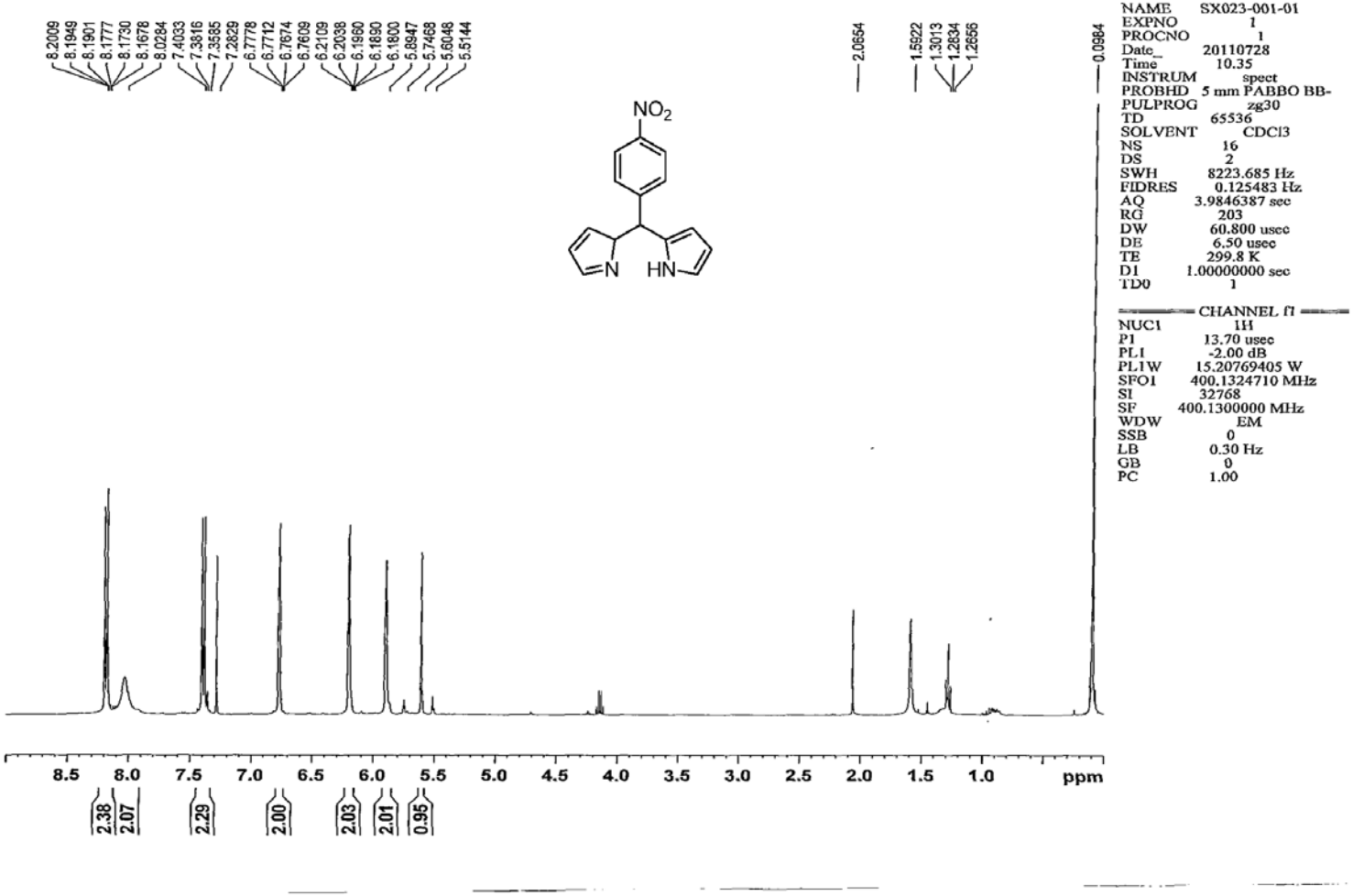
3,5-Dichloro-8-(4'-nitrophenyl)-4,4-difluoro-4-bora-3a,4a-diaza-s-indacence (1c)

The reaction procedure of ***1c*** is according to literature[2]. (89%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42(d, 2H, J = 8.7 Hz), 7.72(d, 2H, J = 8.6 Hz), 6.78 (d, 2H, J = 4.3 Hz), 6.50 (d, 2H, J = 4.4 Hz).

4,4-Difluoro-3,5-dipiperidine-8-(4-nitrophenyl)-4-bora-3a,4a-diaza-s-indacene (NPBDP)[3]

To a solution of compound ***1c*** in CH_3CN was added 4.2eq piperidine, the solution was heated to 140℃ for 3hs when it gradually turned from red to blue. The reaction was monitored by thin layer chromatography until it showed the raw

material was completely consumed. Water was added to the cooled solution and ethyl acetate was used to extract. On removing the solvent by evaporation in vacuum, a dark blue powder was obtained via chromatography on silica gel column, with the eluting solvent of 3:1 petroleum ether/ ethyl acetate. ***1d*** was the by-product of the reaction accompanying the main product ***NPBDP. NPBDP*: (83%)** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (d, 2H, J=8.6Hz), 7.64(d, 2H, J=8.7Hz), 6.42(d, 2H, J=4.5Hz), 6.02(d, 2H, J = 4.5 Hz), 3.56(t, 8H, J=5.3 Hz), 1.78(t, 8H, J=5.7 Hz), 1.71(t, 4H, J=13.8Hz); ESI-MS, m/z (ES) (M^+): 480.44. ***1d*: (10%)**^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (d, 2H, J=8.7Hz), 7.63 (d, 2H, J=8.7Hz), 6.75 (d, 1H, J=5.3Hz), 6.40 (d, 1H, J = 5.3 Hz), 6.22 (d, 1H, J=3.8 Hz), 6.16 (d, 1H, J=3.8 Hz), 3.99 (t, 4H J=5.1Hz), 1.84 (m, 6H); ESI-MS, m/z (ES) (M^+): 432.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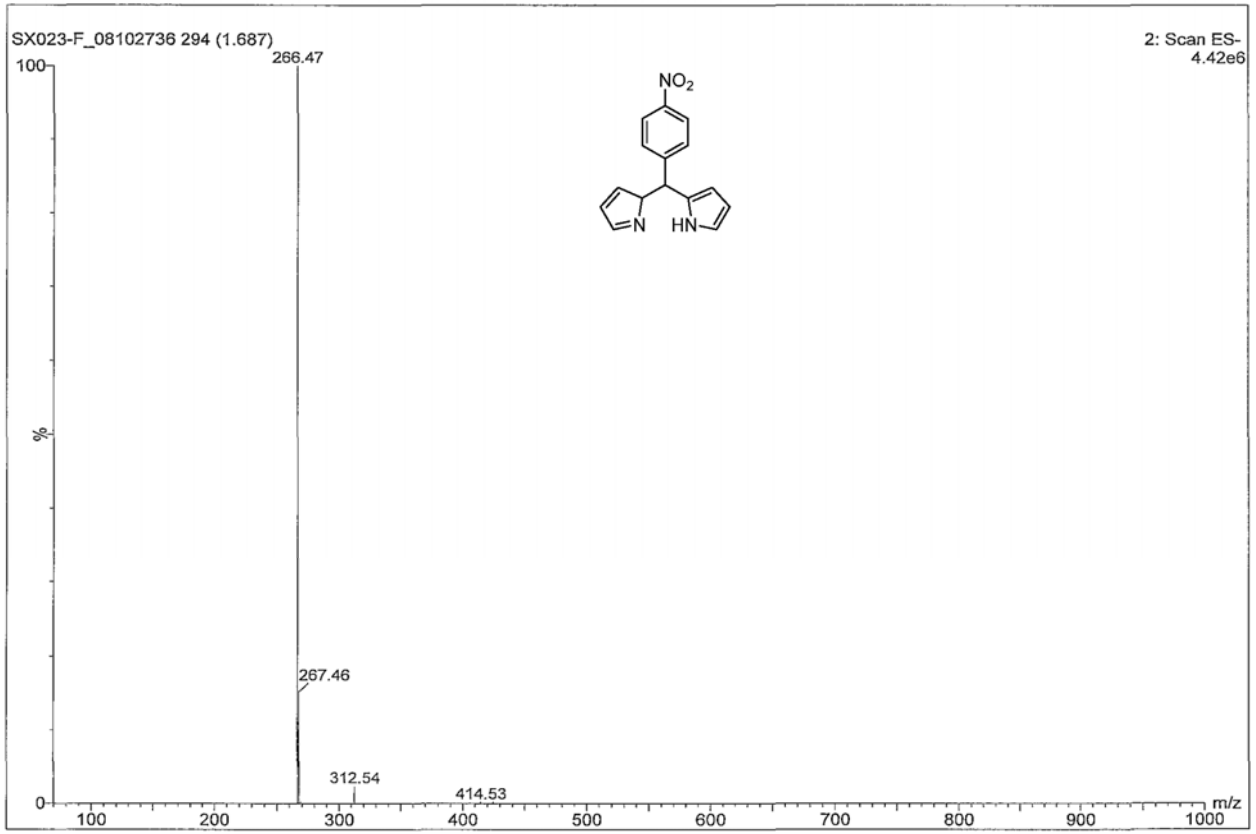
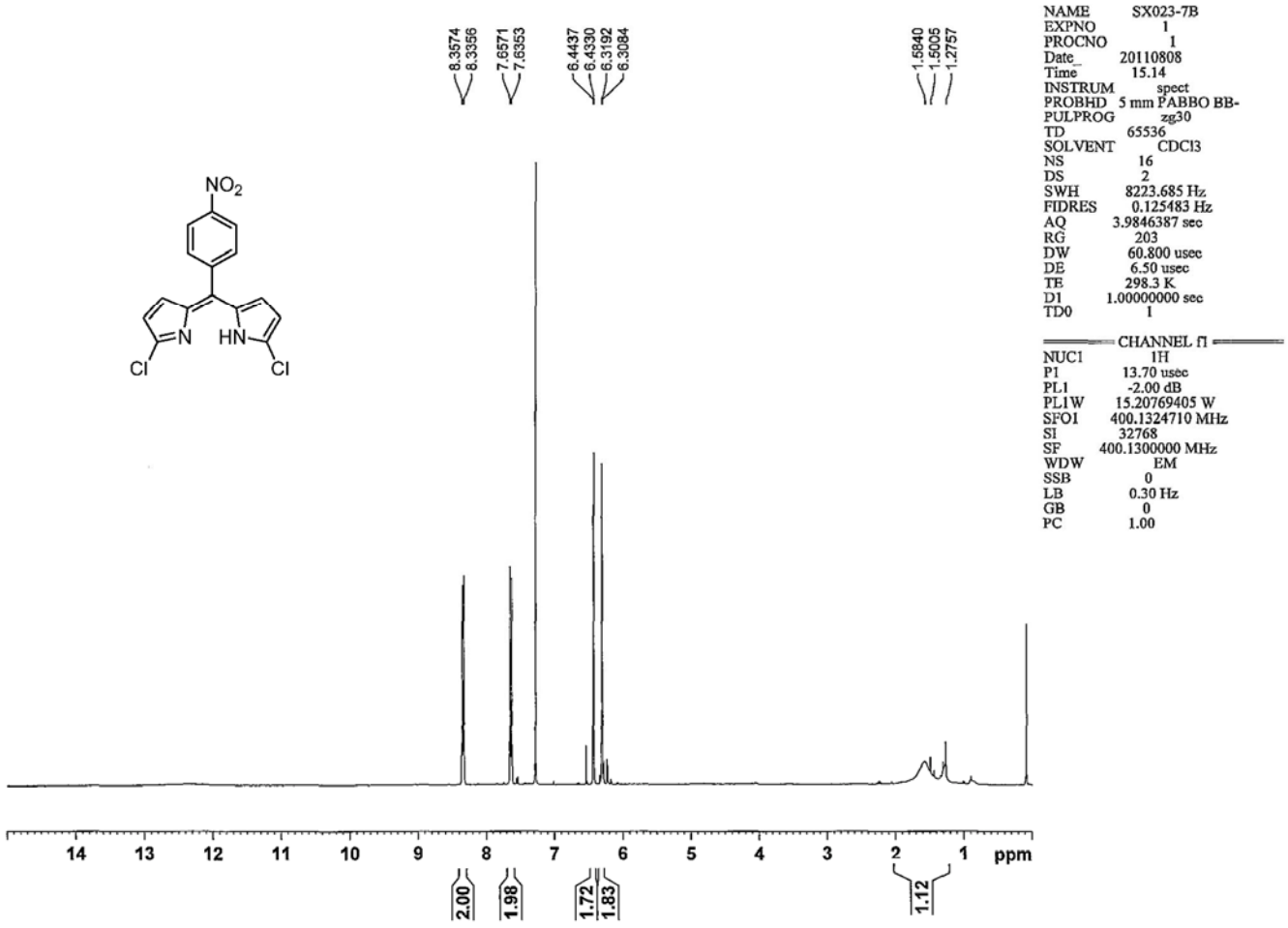


Figure S1. ^{1}H NMR (400 MHz, $CDCl_3$) and mass spetra (acetonitrile) of *1a*



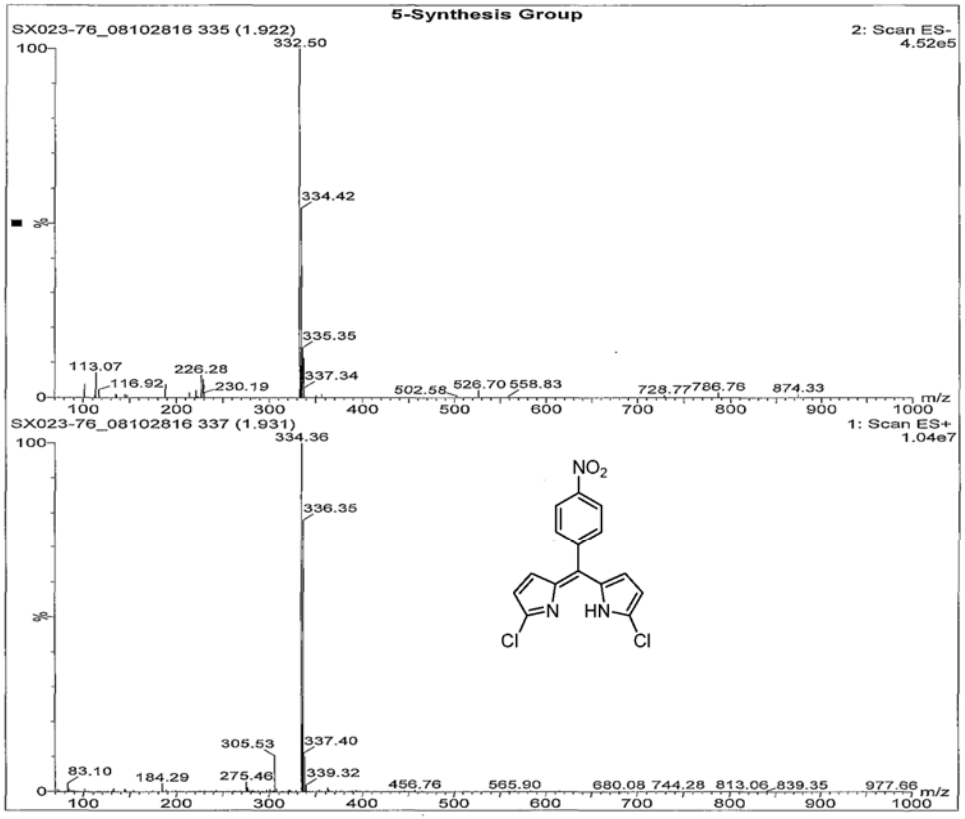


Figure S2. ^{1}H NMR (400 MHz, $CDCl_3$) and mass spetra (acetonitrile) of *1b*

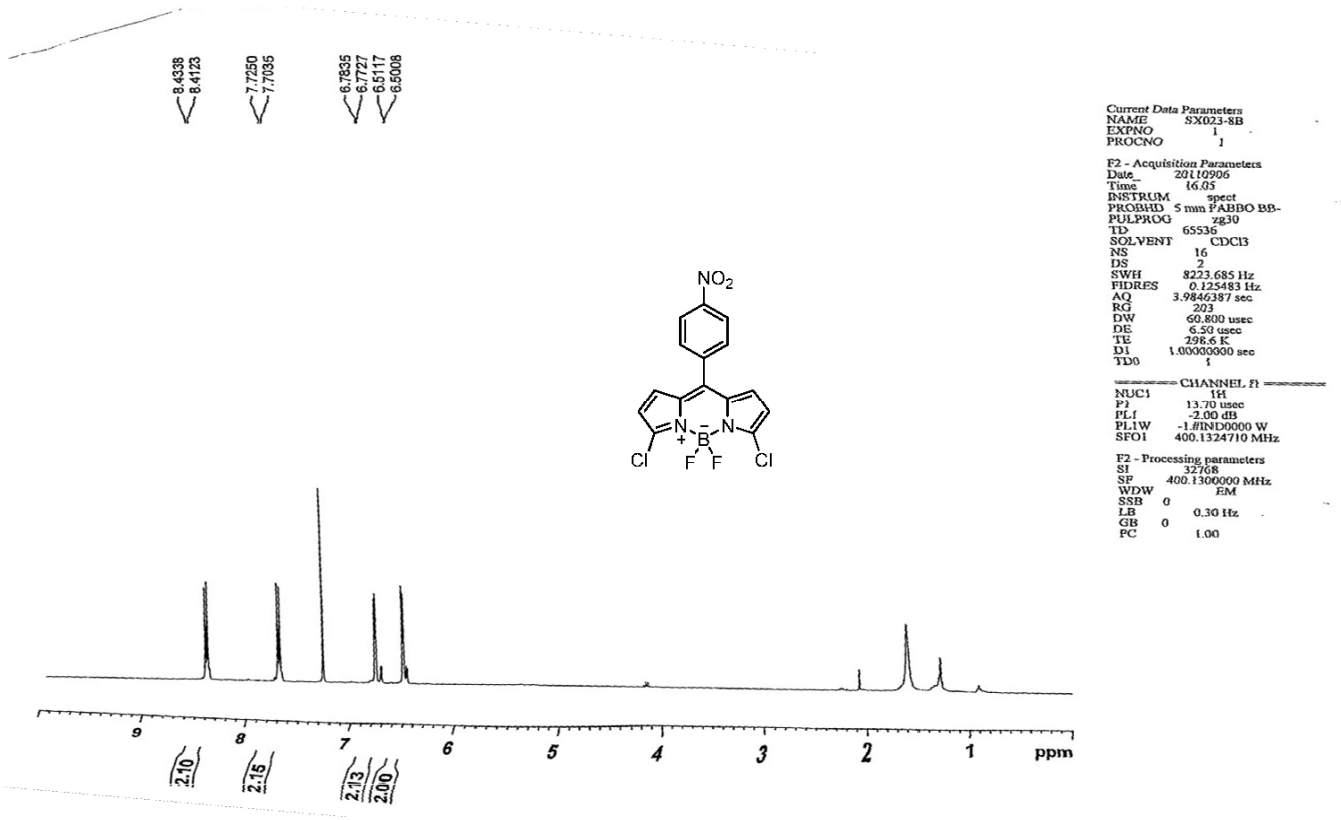
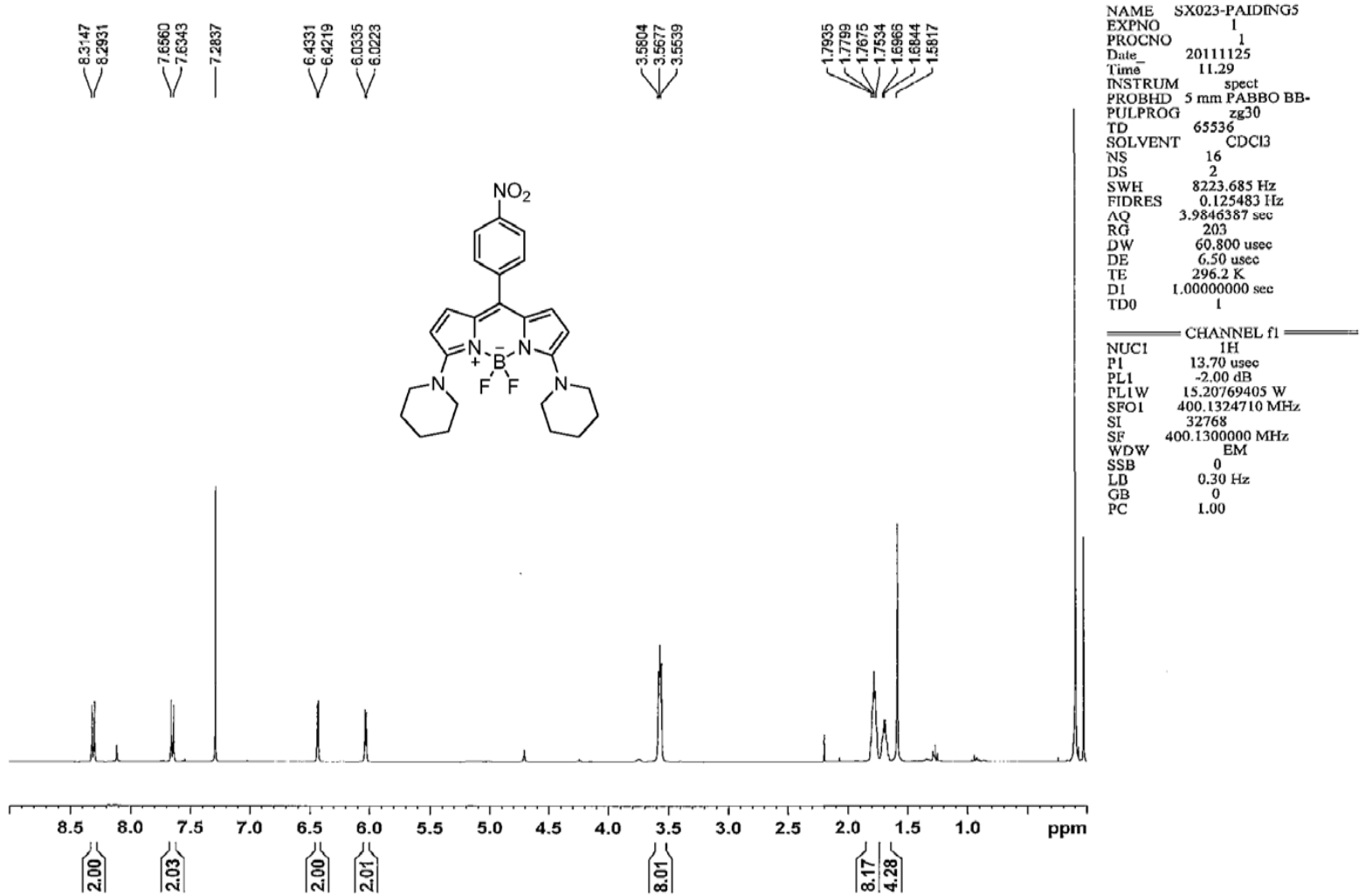


Figure S3. ^{1}H NMR (400 MHz, $CDCl_3$) of *1c*



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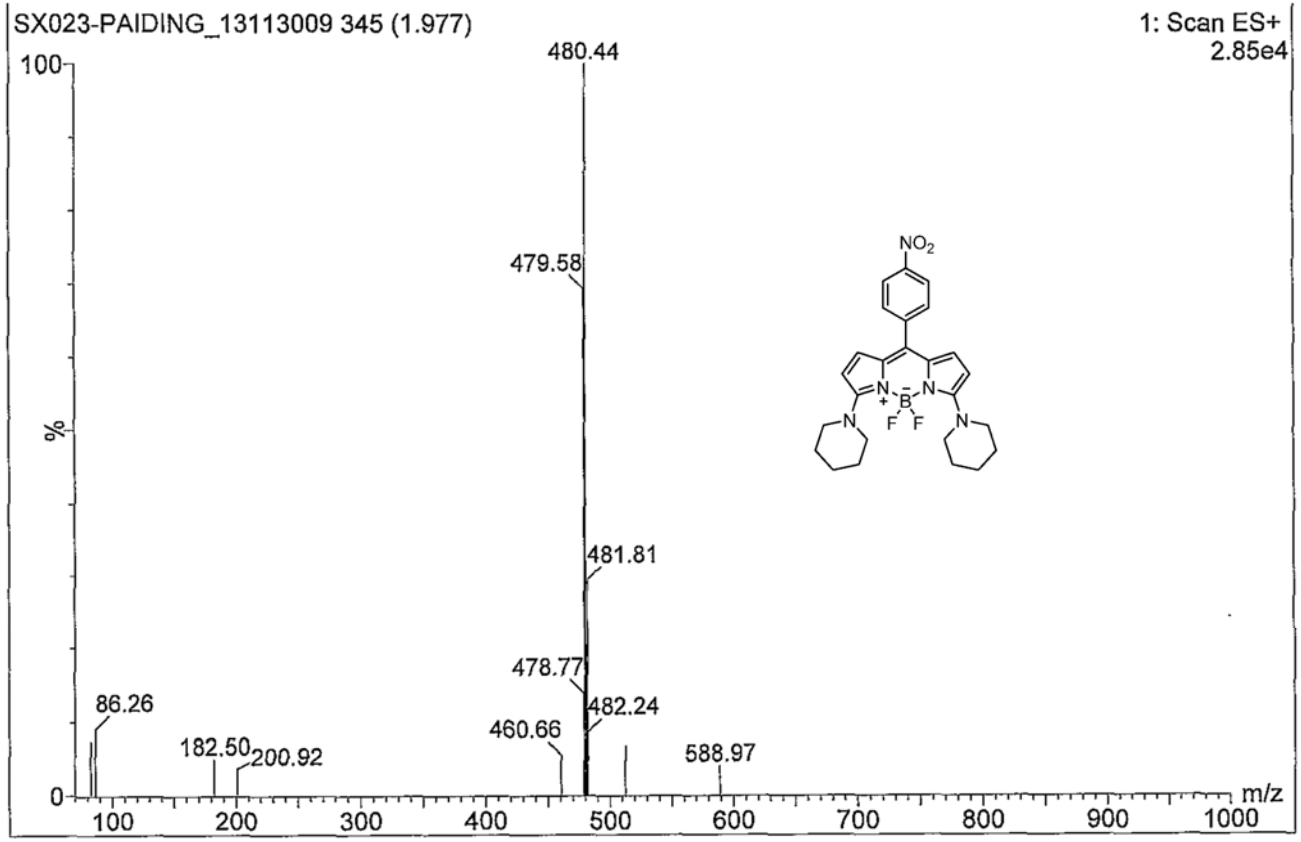
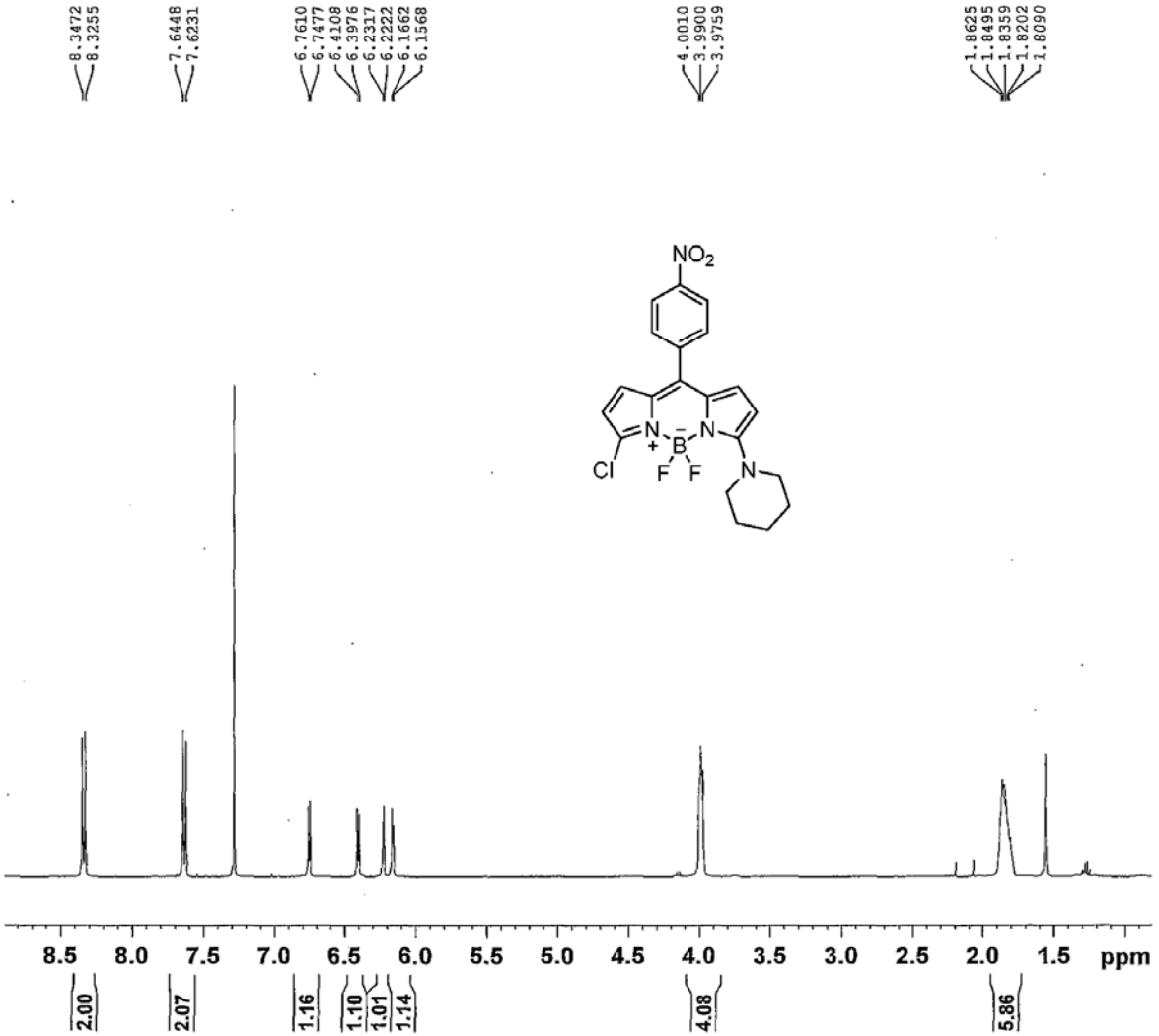


Figure S4. ^{1}H NMR (400 MHz, $CDCl_3$) and mass spetra (acetonitrile) of *NPBDP*

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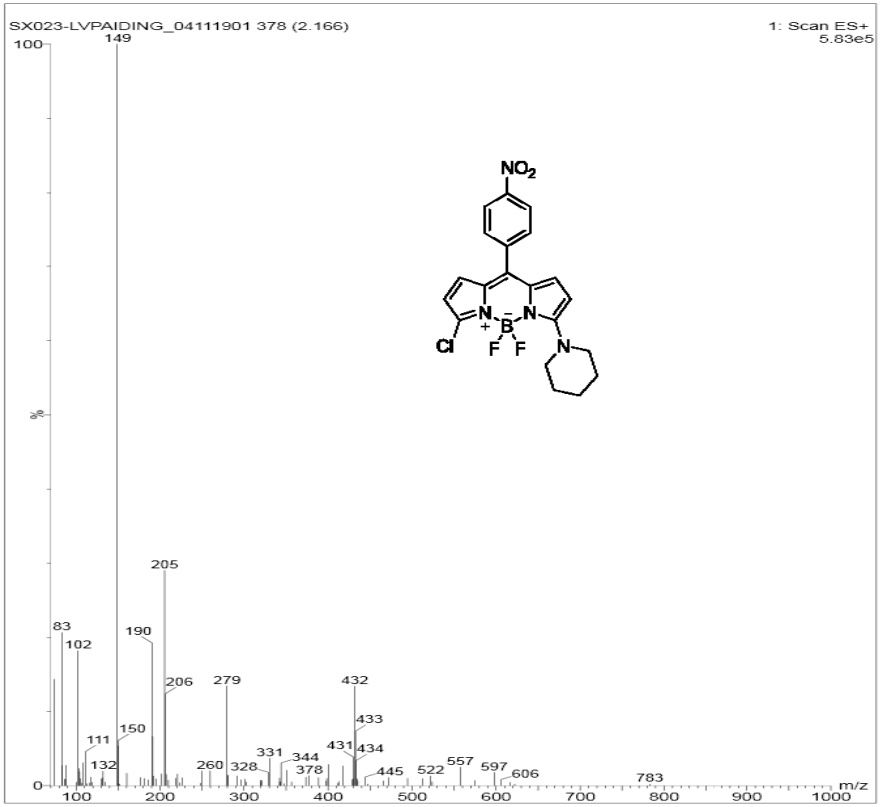

Figure S5. ^{1}H NMR (400 MHz, $CDCl_3$) and mass spetra (acetonitrile) of *1d*

Experimental Section

Reagents and Instrumentation

High molecular weight poly(vinyl chloride) (PVC), bis (2-ethylhexyl) sebacate (DOS) and tetrahydrofuran (THF) were purchased from Fluka (Switzerland). 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (lipid DPPC) and tripopyl phosphate (TPP) were purchased from Sigma-Aldrich (Switzerland). Dimethyl phthalate (DMP) and dibutyl phthalate (DBP) were purchased from Alfa Aesar. Acetonitrile, ethanol, dichloromethane, cyclohexane, toluene were purchased from Nanjing. 3,3′-dioctadecyloxacarbocyanine perchlorate(DiO or $DiOC_{18}(3)$) was purchased from Beyotime Institute of Biotechnology.

Perkin-Elmer LS50B fluorescence spectrometer was used for fluorescence measurement of **NPBDP**. Zeiss LSM710 laser-scanning confocal microscope and Prism and Reflector Imaging Spectroscopy System (PARISS) with Olympus BX53 microscope were used to obtain the fluorescence imaging and spectra of the probe in the lipid bilayer and MCF7 cells. The preparation of **NPBDP** is depicted in scheme 1; the syntheses and characterizations have been described in detail in the Supporting Information.

Homogeneous Solution Detection

Fluorescent **NPBDP** was dissolved in various solvents including acetonitrile, ethanol, dichloromethane, cyclohexane, toluene with a concentration of 5uM for homogeneous fluorescence detection. Then 3.5 mL of the mixture was transferred into a quartz cuvette for fluorescence measurement. The maximum excitation and maximum emission wavelengths of **NPBDP** in cyclohexane are as follows: λ_{ex} = 618 nm, λ_{em} = 677 nm (excitation slit width, 15 nm; emission slit width, 15 nm; scanning speed, 100 nm/min).

Membrane probe $DiOC_{18}$ was dissolved in cyclohexane and ethanol to obtain the concentration of 10^{-7} M. The excitation wavelength is λ_{ex} = 485 nm, the maximum emission wavelengths of the $DiOC_{18}$ in ethanol and cyclohexane are λ_{em} = 501 nm and λ_{em} = 513 nm, respectively (excitation slit width, 10 nm; emission slit width, 10 nm; scanning speed, 100 nm/min, 1% T attenuator).

Preparation of Polymeric Measurements

A total amount 100 mg of mixture containing 3mmol/kg **NPBDP** or $DiOC_{18}$, PVC and the plasticizer (DOS, DBP, DMP, TPP) according to 1:2 by weight was prepared and dissolved in 1 mL of THF. After complete dissolution, 50 μL

cocktail was deposited with a pipet onto quartz slides (44 mm ×11 mm) and remaining solvent was left to evaporate in a draft hood for at least 0.5 h prior to measurements.

In all measurements, the excitation wavelength of polymeric membrane containing **NPBDP** was chosen at 612 nm (excitation slit width, 11 nm; emission slit width, 11 nm; scanning speed, 100 nm/min).

The excitation wavelength of polymeric membrane containing **$DiOC_{18}$** was chosen at 489 nm (excitation slit width, 7 nm; emission slit width, 7 nm; scanning speed, 100 nm/min, 1% T attenuator).

Preparation the lipid bilayers

Lipid was mixed with 0.4μmol fluorescent probes in chloroform and dried under N_2 followed by desiccation under house vacuum for at least 2 h. The mixtures were reconstituted in 1 ml sodium phosphate solutions pH 7.4. Suspension of the lipids in solution was achieved by vortexing for several minutes at low speed. Small unilamellar vesicles were formed by probe sonication. Then, 10μL small unilamellar vesicles solution was dropped on the 0.17 mm thickness microscope cover glass. Let the water evaporate at least 2 h prior to measurements.

Preparation and Staining of Cell Cultures

MCF 7 cells were cultured in high-glucose Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum (FBS, Invitrogen) and 1% penicillin/streptomycin (Invitrogen). One day before imaging, cells were passed and plated in 35 mm diameter confocal petri dish or plated on 0.17mm thickness microscope cover glass. Before the experiments, cells were washed three times with PBS buffer (pH 7.4), incubated with 10μM probe in medium, and imaged.

Fluorescence Imaging Experiments

Confocal fluorescence imaging was performed with a Zeiss LSM710 laser-scanning confocal microscope and 63×oil objective lens. Excitation of **NPBDP** -loaded MCF7 cells and lipid bilayer were carried out at 543 nm HeNe laser and emission was collected in a window from 571 nm to 684 nm. Prism And Reflector Imaging Spectroscopy System (PARISS) and Olympus BX53 microscope were used to obtain the fluorescence imaging and spectra of the probe (excited with green light) in the lipid bilayer and MCF7 cells. Emission spectral and images were obtained from spectrum camera CCD and image camera CCD respectively.

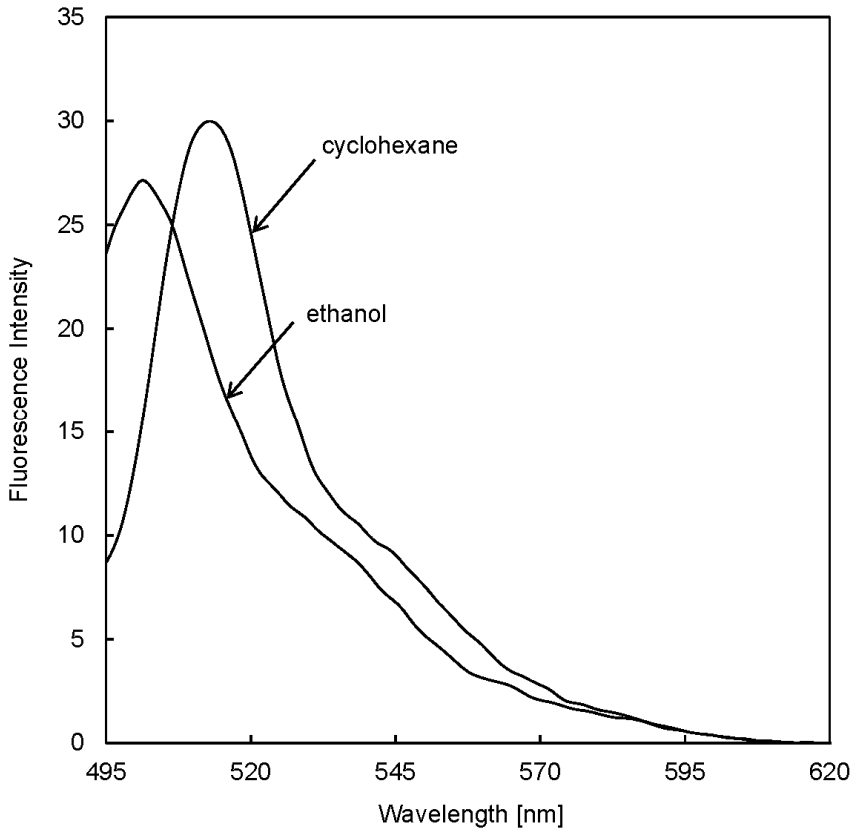

Figure S6. Fluorescence spectra of 10-7 M DiOC18 in ethanol and cyclohexane. The excitation wavelength is λ_{ex} = 485 nm, the maximum emission wavelengths of the $DiOC_{18}$ in ethanol and cyclohexane are λ_{em} = 501nm and λ_{em} = 513 nm, respectively.

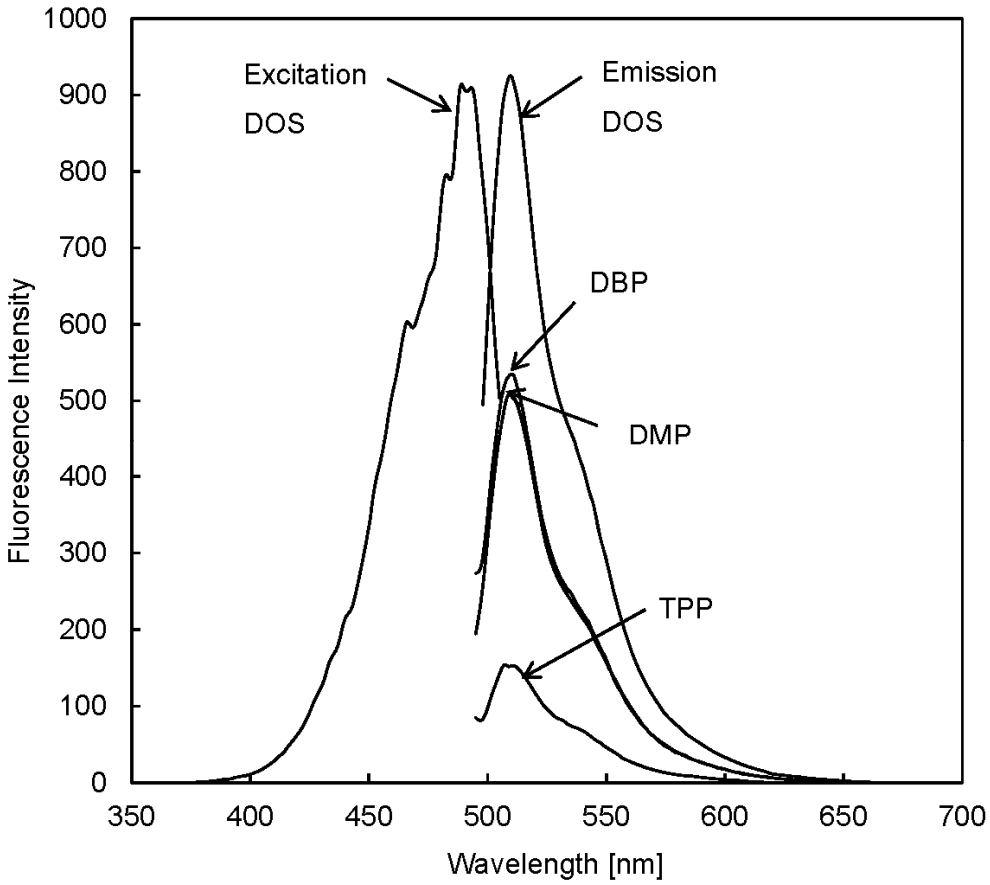

Figure S7. Fluorescence spectra of PVC- $DiOC_{18}$ (3mmol/kg) with different plasticizers DOS , DBP, DMP, TPP. λ_{ex} = 489 nm.

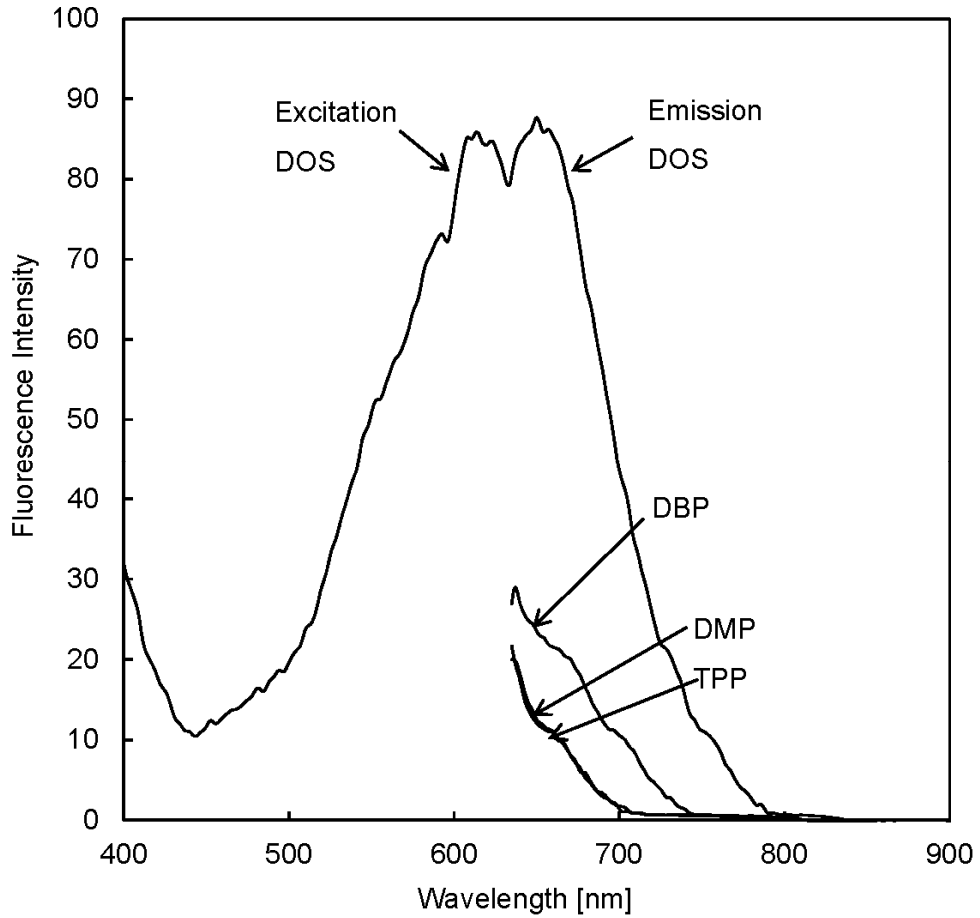

Figure S8. Fluorescence spectra of PVC- NPBDP (3mmol/kg) with different plasticizers DOS , DBP, DMP, TPP. λ_{ex} = 612 nm.

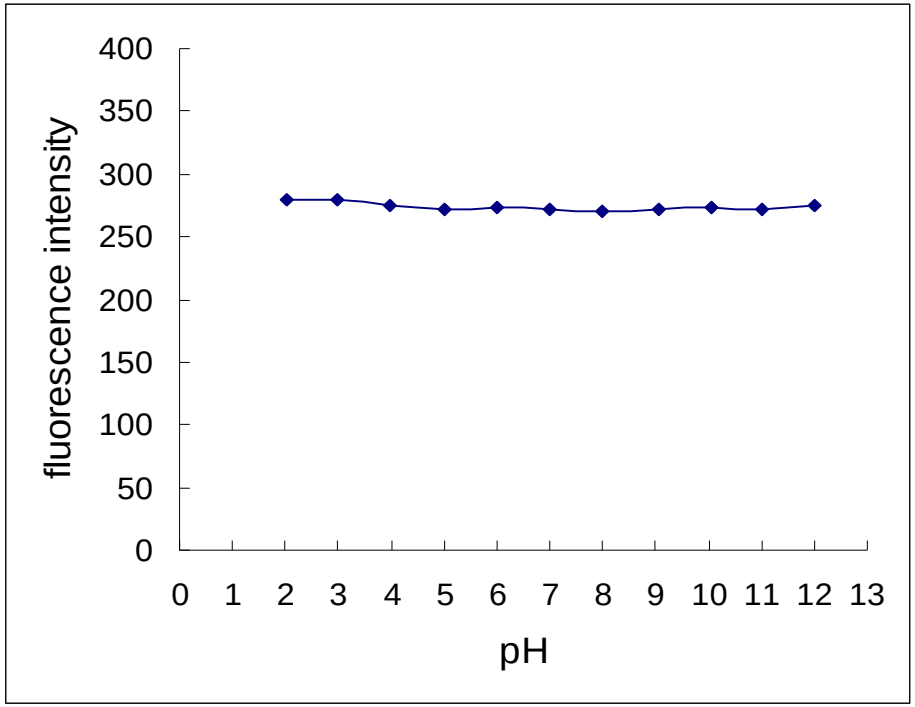

Figure S9. pH response of NPBDP

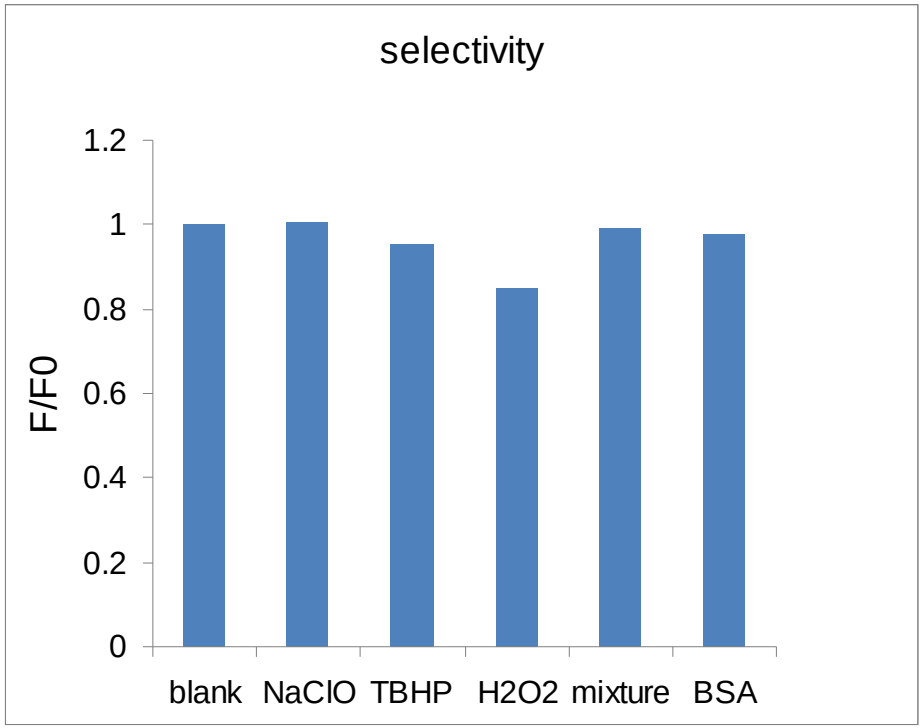

Figure S10. Selectivity of NPBDP. Mixture is the solution containing NaCl, KCl, amino acid and glucose.

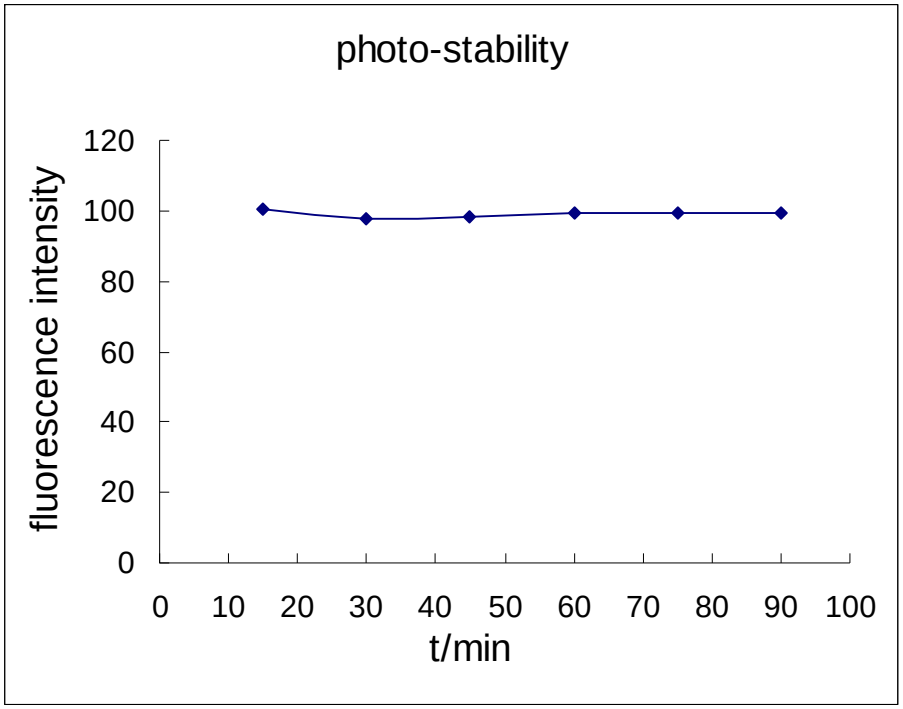

Figure S11. Photo stability of NPBDP in the cell

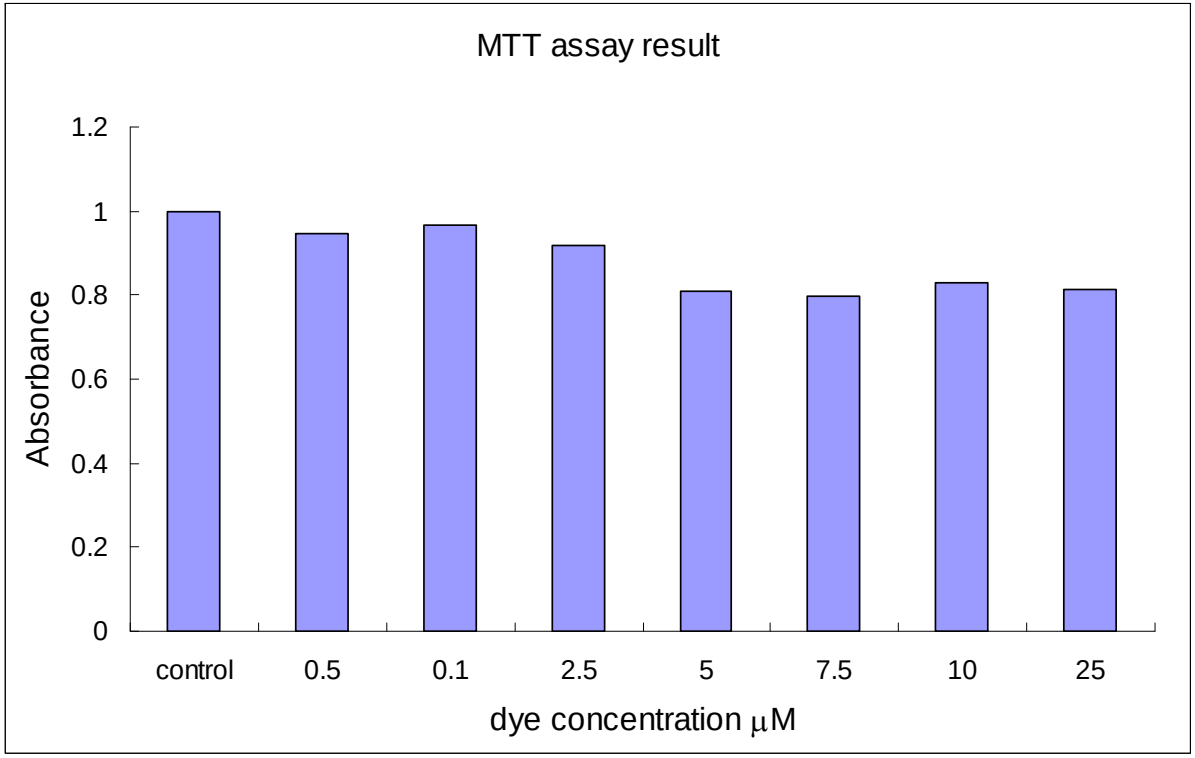

Figure S12. The MTT assay to get the activity of the cells by using ELIASA to detect the absorption at 570 nm.

Table 1. The ratios of maximum intensity of dyes in DOS to other different plasticizers. NPBDP: λ_{ex} = 612 nm, λ_{em} = 650 nm; $DiOC_{18}$: λ_{ex} = 489 nm, λ_{em} = 510 nm.

Plasticizer	PVC-NPBDP	PVC-$DiOC_{18}$
	DOS/plasticizers	DOS/plasticizers
DOS	1.0	1.0
DBP	3.7	1.7
DMP	7.2	1.8
TPP	7.0	6.0

(1) Littler, B. J.; Miller, M. A.; Hung, C. H.; Wagner, R. W.; O'Shea, D. F.; Boyle, P. D.; Lindsey, J. S. *J. Org. Chem.* **1999**, *64*, 1391-1396.

(2) Li, L.; Han, J.; Nguyen, B.; Burgess, K. *J. Org. Chem.* **2008**, *73*, 1963-1970.

(3) Rohand, T.; Baruah, M.; Qin, W.; Boens, N.; Dehaen, W. *Chem. Commun.* **2006**, 266-268.