Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry
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Supporting Information

Development of a fluorescent nanoprobe based on an amphiphilic single-benzene-based fluorophore for lipid droplets detection and its practical applications

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This PDF file includes:

1. Materials and Methods

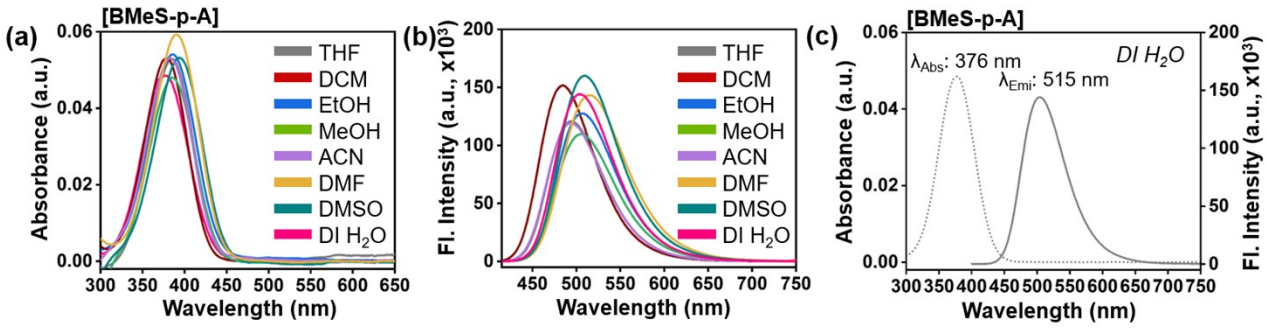
General information

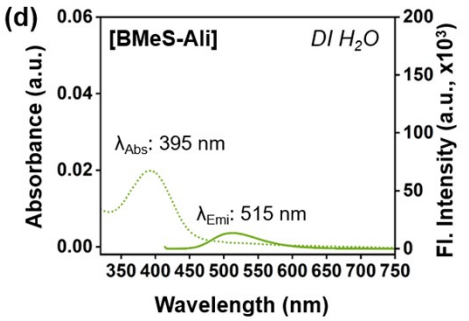
The chemical reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA), TCI (Tokyo, Japan), Alfa Aesar (Haverhill, MA, USA), Acros Organics (Geel, Flanders, Belgium), and Merck (Darmstadt, Germany). Commercial chemicals and solvents were used without further purification. 1-bromododecane, potassium hydroxide, and *N,N*-Dimethylmethanamide (DMF) (Product No. 41820) were purchased from Alfa Aesar (Haverhill, MA, USA). Dimethyl sulfoxide (DMSO, Product No. 1.02952.1000) was purchased from Merck (Darmstadt, Germany). The reagents used to evaluate the screening of various ions and biomolecules were Cu(II), Co(II), Zn(II), Fe(II), Ca(II), Mg(II), K(I), Na(I), lysine (Lys), glutamine (Gln), homocysteine (Hcy), glutathione (GSH), cysteine (Cys), methionine (Met), and phenylalanine (Phe). Phosphate-buffered saline (PBS, pH 7.4, 10x solution, Product No. 70011044) was purchased from Gibco TM (Paisley, Scotland, UK). Nile red (Product No. N0659) and LipidSpot™ 610 (Product No. 70069) were purchased from TCI (Tokyo, Japan) and Biotium (Fremont, CA, USA). The cellulose-based filter paper (Grade 2, pore size 8 μm) was purchased from Whatman® (Maidstone, UK). Organic reactions were performed under an argon atmosphere. UV/vis absorption (Product No. 108-000-10-40) and fluorescence measurement cuvettes (Product No. 101-10-40) were purchased from Hellma Analytics (Müllheim, Germany). UV 365 nm LED light 3 W (Product No. RM104) and 10 W (Product No. SV003) were purchased from Rayman (Seoul, Rep. of Korea) and Alonefire (Guangdong, China) respectively. TLC Silica gel 60F-254 (Product No. 105549) was purchased from Merck (Darmstadt, Germany). ^{1}H and ^{13}C NMR spectra were acquired from JEOL JNM NMR instruments (500 MHz, Tokyo, Japan). In the NMR spectra, the chemical shifts (δ) are reported in parts per million (ppm) relative to the signal (0.00 ppm), with an internal standard tetramethylsilane (TMS) for the solution in DMSO-*d6* (2.50 ppm for ^{1}H) and $CDCl_3$ (77.16 ppm for ^{13}C). Multiplicity is indicated by s (singlet), d (doublet), dd (doublet of doublet), and m (multiplet). High-resolution mass spectra were recorded on a JEOL JMS-700 spectrometer (JEOL, Tokyo, Japan) at Korea Basic Science Center and Kyungpook National University. Value is reported in units of mass to charge (*m/z*). Size and zeta potential were obtained using attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscope instrument (Thermo Scientific Nicolet™ iS™ 5 FT-IR spectrometer, 16 scans, Waltham, MA, USA). The melting point was obtained using Stuart SMP20 melting point apparatus (Stuart, UK, Staffordshire).

Synthesis of BMeS-Ali

The synthesis of **BMeS-Ali** (*N*^{1}-dodecyl-2,5-bis(methylsulfonyl)benzene-1,4-diamine) was conducted in a round-bottom flask, which was flame-dried, under an argon atmosphere. **BMeS-p-A** (60 mg, 0.228 mmol), potassium carbonate (39.6 mg, 0.286 mmol), and potassium iodide (catalytic amount) were dissolved in anhydrous *N,N*-dimethylformamide (DMF, 3 mL), and the mixture was slowly warmed-up to 90 °C with stirring (300 rpm). 1-bromododecane (65.8 μL, 0.274 mmol) was added dropwise under the same condition and the reaction was allowed to be maintained for 12 h under reflux condition. The reaction mixture was then cooled down to room temperature. The resulting solution was washed with ethyl acetate (EtOAc, 10 mL, 3 times) and deionized water (DI H_2O, 10 mL, 3 times). The organic phase was washed using brine and dried over anhydrous sodium sulfate (Na_2SO_4). The resulting residue was concentrated under vacuo and purified by silica gel column chromatography (n-hex/EtOAc=6:4, *v/v*). **BMeS-Ali** was collected with a 33% yield (bright green powder). Melting point: 158 °C. ^{1}H NMR (500 MHz, DMSO-*d6*): δ 7.41 (s, 1H), 7.02 (s, 1H), 5.61 (s, 2H), 5.35 (t, 1H), 3.18 (d, 5H), 3.19 (m, 2H), 1.24 (m, 20H), 0.85 (t, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 139.36, 135.84, 129.24, 128.29, 119.43, 113.05, 43.86, 41.56, 41.48, 31.91, 29.55, 29.53, 29.59, 29.55, 29.35, 29.05, 27.15, 22.70, 14.14. HRMS (m/z): calcd for $C_{20}H_{36}N_2O_4S_2$, 494.2116; found, 432.2117.

2. Supporting Figures





(e)

Solvent	**BMeS-p-A** $\lambda_{abs\ max}/\lambda_{emi\ max}$ (nm)	**BMeS-Ali** $\lambda_{abs\ max}/\lambda_{emi\ max}$ (nm)
THF	384/494	395/509
DCM	376/486	396/510
EtOH	385/506	399/519
MeOH	400/506	384/520
ACN	403/495	398/515
DMF	390/504	403/517
DMSO	394/509	407/523
DI H_2O	378/515	395/515

Fig. S1. (a) UV/Vis absorption and (b) emission spectra of **BMeS-p-A** (10 μM) in various solvents (THF, DCM, EtOH, MeOH, ACN, DMF, DMSO, and DI H_2O). [Abbreviations] THF: tetrahydrofuran, DCM: dichloromethane, EtOH: ethanol, MeOH: methanol, ACN: acetonitrile, DMF: *N,N*-dimethyl formamide, DMSO: dimethyl sulfoxide, and DI H_2O: deionized water. (c, d) UV/Vis absorption and emission spectra of **BMeS-p-A** (10 μM) and **BMeS-Ali** (10 μM) in DI H_2O. The UV/Vis absorption and emission spectra were recorded within 1 min after mixing at 25 °C. The emission spectra were measured under excitation at the maximum absorption wavelength in each solvent. (e) Table of maximum excitation and emission wavelength of **BMeS-p-A** and **BMeS-Ali** in each solvent.

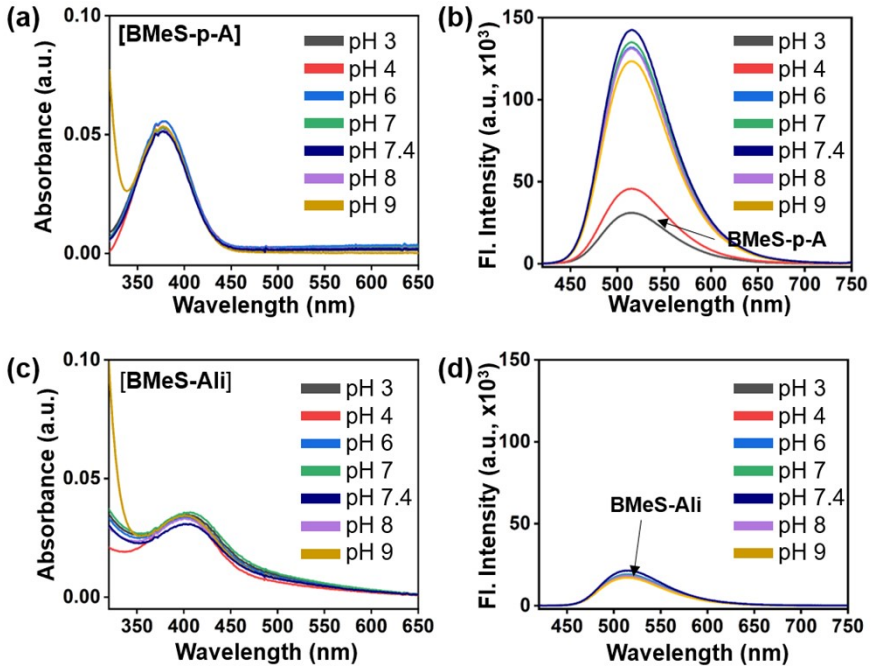


Fig. S2. (a, c) UV/Vis absorption and (b, d) emission spectra of **BMeS-p-A** (10 μM) and **BMeS-Ali** (10 μM) in various pH buffers (pH 3, 4, 6, 7, 8, 9) including biological pH 7.4. The fluorescence intensity was measured under excitation at 395 nm. The intensity was recorded within 1 min after mixing at 25 ˚C.

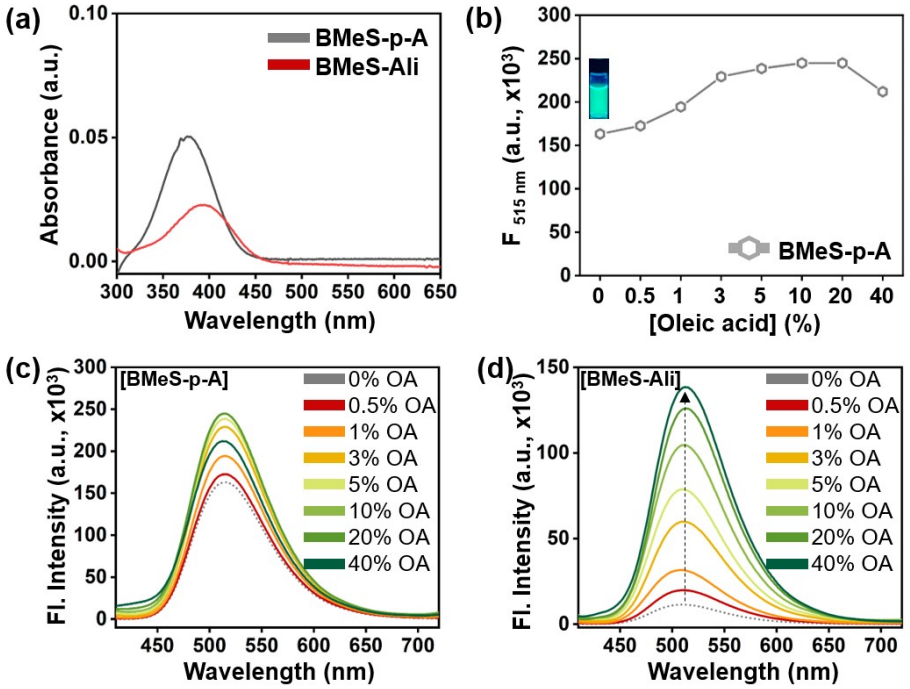


Fig. S3. (a) UV/Vis absorption spectra of **BMeS-p-A** (10 μM) and **BMeS-Ali** (10 μM) in DI H_2O. (b) Emission intensity plot (peak height at 515 nm) and (c) emission spectra of **BMeS-p-A** (10 μM) with various concentrations of OA (0–40%) in DI H_2O. (d) Emission spectra of **BMeS-Ali** (10 μM) with various concentrations of OA (0–40%) in DI H_2O. Each spectrum was recorded within 1 min after mixing at 25 °C. The emission spectra were measured under excitation at the maximum absorption wavelength in the 0% OA condition.

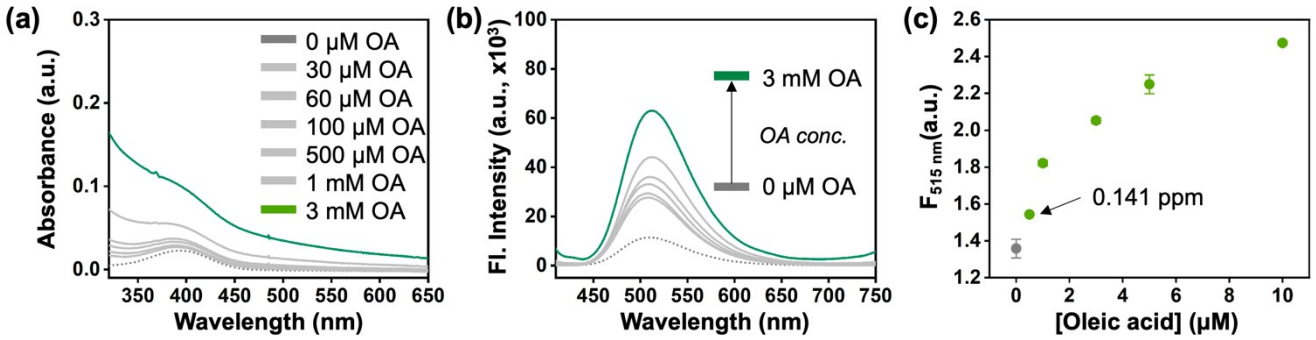


Fig. S4. (a) UV/Vis absorption (b) emission spectra of **BMeS-Ali** (10 μM) with various concentrations (0 μM–3 mM) of OA in DI H_2O, measured within 1 min after mixing at 25 ˚C. (c) Emission intensity plot (peak height at 515 nm) of **BMeS-Ali** (1 μM) with various concentrations (0 μM–10 μM) of OA in DI H_2O, measured within 1 min after mixing at 25 ˚C.

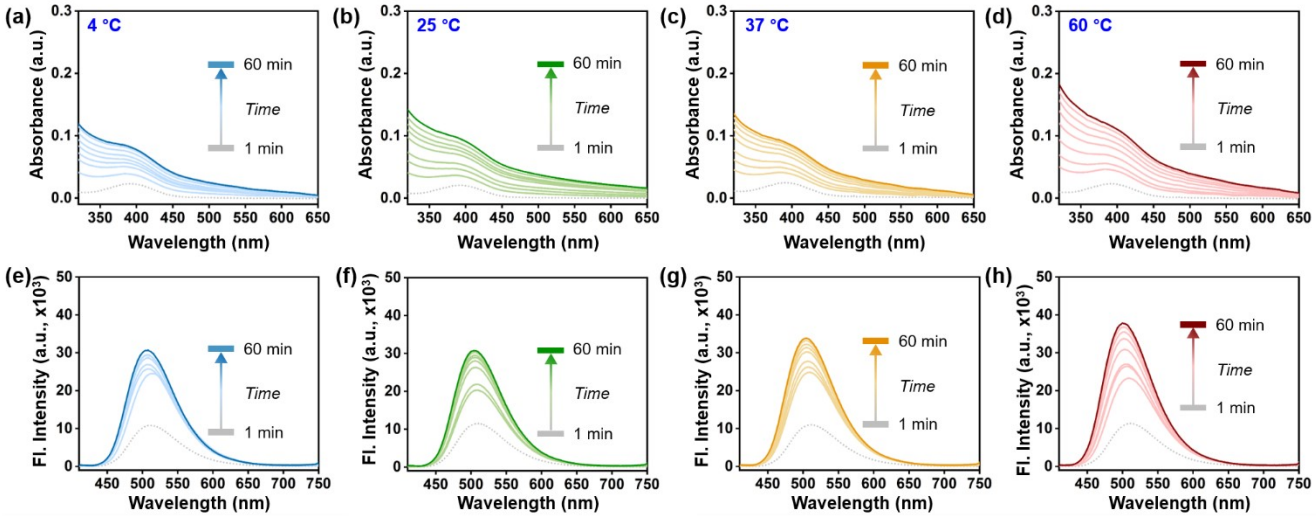


Fig. S5. (a, b, c, d) UV/Vis absorption and (e, f, g, h) emission spectra of **BMeS-Ali** (10 μM) after adding oleic acid (100 μM) in DI H_2O at various temperatures (4 ˚C, 25 ˚C, 37 ˚C, and 60 ˚C). The absorption and emission spectra were recorded within 1 min. The emission spectra were measured under excitation at the maximum absorption wavelength.

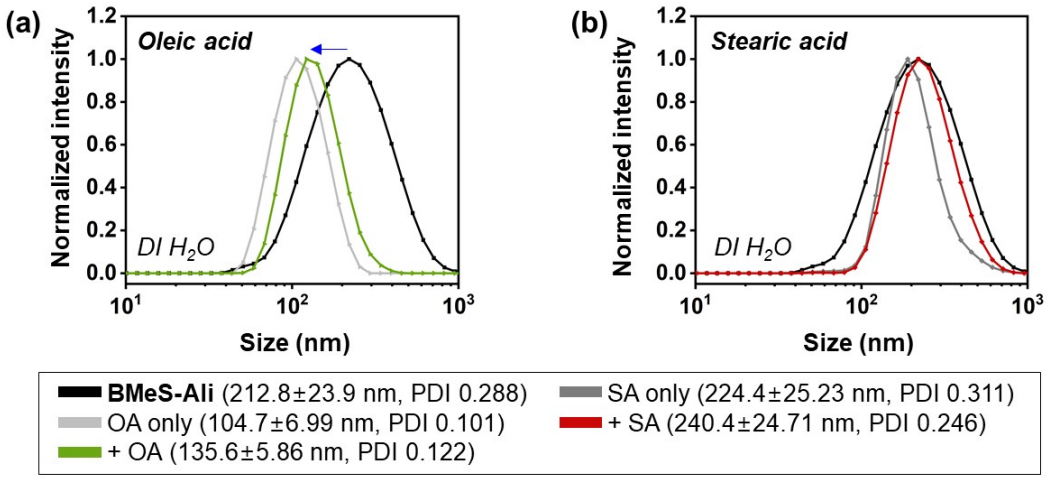


Fig. S6. Comparison of mean hydrodynamic diameter of (a) reassembled **BMeS-Ali** (20 μM) with oleic acid (OA, 200 μM, green line), and (b) reassembled **BMeS-Ali** with stearic acid (SA, 200 μM, red line) in DI H_2O, measured by dynamic light scattering (DLS).

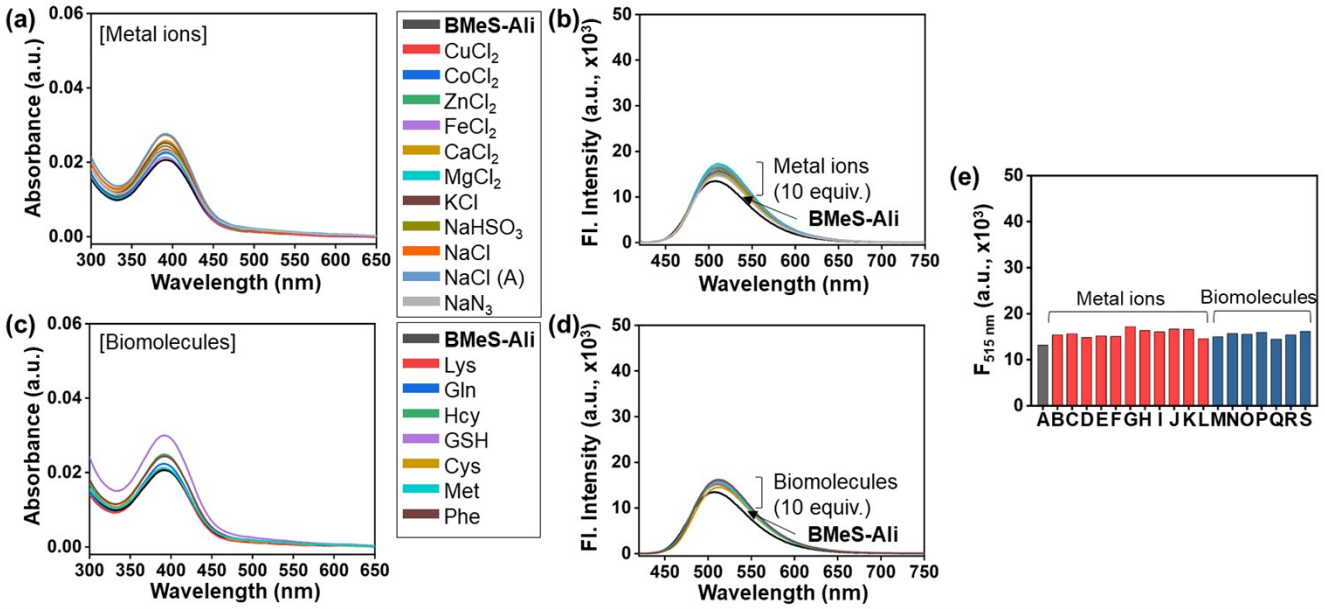


Fig. S7. (a, c) UV/Vis absorption and (b, d) emission spectra of **BMeS-Ali** (10 μM) with metal ions ($CuCl_2$, $CoCl_2$, $ZnCl_2$, $FeCl_2$, $CaCl_2$, $MgCl_2$, KCl, $NaHSO_3$, NaCl, NaCl anion, and NaN_3) and biomolecules (Lys, Glu, Hcy, GSH, Cys, Met, and Phe). [Abbreviations] Lys: lysine, Gln: glutamine, Hcy: homocysteine, GSH: glutathione, Cys: cysteine, Met: methionine, and Phe: phenylalanine. (e) Emission intensity plot (peak height at 515 nm) of **BMeS-Ali** (10 μM) after mixing metal ions and biomolecules (100 μM) in DI H_2O. (A) **BMeS-Ali**, (B) **BMeS-Ali** + $CuCl_2$, (C) **BMeS-Ali** + $CoCl_2$, (D) **BMeS-Ali** + $ZnCl_2$, (E) **BMeS-Ali** + $FeCl_2$, (F) **BMeS-Ali** + $CaCl_2$, (G) **BMeS-Ali** + $MgCl_2$, (H) **BMeS-Ali** + KCl, (I) **BMeS-Ali** + $NaHSO_3$, (J) **BMeS-Ali** + NaCl, (K) **BMeS-Ali** + NaCl (anion), (L) **BMeS-Ali** + NaN_3, (M) **BMeS-Ali** + Lys, (N) **BMeS-Ali** + Gln, (O) **BMeS-Ali** + Hcy, (P) **BMeS-Ali** + GSH, (Q) **BMeS-Ali** + Cys, (R) **BMeS-Ali** + Met, and (S) **BMeS-Ali** + Phe. All spectra were recorded within 1 min after mixing at 25 °C under excitation at 395 nm.

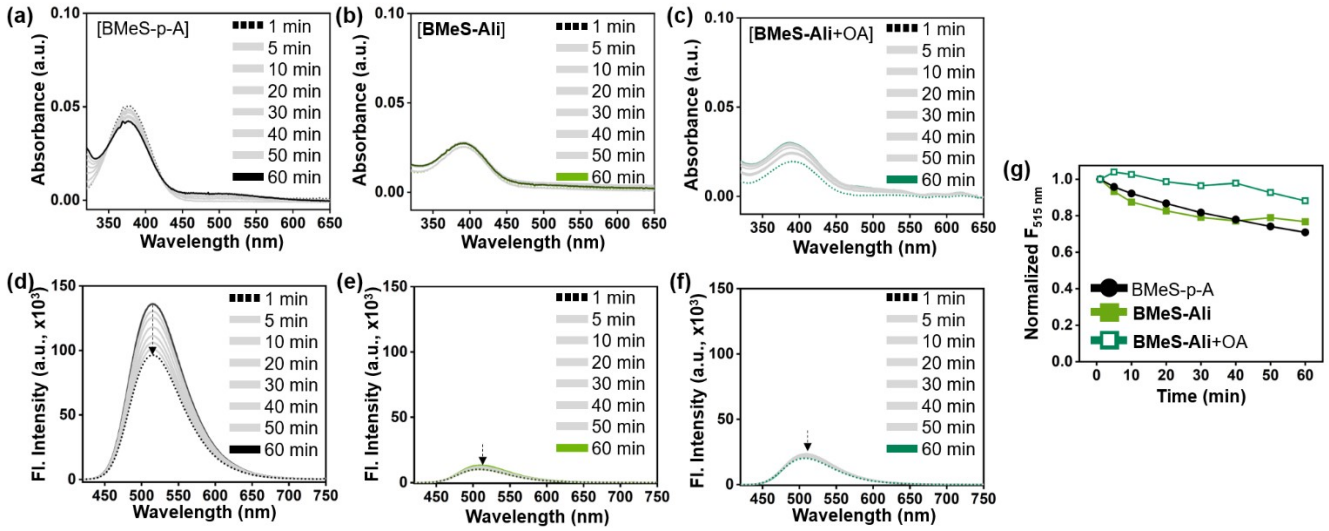


Fig. S8. (a, b, c) UV/Vis absorption and (d, e, f) emission spectra of **BMeS-p-A** (10 μM) and **BMeS-Ali** (10 μM) under continuous UV light exposure (365 nm, 3 W) in DI H_2O. (g) Normalized emission intensity (peak height at 515 nm) plot of **BMeS-p-A** (10 μM, black line), **BMeS-Ali** (10 μM, green line), and **BMeS-Ali** (10 μM, dark green line) with OA (100 μM) under continuous UV light exposure (365 nm, 3 W) in DI H_2O. Incubation time indicates the UV light exposure time (0–60 min).

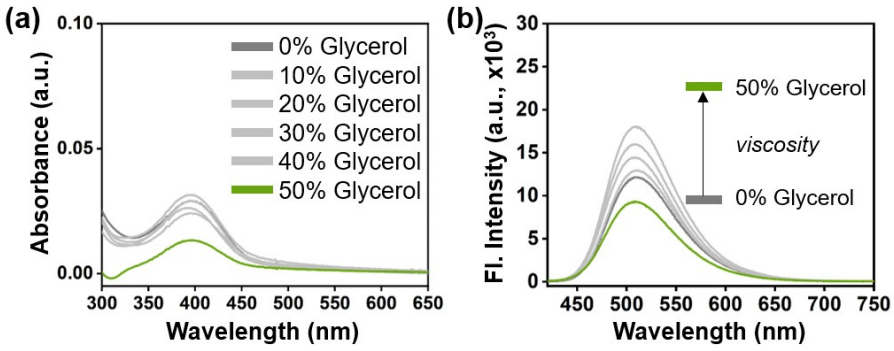


Fig. S9. (a) UV/Vis absorption and (b) emission spectra of **BMeS-Ali** (10 μM) in the mixture of DI H_2O and glycerol (0–50% glycerol). The absorption and emission spectra were recorded within 1 min after mixing at 25 °C. The emission spectra were measured under excitation at 395 nm.

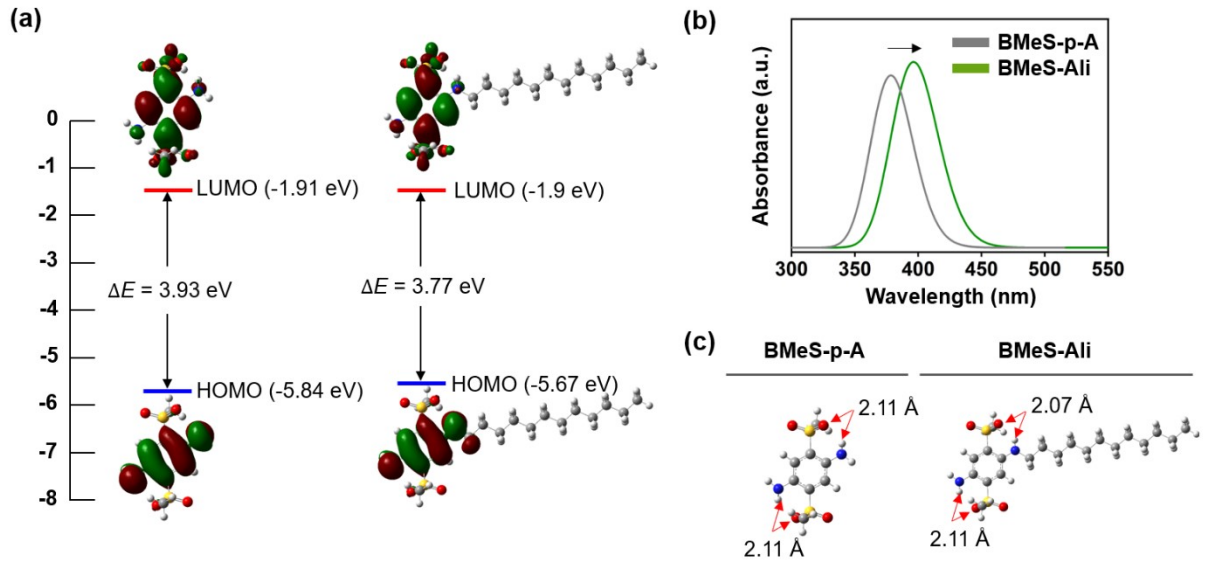


Fig. S10. (a) DFT calculation (B3LYP-d3/6-31+G(d)) results (most stable conformational structure, HOMO-LUMO) of **BMeS-p-A** (left) and **BMeS-Ali** (right). (b) Calculated absorption spectra of **BMeS-p-A** and **BMeS-Ali** in DI H_2O. (c) Optimized molecular structures of **BMeS-p-A** and **BMeS-Ali** with DFT-calculated atomic distance of the optimal geometries at the B3LYP/6-31+G(d)) level.

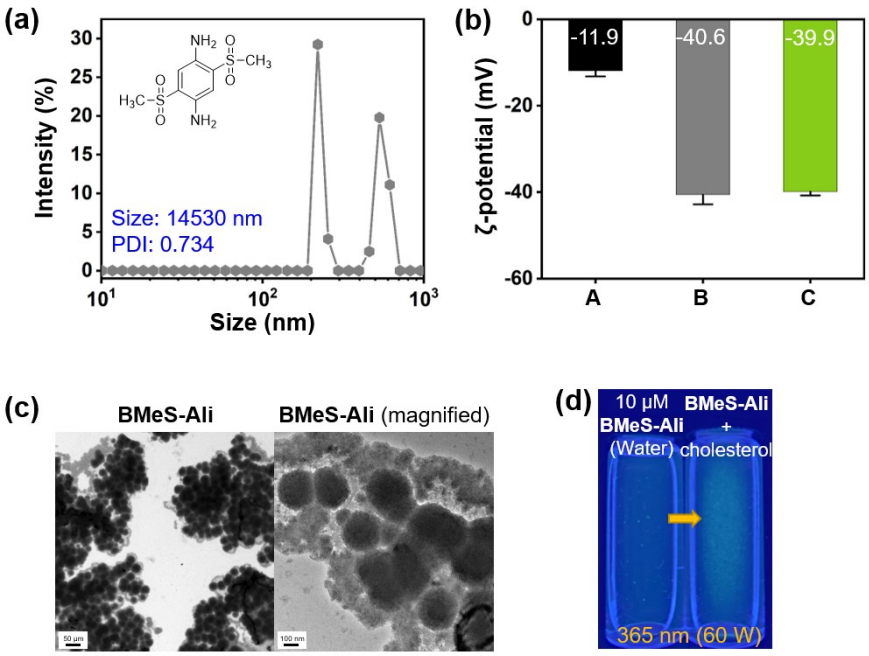


Fig. S11. (a) Mean hydrodynamic diameter of **BMeS-p-A** and (b) zeta-potential value of **BMeS-Ali** in DI H_2O, measured by dynamic light scattering (DLS). A: **BMeS-Ali**. B: oleic acid (OA). C: **BMeS-Ali** + OA. (c) Transmission electron microscopy (TEM) images of **BMeS-Ali** (left, 50 μm scale bar) and magnified **BMeS-Ali** (right, 100 nm scale bar). (d) Photo of **BMeS-Ali** (left) in DI H_2O and after adding cholesterol (500 μM, right) under 365 nm UV light.

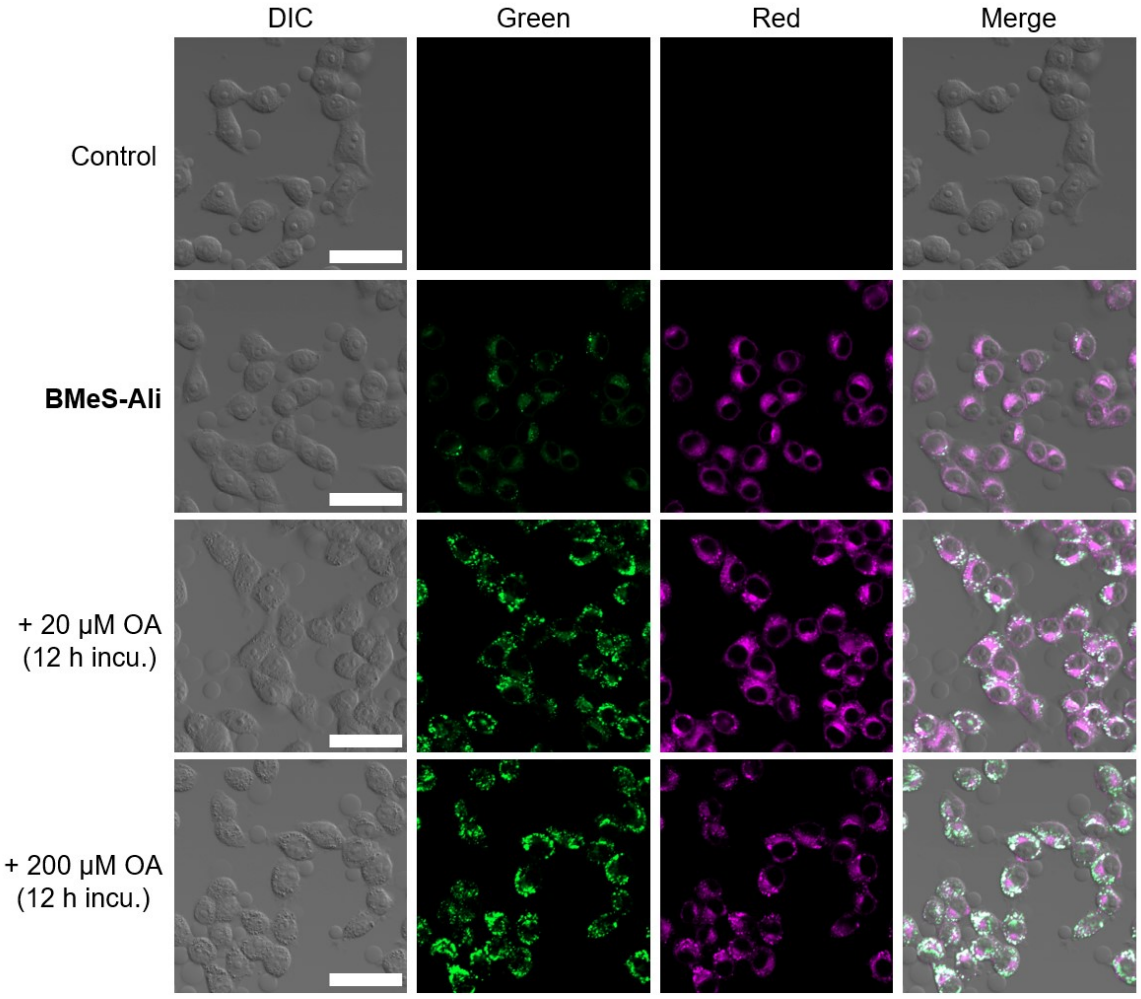


Fig. S12. CLSM images of HeLa cells co-incubated with **BMeS-Ali** (20 μM) and Nile Red (300 nM). [OA]: 20 μM and 200 μM of OA were pretreated respectively and incubated for 12 h at 37 ˚C; [**BMeS-Ali**]: 20 μM was treated and incubated for 1 h at 37 ˚C, and then incubated with Nile Red (300 nM) for 15 min at 37 ˚C. The scale bar is 20 μm. Excitation wavelength and detection channel: green (488 nm, 495–575 nm), red (561 nm, 585–700 nm).

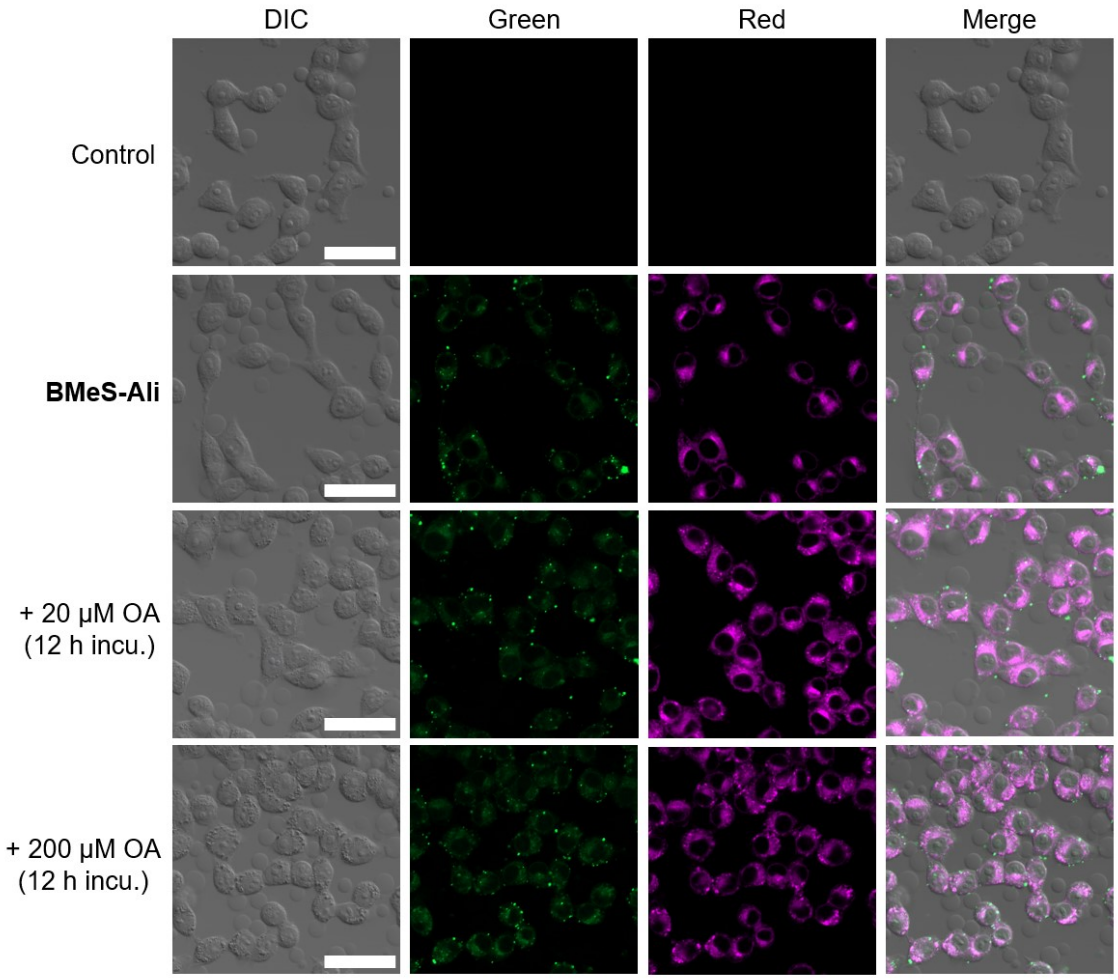


Fig. S13. CLSM images of HeLa cells co-incubated with **BMeS-Ali** (20 μM), Nile Red (300 nM). [OA]: 20 μM and 200 μM of OA were pretreated respectively and incubated for 12 h at 37 ˚C; [**BMeS-Ali**]: 20 μM was treated and incubated for 1 h at 37 ˚C, and then incubated with Nile red (300 nM) for 15 min at 37 ˚C. The scale bar is 20 μm. Excitation wavelength and detection channel: green (405 nm, 415–575 nm), red (561 nm, 585–700 nm).

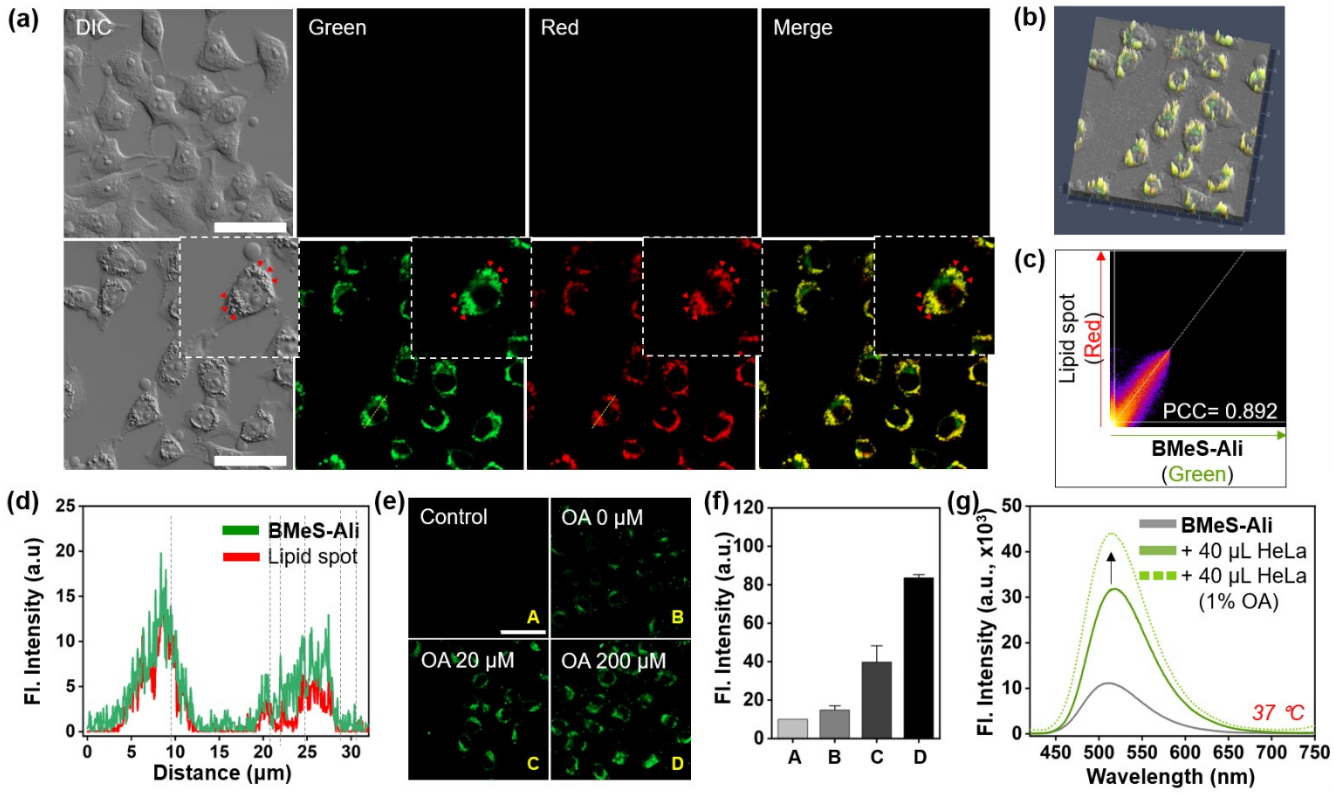


Fig. S14. (a) CLSM images of HeLa cells co-incubated with **BMeS-Ali** (20 μM) and LipidSpot™ 610 (1000ᔓ). [OA]: 200 μM of OA was pretreated and incubated for 12 h at 37 ˚C; [**BMeS-Ali**]: 20 μM of **BMeS-Ali** was treated and incubated for 1 h at 37 ˚C, and then incubated with LipidSpot™ 610 (1000ᔓ) for 30 min at 37 ˚C. The scale bar is 20 μm. Excitation wavelength and detection channel: green (405 nm, 410–585 nm), red (561 nm, 595–700 nm). (b) 2.5D merged CLSM image of HeLa cells. (c) The Pearson's correlation coefficient (PCC) values (**BMeS-Ali** vs. LipidSpot™ 610) were calculated using Image-J software. (d) Fluorescence intensity plot along the yellow dotted line on the images in panel (a). (e) CLSM images of HeLa cells with **BMeS-Ali** (20 μM) after treatment of OA (0 μM, 20 μM, 200 μM) with 12 h incubation at 37 ˚C. (f) The relative fluorescence intensity plot of cell images in panel (e). The fluorescent signals in the images were measured using Image-J software by extracting the ROI (region of interest) value over the entire cells. (A: Control, B: OA 0 μM, C: OA 20 μM, D: OA 200 μM). (g) Emission spectra of **BMeS-Ali** (10 μM), **BMeS-Ali** (10 μM) + cell lysate solution (40 μL), and **BMeS-Ali** (10 μM) + cell lysate solution (40 μL) + OA (1%) in DI H_2O.

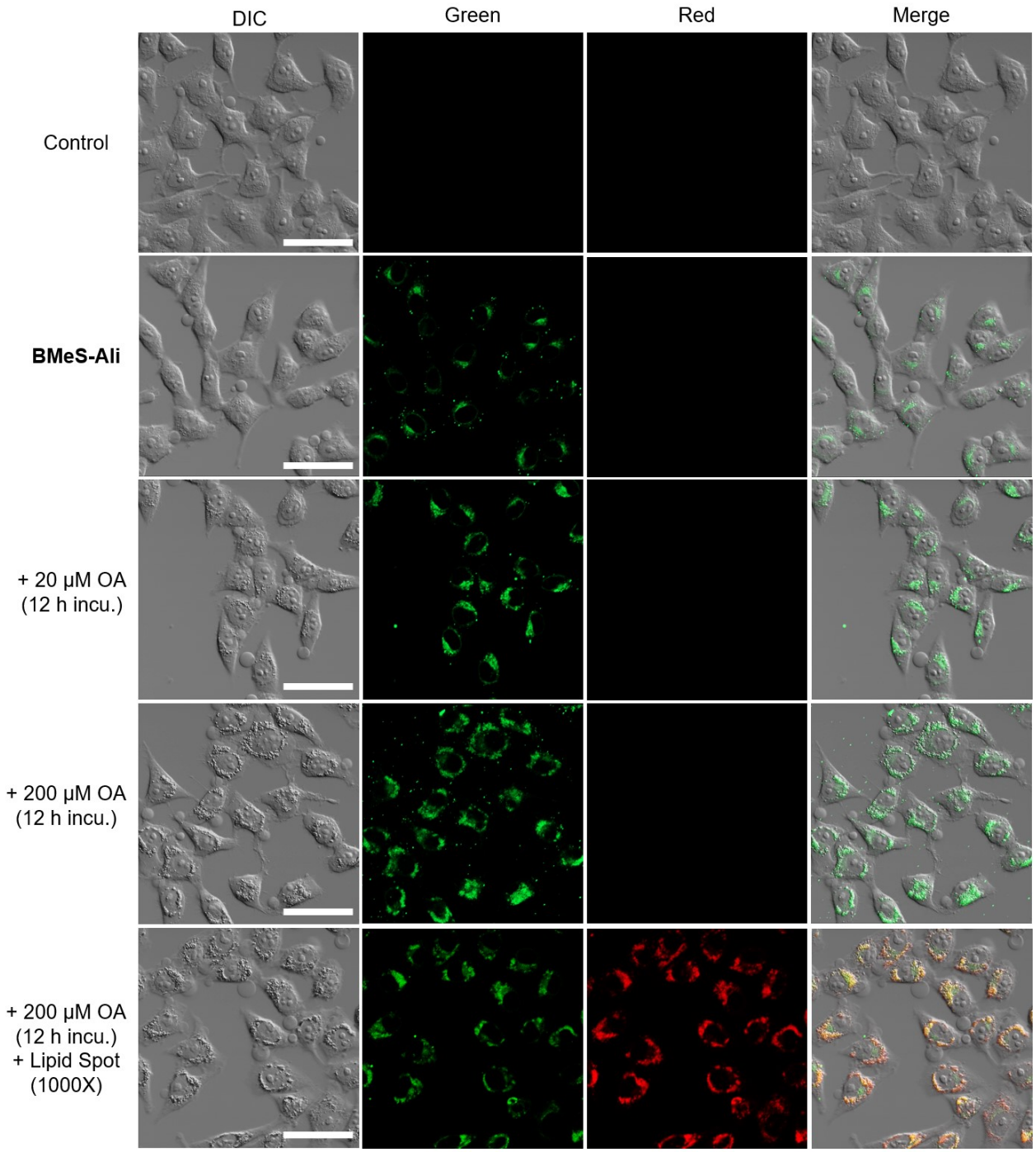


Fig. S15. CLSM images of HeLa cells co-incubated with **BMeS-Ali** (20 μM), LipidSpot™ 610 (1000✂). [OA]: 20 μM and 200 μM of OA were pretreated respectively and incubated for 12 h at 37 ˚C; [**BMeS-Ali**]: 20 μM was treated and incubated for 1 h at 37 ˚C, and then incubated with LipidSpot™ 610 (1000✂) for 30 min at 37 ˚C. The scale bar is 20 μm. Excitation wavelength and detection channel: green (405 nm, 410–585 nm), red (561 nm, 595–700 nm).

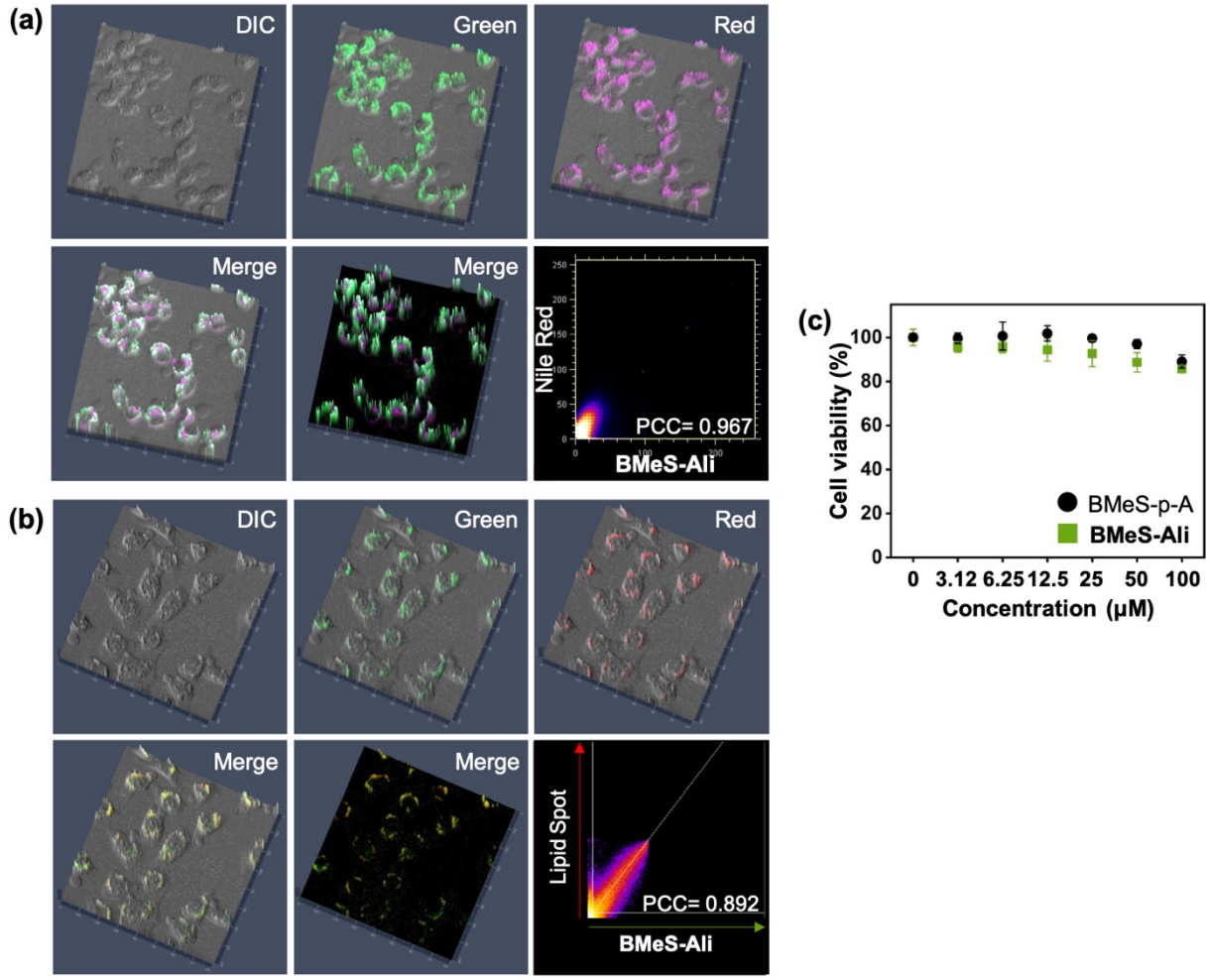


Fig. S16. 2.5D CLSM images of HeLa cells after co-treated with (a) **BMeS-Ali** (20 μM) and Nile Red (300 nM), (b) **BMeS-Ali** (20 μM) and LipidSpot™ 610 (1000ᔕ). (c) Cell viability of **BMeS-p-A** and **BMeS-Ali**. HeLa cells were incubated with 0, 3.12, 6.25, 12.5, 25, 50, and 100 μM of **BMeS-p-A** (black dot) and **BMeS-Ali** (green dot) for 1 h. The cell viability was measured using the Cell Counting Kit-8 (CCK-8). Means and standard deviations were calculated using triplicate measurements.

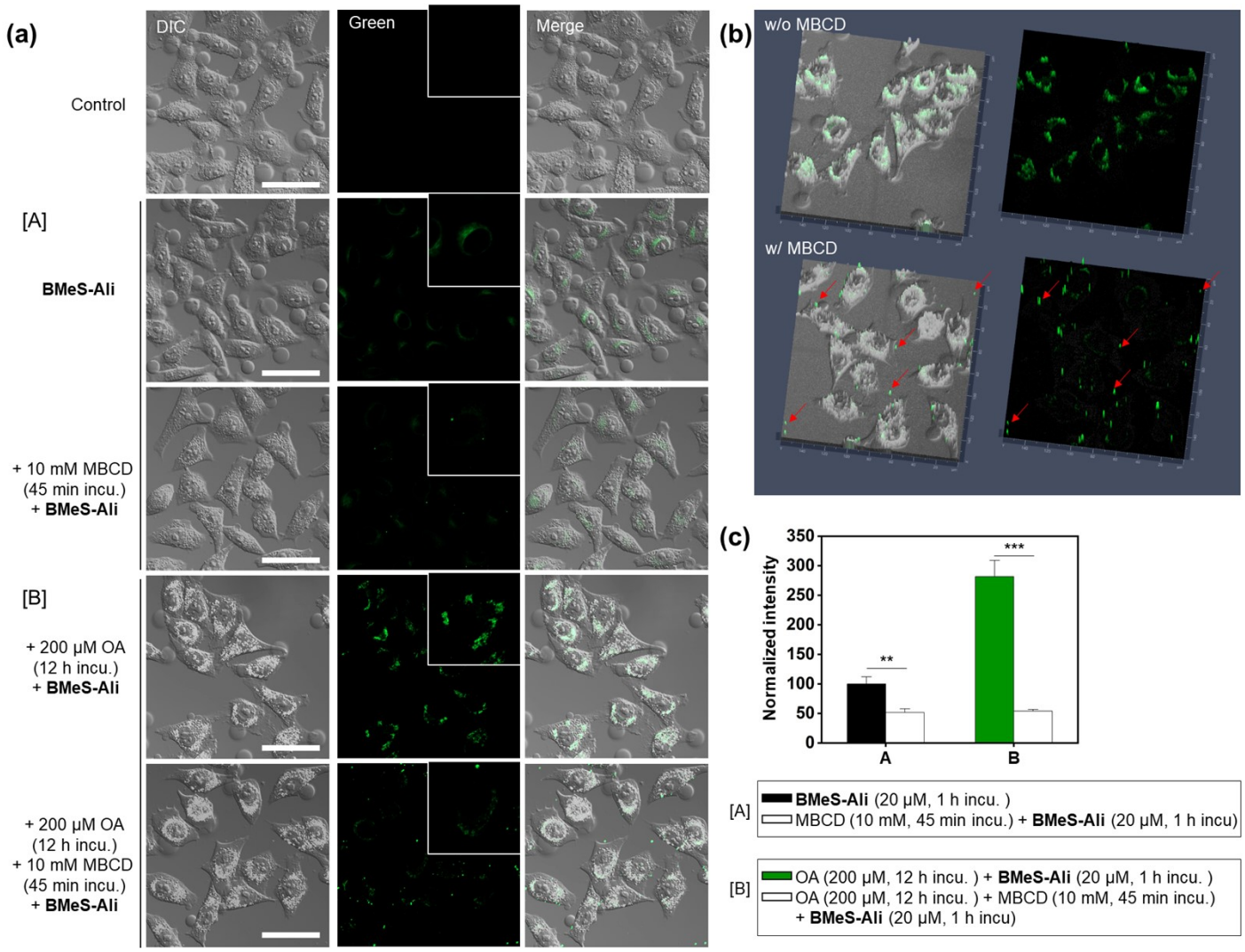


Fig. S17. (a) CLSM images of HeLa cells that inhibited lipid-rafts mediated endocytosis with **BMeS-Ali**. [A] 20 μM of **BMeS-Ali** was treated and incubated for 1 h at 37 ˚C (top); 10 mM MBCD was treated and incubated for 45 min at 37 ˚C, and **BMeS-Ali** was further incubated (bottom). [B] 200 μM of OA were pretreated respectively and incubated for 12 h at 37 ˚C; **BMeS-Ali** was added (top); after OA treatment, 10 mM MBCD was treated and incubated for 45 min at 37 ˚C, and **BMeS-Ali** was further incubated (bottom). The scale bar is 20 μm. Excitation wavelength and detection channel: green (405 nm, 410–585 nm), red (561 nm, 595–700 nm). (b) 2.5D CLSM images of [B]. Red arrow: extracellular **BMeS-Ali**. (c) Normalized fluorescence signal intensity of **BMeS-Ali** from [A] and [B] (green channel images). The fluorescence signal intensities were measured using Image-J software. Means and standard deviations were calculated using triplicate measurements. MBCD: methyl-β-cyclodextrin.

3. Supporting Tables

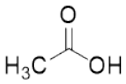
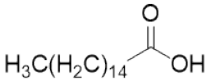
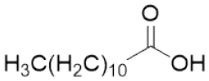
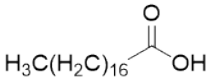
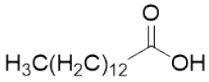
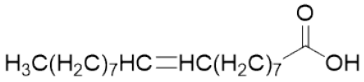
Table S1. Photophysical properties (UV/Vis absorption, emission, Stokes shift, quantum yield) of **BMeS-p-A** (10 μM) in various solvents (THF: tetrahydrofuran, DCM: dichloromethane, EtOH: ethanol, MeOH: methanol, ACN: acetonitrile, DMF: *N,N*-dimethyl formamide, DMSO: dimethyl sulfoxide, and DI H_2O: deionized water). λ_{abs}: absorption maximum wavelength. ε: molar extinction coefficient. λ_{emi}: emission maximum wavelength. QY: quantum yield.

Compound	Solvents	λ_{abs} (nm)	ε (L mol^{-1} cm^{-1})	λ_{emi} (nm)	Stokes shift	QY
BMeS-p-A	THF	384	4545.50	494	110	0.38
	DCM	376	4215.00	486	110	0.39
	EtOH	385	4225.50	506	121	0.52
	MeOH	400	3986.00	506	106	0.47
	ACN	403	3379.50	495	92	0.45
	DMF	390	5138.00	504	114	0.61
	DMSO	394	4374.50	509	115	0.80
	DI H_2O	378	4118.50	515	137	0.49

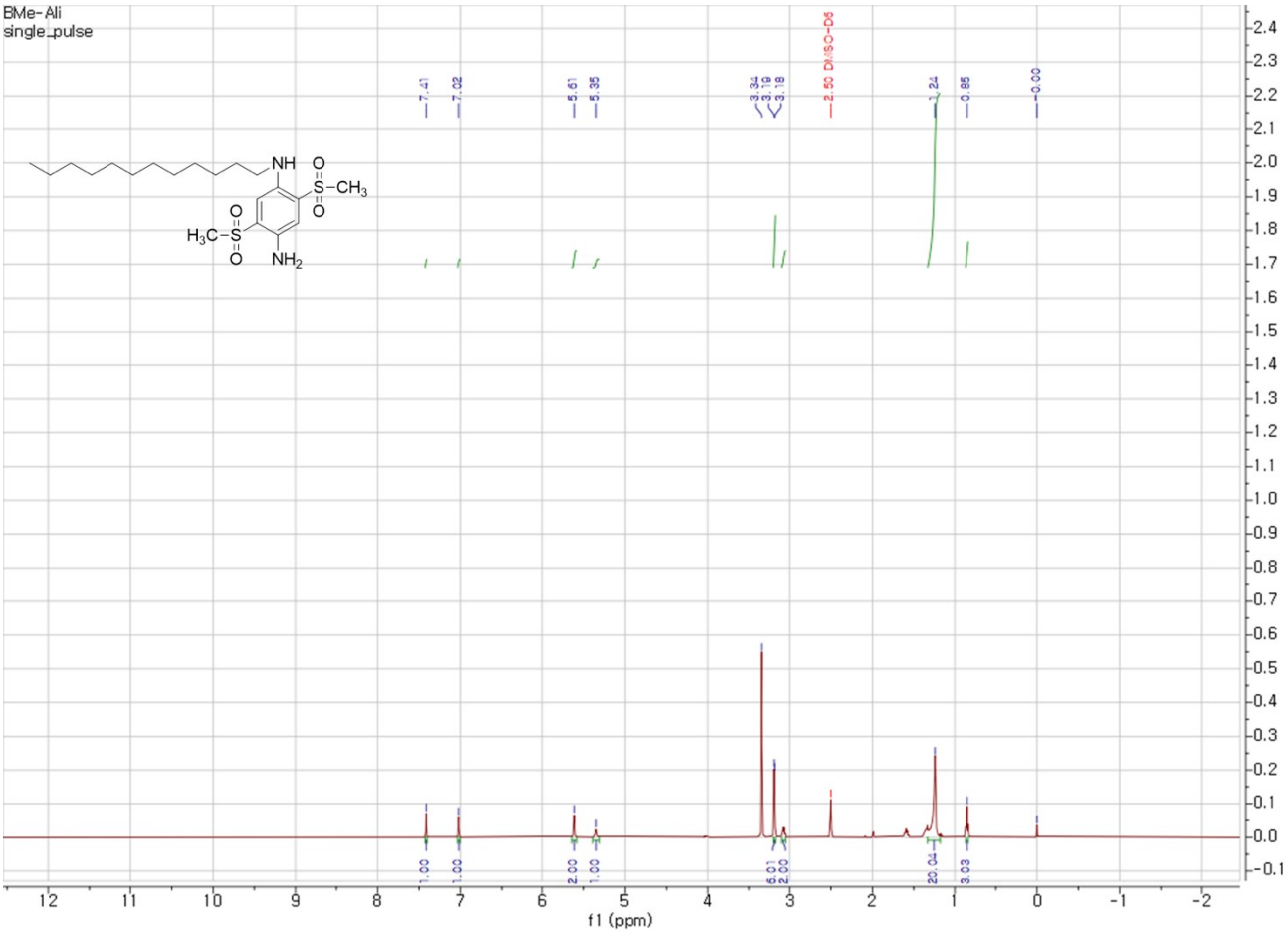
Table S2. Photophysical properties (UV/Vis absorption, emission, Stokes shift, quantum yield) of **BMeS-Ali** (10 μM) in various solvents (THF: tetrahydrofuran, DCM: dichloromethane, EtOH: ethanol, MeOH: methanol, ACN: acetonitrile, DMF: *N,N*-dimethyl formamide, DMSO: dimethyl sulfoxide, and DI H_2O: deionized water). λ_{abs}: absorption maximum wavelength. ε: molar extinction coefficient. λ_{emi}: emission maximum wavelength. QY: quantum yield.

Compound	Solvents	λ_{abs} (nm)	ε (L mol^{-1} cm^{-1})	λ_{emi} (nm)	Stokes shift	QY
BMeS-Ali	THF	395	3783.00	509	114	0.42
	DCM	396	3357.50	510	114	0.37
	EtOH	399	3273.00	519	120	0.50
	MeOH	384	2852.00	520	136	0.44
	ACN	398	3763.00	515	117	0.13
	DMF	403	3605.00	517	114	0.52
	DMSO	407	3468.50	523	116	0.72
	DI H_2O	395	3548.50	515	120	0.03

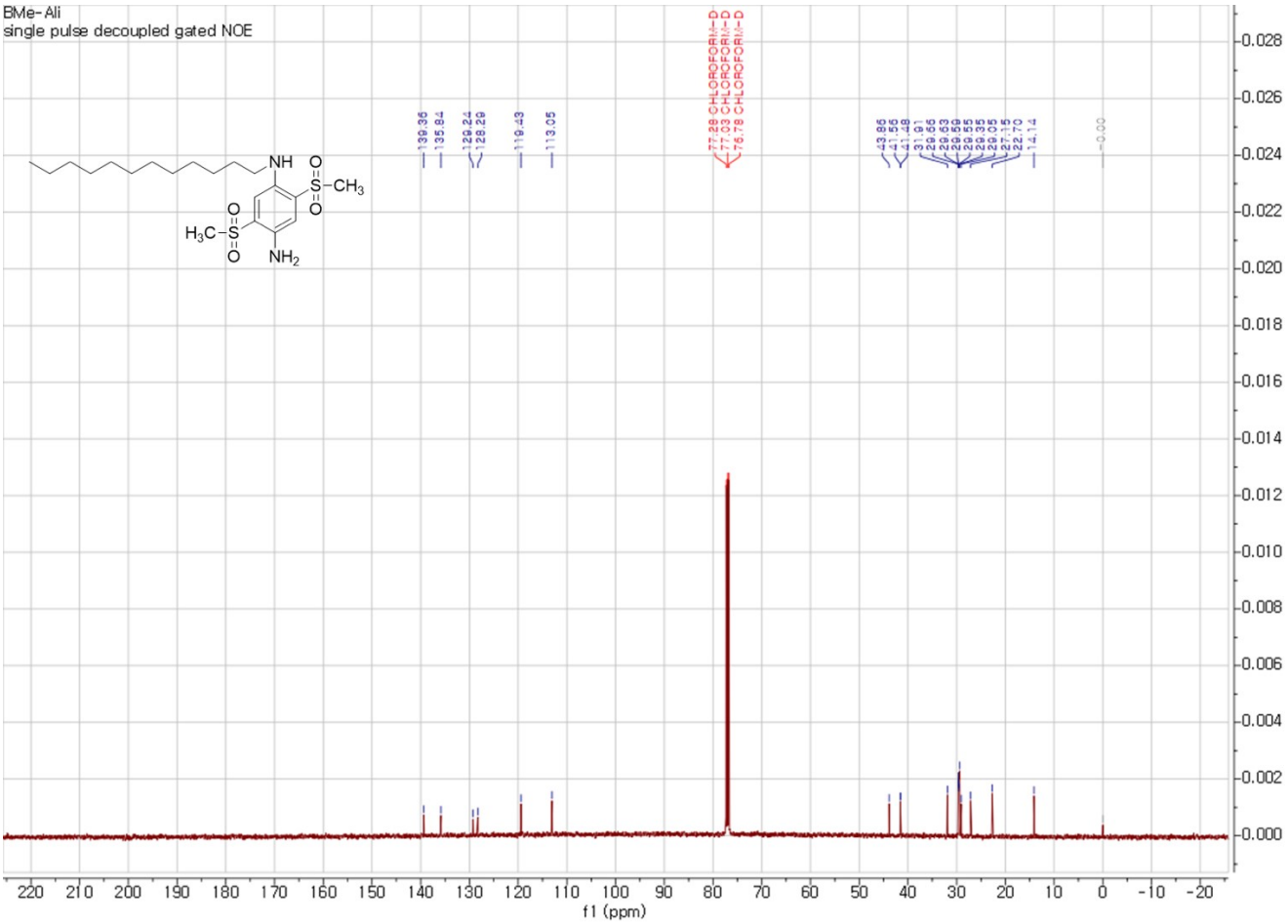
Table S3. Chemical structure of fatty acids.

Name	Structure	Name	Structure
(a) Acetic acid		(d) Palmitic acid	
(b) Lauric acid		(e) Stearic acid	
(c) Myristic acid		(f) Oleic acid	

[1]H Spectra of BMeS-Ali



^{13}C Spectra of BMeS-Ali



HR–mass spectra of BMeS-Ali