Supporting Information

Simultaneous dual-colour tracking lipid droplets and lysosomes dynamics using a fluorescent probe

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1) Experimental details

General Information

All the commercially available chemicals were directly used without further purification unless otherwise stated. All the solvents are of analytical grade and were freshly distilled prior to use. Nuclear magnetic resonance (NMR) spectra were recorded at ambient temperature using Bruker AVANCE III 400/500 spectrometers, with working frequencies of 400/500 and 100/125 MHz for 1H and 13C, respectively. Chemical shifts were recorded in units of ppm with TMS as the internal standard. High-resolution mass spectra (HRMS) were recorded on a Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS). PL emission spectra of both solution and thin-film samples were recorded on a Perkin-Elmer LS55 fluorescence spectrophotometer at 298 K. The UV-Vis absorption spectra were measured on a Perkin-Elmer Lambda 950 scanning spectrophotometer.

Cell Culture

All the cell lines (HeLa, HepG2, Hep3B and IMCD3) were cultured in DMEM supplemented with 10% FBS and 1% penicillin–streptomycin and incubated in a humidified 5% CO2 incubator at 37 °C with the medium changed every other day.

Cytotoxicity measurement of NIM-7 in cell lines

A standard CCK-8 assay was applied to measure the cytotoxicity of **NIM-7** in HeLa and IMCD3 cells, following the manufacturer's instructions. Briefly, cells were seeded in 96-well plates (1×10^4 cells per well), and after 12 h culture, cells were incubated with **NIM-7** at varied concentrations for another 24 h. Then, CCK-8 solution (15 μL) was added to each well of the plates and the plates were incubated for another 2 h in a CO_2 incubator. The absorbance at 450 nm was measured using a microplate reader (Bio-Rad, USA).

Photostability of NIM-7 during cell imaging

HeLa cells were seeded and cultured as described before. After 24 h, the cells were stained with 0.5 μM **NIM-7** for 1 h in a CO_2 incubator. Then the medium was replaced with fresh medium. Under a CLSM, **NIM-7** was excited at 488 nm and 561 nm. The cells were scanned every 1 min (totally 15 min) with 5% laser power. Meanwhile images before and after scanning were captured.

Toxicity assessment of NIM-7 on zebrafish embryos

Living zebrafish embryos (3 dpf) were reared in 6-well plates at density of 5 per well for each concentration. E3 embryo medium containing different concentrations of **NIM-7** was added into the plate. Survival rate of each group was recorded everyday for 4 days after treatment. Fish was identified as dead if it showed no moving and the heart stopped beating.

Cellular imaging and co-localization under CLSM

Cells were seeded on 35 mm glass-bottom culture dishes at a density of 1×10^5 cells per dish and incubated in a CO_2 incubator. After 12 h incubation, cells were incubated with 0.5 μM NIM-7 for 0.5 h, then 50 nM LysoTracker® Blue DND-22, HCS LipidTOX™ Deep Red Neutral Lipid Stain (1:1000 dilution) or 100 nM ER-Tracker™ Blue-White DPX were added and kept for another 0.5 h. After that, the medium was removed and cells were washed with PBS twice, then 1.5 mL growth medium was added into the dishes. Under a confocal laser scanning microscope (Leica, Germany), **NIM-7** was excited at 488 nm and 561 nm, while LysoTracker® Blue DND-22 and ER-Tracker™ Blue-White DPX were excited at 405 nm, and HCS LipidTOX™ Deep Red Neutral Lipid Stain was excited at 633 nm. The range of two emission channels are 530~570 nm and 640~680 nm for lipid droplets and lysosomes are, respectively. For commercial probes, the emission channels were chosen in accord with the manufacturer's instructions. The pictures of cellular imaging were captured. For 3D imaging, the images were harvested every 0.5 um on the z-axis, and the 3D animation pictures were generated with packed software.

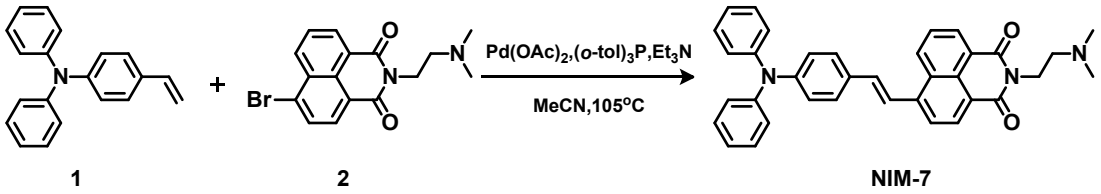
Super-resolution microscope imaging

HeLa, HepG2, IMCD3 and TTF cells were seeded on 35 mm glass-bottom culture dishes at a density of 1×10^5 cells per dish and incubated in a CO_2 incubator. After 12 h incubation, cells were incubated with 0.5 μM **NIM-7** for 1 h. After that, the medium was removed and cells were washed with PBS twice, then 1.5 mL growth medium was added into the dishes. Under a structured illumination microscope (Nikon, Japan), **NIM-7** was excited and the emission signals were harvested as described above, and the pictures of cellular imaging were captured.

Real-time tracking LDs and lysosomes by NIM-7

HeLa cells were cultured, seeded and stained as described above. **NIM-7** was excited and the emission signals were harvested as described above. The data were acquired from a live-cell imaging workstation (Leica AS MDW).

Synthetic procedures and characterization data



Scheme S1. Synthetic route of **NIM-7**.

The intermediates, *N*,*N*-diphenyl-4-vinylaniline (**1**),[1] and 6-bromo-2-(2-(dimethylamino)ethyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**2**)[2] were synthesized according to reported procedures.

2-(2-(Dimethylamino)ethyl)-6-(4-(diphenylamino)styryl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**NIM-7**):

A high pressure bottle was charged with a mixture of **1** (0.41 g, 1.5 mmol), **2** (0.33 g, 1.0 mmol), $Pd(OAc)_2$ (11.2 mg, 0.05 mmol), (*o*-tolyl)$_3$P (30.4 mg, 0.1 mmol), triethylamine (1.42 g, 14 mmol) and MeCN (10 mL). The reaction mixture was stirred at 105 °C for 48 h under argon. After cooled to room temperature, the mixture was poured into water (20 mL). The resulting red solid was collected, washed with water and dried in vacuo. The crude product was purified by column chromatography (eluent: acetone/hexanes = 1/2, v/v). The red solid was then obtained by recrystallizing with MeOH/DCM (yield: 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (dd, *J* = 7.2, 0.8 Hz, 1H), 8.56 (m, 2H), 7.96 (d, *J* = 8.0 Hz, 1H), 7.80–7.70 (m, 2H), 7.51 (d, *J* = 8.4 Hz, 2H), 7.33–7.27 (m, 5H), 7.15 (m, 4H), 7.08 (m, 4H), 4.39–4.29 (t, *J* = 6.8 Hz, 2H), 2.73–2.63 (t, *J* = 6.8 Hz, 2H), 2.38 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.4, 164.1, 148.6, 147.2, 141.9, 135.0, 131.2, 130.1, 129.5, 129.4, 128.8, 128.1, 126.6, 125.0, 123.6, 123.5, 123.0, 122.8, 121.2, 120.9, 56.9, 45.7, 38.1. HRMS (ESI) for $C_{36}H_{31}N_3O_2$ $(M+H)^+$ Cacld: 538.2495, Found: 538.2461.

2) Spectroscopic and cell imaging properties of NIM-7

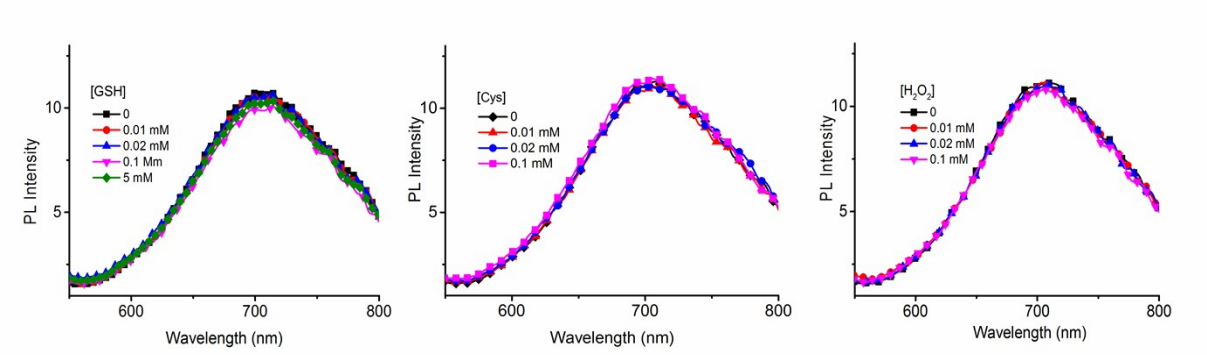


Figure S1. Fluorescence spectra of **NIM-7** (2 μM) in DMSO/H_2O (4/1, v/v) with intracellular nucleophiles (a) GSH, (b) Cys, and (c) H_2O_2 under an excitation of 450 nm.

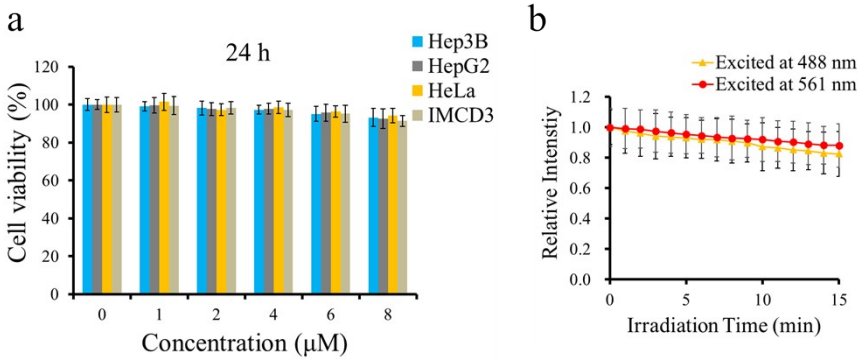


Figure S2. Cytotoxicity and photostability of **NIM-7** in cellular imaging. (a) Cytotoxicity of **NIM-7** on Hep3B, HepG2, HeLa and IMCD3 cells was evaluated by CCK-8 assay. Cells were incubated with varied concentrations of **NIM-7** for 24 hrs, n = 5, error bars represent SEM (standard error of mean); (b) Photostability of **NIM-7** in cellular imaging. Signal intensity of **NIM-7** in HeLa cells (0.5 μM, 1 h) under different light irradiation time was detected by CLSM. n = 5, error bars represent standard deviation (SD).

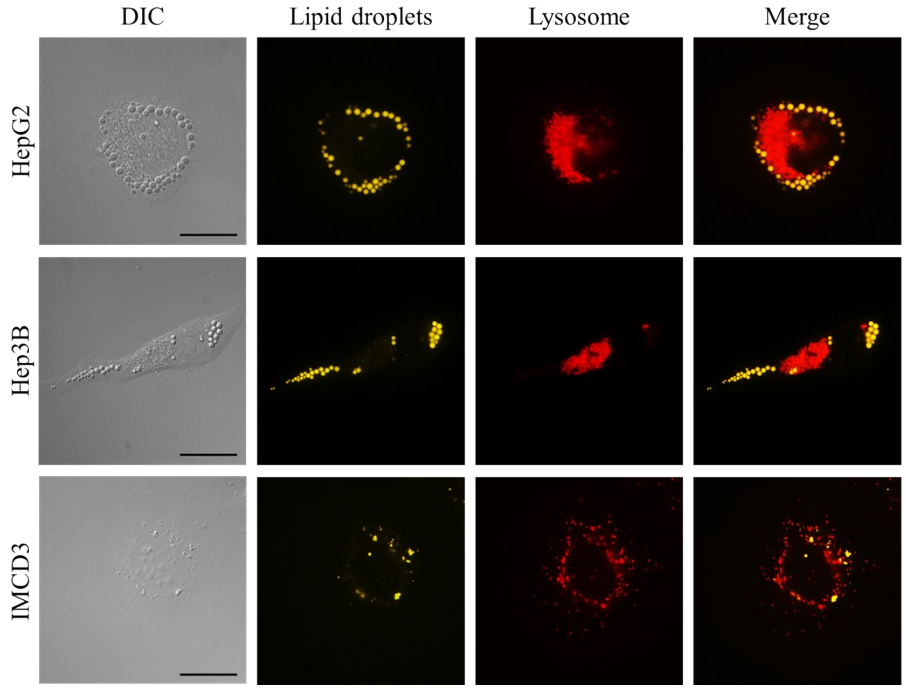


Figure S3. Confocal microscopy images of HepG2, Hep3B and IMCD3 cells stained with **NIM-7** (0.5 μM, 1 h). **NIM-7** was excited at 488 nm and 561 nm, respectively. Scale bar: 20 μm.

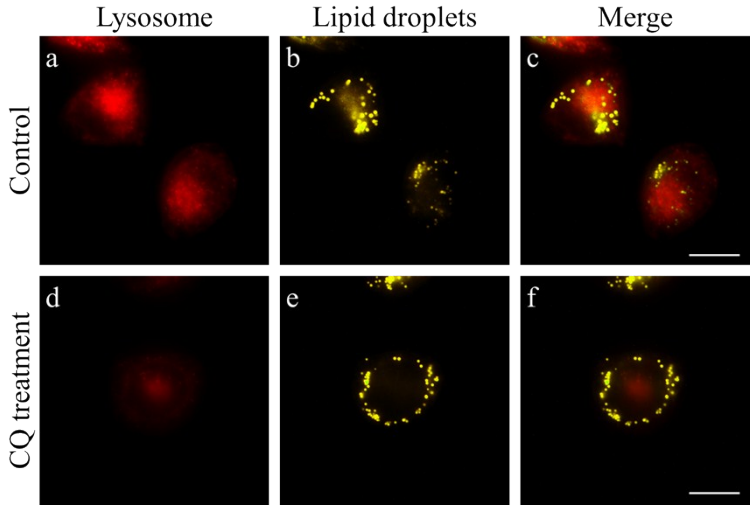


Figure S4. Disruption of acid microenvironment of lysosome decreases the localization of **NIM-7** in lysosomes. (a-c) Cells were stained with 0.5 μM **NIM-7** for 1 h. (d-e) Cells were pre-incubated with 50 μM chloroquine (CQ) for 0.5 h followed by 0.5 μM **NIM-7** for another 1 h. (a) and (d) Excited at 561 nm; (b) and (e) Excited at 488 nm; (c) and (f) Merge of (a) and (b), (d) and (e). Scale bar: 20 μm.

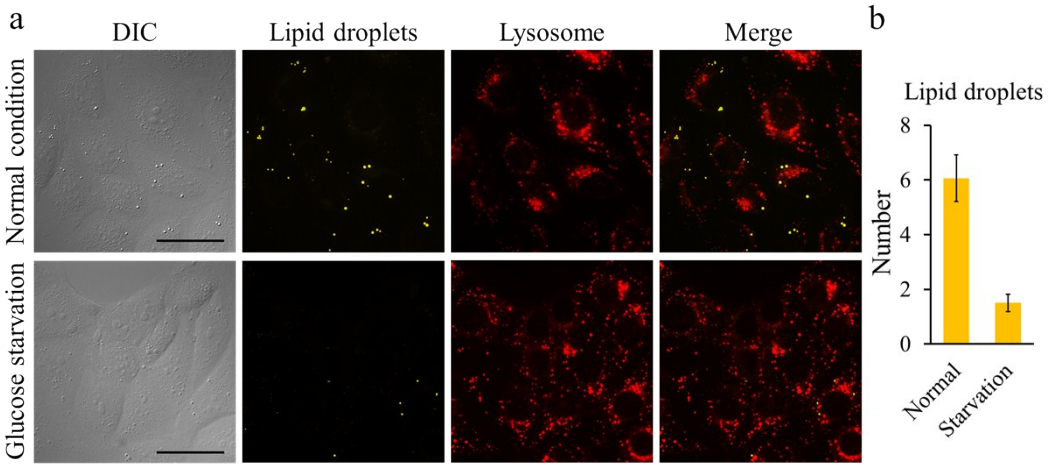


Figure S5. Glucose starvation decreases lipid droplets. (a) IMCD3 cells were incubated under normal growth condition or under glucose starvation conditions for 24 h followed by **NIM-7** staining (0.5 μM) for another 1 h. **NIM-7** was excited under blue channel (lipid droplets) and green channel (lysosome), respectively, through a fluorescence microscope. (b) Quantifications of LDs number per cell (n = 50). Bars represent SEM. Scale bar: 50 μm.

Table S1 Photophysical properties of NIM-7 in solvents with different polarities

Solvent	λ_{ab}[a] (nm)	λ_{PLmax}[b] (nm)	φ_{PL}[c][3]
Tol	448	552	0.81
EA	446	604	0.77
$CHCl_3$	461	624	0.36
ACE	448	658	0.060
DMSO	461	674	0.015

[a] λ_{ab}: absorption maximum. [b] λ_{PLmax}: fluorescence maximum. [c] φ_{PL}: PL quantum yield. Here, Tol denotes toluene; EA denotes ethyl acetate; $CHCl_3$ denotes chloroform; ACE denotes acetone; and DMSO denotes dimethyl sulfoxide.

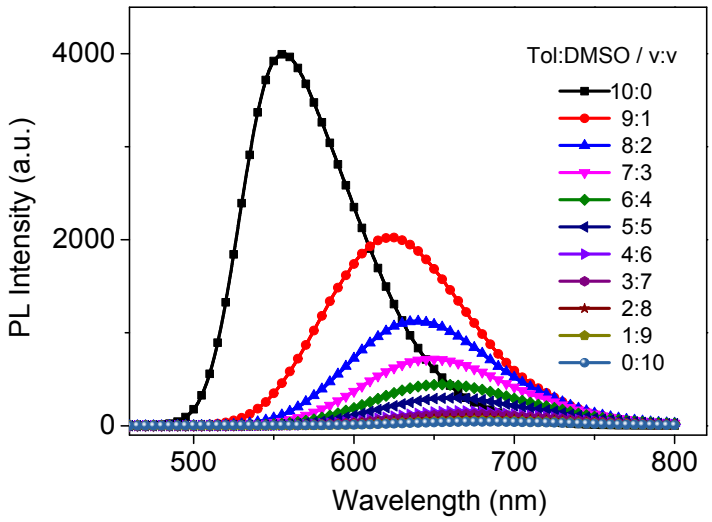


Figure S6. Fluorescence spectra of **NIM-7** in DMSO−toluene mixtures with different volume ratios (From 0:10 to 10:0). The excitation wavelength was 450 nm.

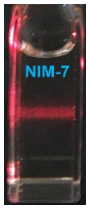


Figure S7. Light scattering experiments involving **NIM-7** in DMSO/H_2O (1/250, v/v).

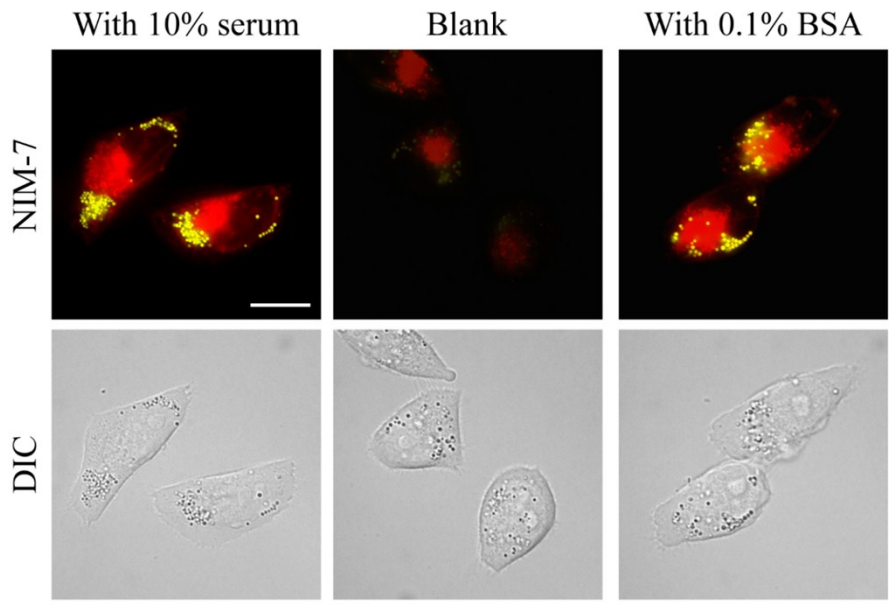


Figure S8. HeLa cells were incubated with DMEM containing 0.5 μM **NIM-7** with or without serum or BSA for 1 h. For lipid droplets (yellow fluorescence) and lysosome (red fluorescence) imaging, the cells were excited at 488 nm and 561 nm, respectively. The upper panel shows the merged images; the lower panel shows the corresponding DIC images. Scale bar: 20 μm.

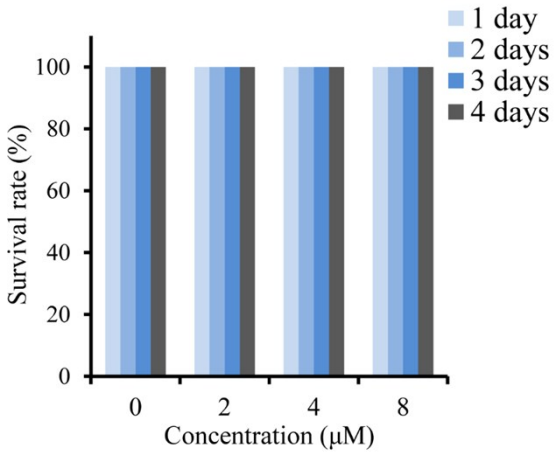


Figure S9. Survival rates of zebrafish embryos (3 dpf) incubated with **NIM-7** under different conditions. n = 5.

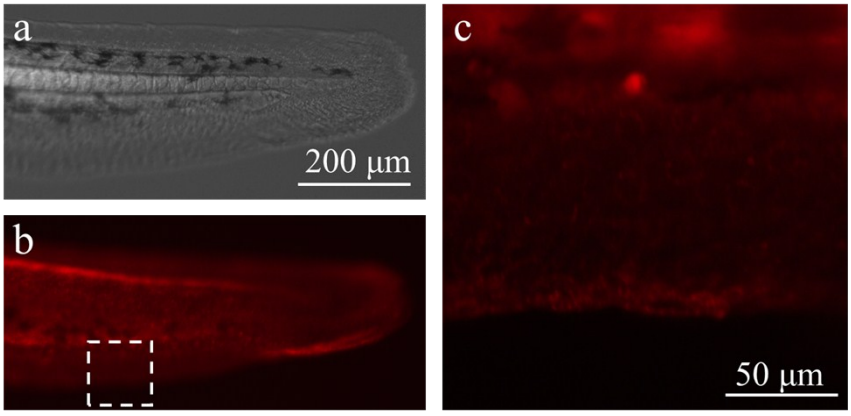


Figure S10. Zebrafish embryos imaging by **NIM-7** after CQ (200 μM) treatment. (a) white field; (b) **NIM-7** was excited at green channel; (c) the enlarged zone form (b).

3) ^{1}H and ^{13}C NMR spectra, HRMS data, and HPLC traces

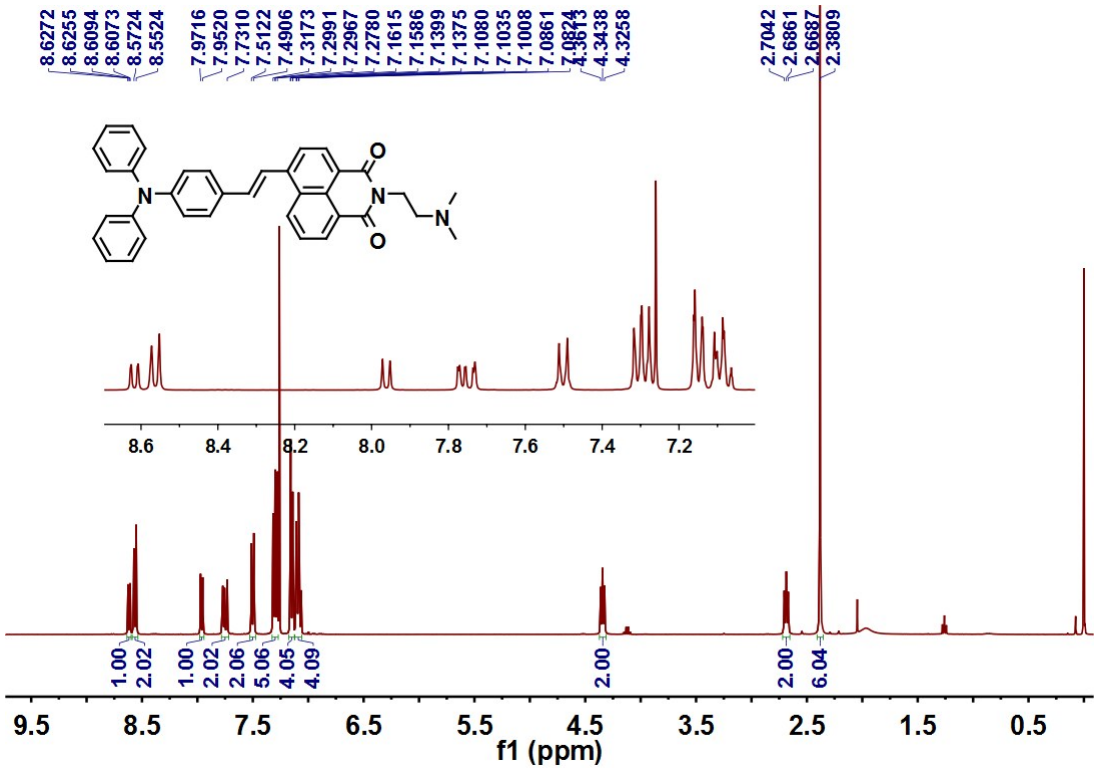


Figure S11. ^{1}H-NMR spectrum of **NIM-7**.

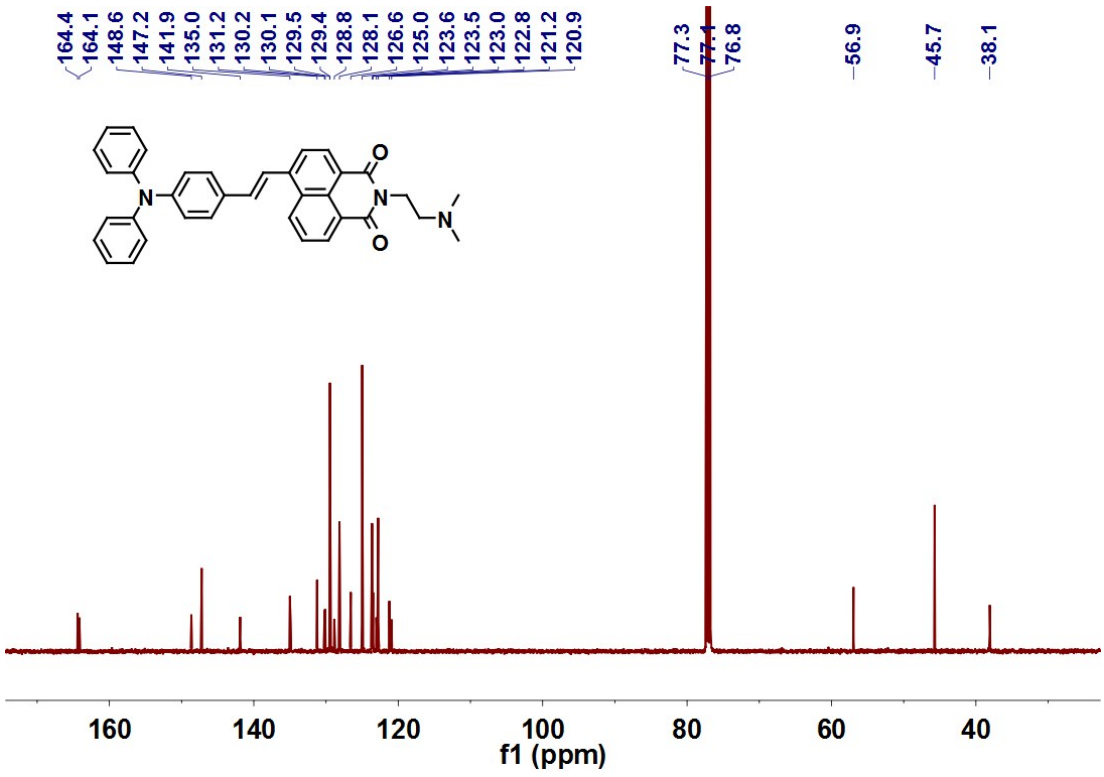


Figure S12. ^{13}C-NMR spectrum of **NIM-7**.

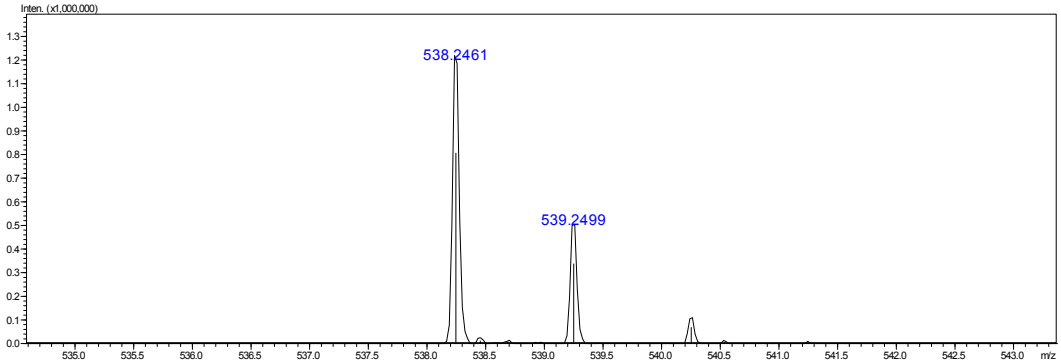


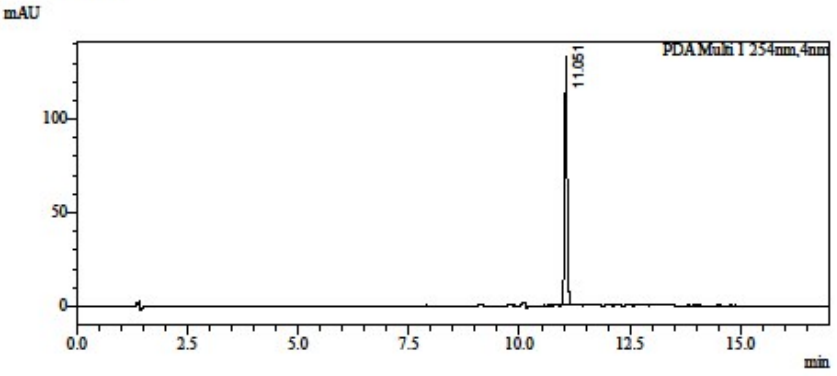
Figure S13. HRMS spectrum of **NIM-7**.

HPLC Report

<Sample Information>

Sample Name	: 1223-1	Batch ID:	
Project ID	: 0024	Compound ID:	
Vial #	: 1-82	Injection Volume	: 20 uL
Date Acquired	: 2016/12/23 17:14:29	Acquired by	:
Date Processed	: 2016/12/26 12:21:41	Processed by	:
Data Filename	:C:\LabSolutions\Data\20161223\HG010022-1223-1_0024_HPLC-2_04.lcd		
Method	: Column: XUnion C18 150 mm*4.6 mm 5 um		
	Mobile Phase: A:0.05% TFA Water B: ACN		
	Gradient : B from 10% to 100% in 10 min and hold 100% for 5 min,then turn to 10% in 2 min		
	Flow Rate: 1.5 mL/min; Column Temperature:40 ℃.		

<Chromatogram>



<Chromatogram Peak Table>

Peak Table

PDA Ch1 254nm

Peak	Ret. Time	Area	Height	Talling Factor	Resolution	Peak Purity	Area%
1	11.051	509205	133402	1.093		0.999999	100.000
总计		509205	133402				100.000

Reported by: System

Figure S14. HPLC spectrum of **NIM-7**.

4) Supporting references

[1] J. Zhang, G. Wu, C. He, D. Deng, Y. Li, *J. Mater. Chem.* **2011**, 21, 3768.

[2] X. Zheng, W. Zhu, D. Liu, H. Ai, Y. Huang, Z. Lu, *ACS Appl Mater Interfaces* **2014**, 6, 7996.

[3] H.-H. Lin, Y.-C. Chan, J.-W. Chen, C.-C. Chang, *J. Mater. Chem.* **2011**, 21, 3170.