Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Control of giant vesicle assemblies by stimuli-responsive lipids

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1. General Information

1.1. Materials

Synthetic precursors, chemical reagents, and solvents were procured from Sigma-Aldrich, Tokyo Chemical Industry Co., Ltd, Thermo Fisher Scientific, or Combi-blocks Inc, and utilized without further purification unless specified otherwise. Thin-layer chromatography (TLC) was performed on E. Merck silica gel 60 F254 analytical plates. The progression of chemical synthesis was tracked through the use of a handheld UV lamp (254 nm) or liquid chromatography analysis. Column chromatography was executed using Merck silica gel (60 Å, 230−400 mesh, particle size 0.040−0.063 mm) with technical-grade solvents.

1.2. Nuclear magnetic resonance (NMR) spectroscopy

^{1}H and ^{13}C NMR spectra were acquired using Bruker AVA-300 or Varian VX-500 MHz instruments. Chemical shifts were expressed in parts per million (δ) and calibrated using the solvent residual signal of chloroform-*d* (7.26 ppm for ^{1}H NMR spectra and 77.16 ppm for ^{13}C NMR spectra).[S1] Multiplicity was recorded as follows: s (singlet), d (doublet), t (triplet), m (multiplet), dd (doublet of doublet), etc. Coupling constants were reported in Hertz (Hz).

1.3. Liquid chromatography and mass analysis

Liquid chromatography-mass spectrometry (LC-MS) analysis was conducted using an Agilent 1260 Infinity LC System coupled with an Agilent 6120 quadrupole mass spectrometer. The LC system was equipped with a diode array detector (DAD), an evaporative light scattering detector (ELSD), and an Eclipse Plus C8 analytical column. The analysis utilized a solvent gradient as follows: Phase A consisted of H_2O with 0.1% formic acid, and Phase B comprised MeOH with 0.1% formic acid. In all experiments, 20 μL of the respective sample was injected. High-resolution mass spectrometry (HRMS) was conducted at the University of California San Diego Molecular Mass Spectrometry Facility using an Agilent 1260 Infinity Binary LC coupled with a 6230 Accurate-Mass TOFMS system for HPLC-HR-ESI/APCI-MS analysis.

1.4. Light sources

Irradiation with 365 nm light was conducted using an EvoluChem™ LED (9 mW/cm^2, HepatoChem) or a handheld 365 nm flashlight (Alonefire SV003). For visible light, a 470 nm LED flashlight (UltraFire H-B3) was utilized. The tungsten-halogen lamp (Osram EVA 64623 HLX) from Olympus microscopy served as the visible light source for capturing real-time videos. We confirmed that the morphological effects of azobenzene assemblies were identical when exposed to either 470 nm LED light or the tungsten-halogen lamp.

1.5. Phase-contrast microscopy

Phase-contrast imaging was conducted using an Olympus BX51 optical microscope equipped with a phase-contrast condenser (Ph3) and a 100x oil immersion objective. Images were captured with an ORCA-spark Digital CMOS camera (C11440-36U, Hamamatsu) using Olympus cellSens 3.1 software. To visualize Nile Red-labeled vesicles, imaging utilized a

fluorescence mercury light source (U-LH100HGAPO, Olympus) combined with a Texas Red filter cube. Typically, samples were prepared by dispensing 1.5 μL of the sample onto a microscope glass slide and covering it with a coverslip. The acquired images were subsequently analyzed using ImageJ software.

1.6. UV-vis spectrometry

The UV-Vis analysis was conducted using a NanoDrop 2000c spectrophotometer (Thermo Scientific). We utilized standard quartz glass cuvettes with a path length of 10 mm and a volume of 3500 μL (Thorlabs, Inc.).

1.7. Dynamic light scattering

Dynamic Light Scattering (DLS) measurements were carried out using the DynaPro® Plate Reader III at the MRSEC Materials Characterization Facility at UC San Diego. A 386-well plate with a sample volume of 30 μL was utilized for the experiments. All measurements were conducted in triplicate as a minimum.

1.8. Differential scanning calorimetry (DSC)

The DSC analysis was performed using a DSC Q200 instrument from TA Instruments at Applied Technical Services (ATS). DSC was conducted according to ASTM D3418-21, standard test method, from -90°C to 200°C under N_2 atmosphere.

1.9. Statistical methods

Statistical analysis of the data was performed using Prism 5 software, and the error bars represent the standard deviation based on a minimum of three replicates

2. Experimental Section

2.1. General self-assembly process of azobenzene products using light-triggered method

In 2 mL Eppendorf tubes, stock solutions of a 2 mM azobenzene in H_2O were prepared by subjecting them to UV treatment for 3 min followed by heating at 50 °C for 3 min. We confirmed that 2 mM of *cis*-azobenzene lipids (compound **1**, **2**, or **2A**) can be readily dissolved in a pure H_2O solution without forming observable aggregates, which was double-checked under a microscope. After cooling to room temperature, the stock solution was combined with salts or buffers, followed by exposure to 470 nm light for 3 min. The total concentration of azobenzene assemblies ranged from 0.5 to 1.0 mM for general imaging purposes. Comparison with the typical thin-film hydration method, which involves the evaporation of lipid-containing organic solvents, followed by tumbling of thin-lipid layered vials in the aqueous media, yielded identical results to the light-triggered method.

2.2. Light illumination

For UV activation, a 365 nm LED light source was positioned over reaction vials at a distance of more than 20 cm for a duration of 3 min. For imaging purposes, a handheld 365 nm or a 470 nm flashlight was set up on a tabletop stand next to the light microscope and directed onto the glass slide containing the sample. The distance between the sample and the flashlights was maintained at over 15 cm. The influence of heat generated from the light source was negligible.

2.3. Analysis of isomeric ratio

To obtain the isomeric ratio of compound **1** and **2A-C_{10}**, the chromatograms obtained by the LC-DAD method were analyzed. The relative ratio was compared using the integrated area under the curve for each isomer. For the precise analysis, it is important to note that measurements should be taken at isosbestic points, where absorptivity between isomers is identical. For compound **1**, the isosbestic point was recorded at 242 nm. For compound **2A-C_{10}**, the point was 250 nm.

2.4. In situ 2A-C_{10} imine formation reaction

The in situ formation of **2A-C_{10}** was conducted by combining 20 µL of 2 mM **2A** in H_2O, 20 µL of blank H_2O, 20 µL of 8 mM decylamine (C_{10}) in HEPES buffer, and 20 µL of 200 mM NaCl in HEPES buffer in glass vials (2 mL). The total concentration of each component in the 80 µL reaction mixture was as follows: 0.5 mM **2A**, 2 mM C_{10}-amine, 25 mM HEPES, and 50 mM NaCl at pH 7.5. The mixture was left to tumble overnight using a tube rotator set at 15 rpm for 24 h. To prepare in situ samples for the UV-induced vesicle merging experiment, 0.2 mM sodium oleate in H_2O was used instead of blank H_2O.

2.5. Light-induced delipiation using photoacid

A merocyanine photoacid with a methoxy and trimethylamine group was prepared following a previous report.[S2] For light-induced acidification using the photoacid, a 16 mM stock solution of photoacid in H_2O was prepared in 2 mL Eppendorf tubes. To an 80 µL photoacid

stock, 80 µL of 100 mM NaCl was added, followed by pH adjustment using a 0.1 M NaOH solution to achieve a final pH range close to 5.5. In general, the final concentration of the solution was as follows: photoacid 7.5–7.6 mM, NaCl 46–47 mM, at pH 5.3–5.7.

To an empty 2 mL glass vial, 20 µL of the isolated 2 mM **2A-C$_{10}$** in methanol stock solution was added, followed by blowing a stream of argon gas to form a thin lipid film. Subsequently, 40 µL of the previously prepared photoacid (pH ~5.5 in NaCl) solution was added to the vial. The mixture was warmed up to 50 °C, followed by gentle tumbling for a minimum of 0.5 h for vesicle formation. A 1.5 µL sample of the prepared mixture was applied to a glass slide covered with a cover slip. Real-time images or movies were obtained under the irradiation of either a 470 nm LED light or an intense halogen lamp from the microscopy setting. Both visible light sources produced identical outcomes.

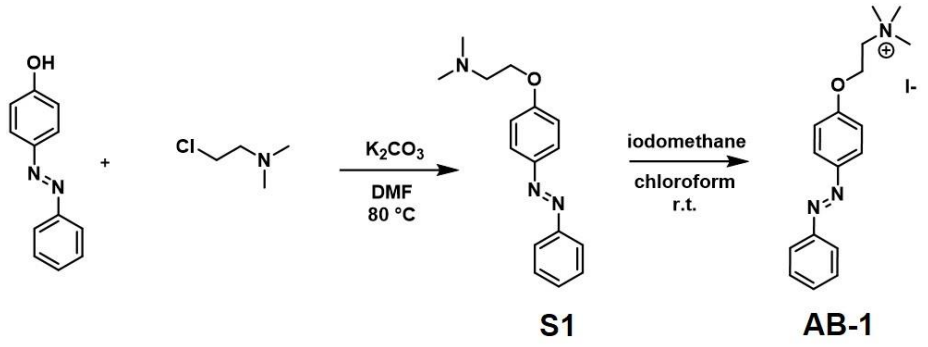
2.6. Light-induced vesicle growth and releasing

An in situ solution of **2A-C$_{10}$** imine, containing 10 mol% sodium oleate, was prepared as outlined in section 2.4. To promote vesicle growth, the resulting in situ solution underwent irradiation with 365 nm light for 3 min, followed by tumbling in the dark for 2 h. The subsequent releasing of vesicles was observed by placing a 1.5 µL sample of enlarged vesicles under the irradiation of 470 nm LED light or a halogen lamp in the microscope setting.

2.7. Cryo-TEM

Cryo-TEM grids (Lacey Carbon Film, Electron Microscopy Sciences #LC200-Cu) were glow-discharged using an Emitech K350 unit at 20 mA for 45 seconds. Next, 3.5 µL of a 1 mM lipid dispersion of the sample was applied to the grids. The grids were immediately vitrified using a Vitrobot Mark IV (Thermo Fisher Scientific) with 4 second blot times and a blot-force setting of 4 at 4 ºC and 100% humidity. Plunge-freezing into liquid ethane cooled by liquid nitrogen was employed for vitrification. The samples were stored in liquid nitrogen until use. Images were acquired on a Talos Arctica (FEI) operated at 200 kV, collected with a total dose of 40 e/Å^2 at 1.55 Å/pixel, and a 3 µm nominal defocus.

3. Chemical Synthesis and Characterization



Synthesis of **S1**:

To a solution of 4-phenylazophenol (100 mg, 0.50 mmol, 1 equiv.) in dimethylformamide (DMF, 2 mL) were added 2-chloro-*N*,*N*-dimethylethylamine hydrochloride (145 mg, 1.00 mmol, 2 equiv.) and potassium carbonate (348 mg, 2.52 mmol, 5 equiv.). The mixture was stirred at 80 °C overnight (>20 h). The resulting mixture was washed with brine and extracted using dichloromethane (DCM). The collected organic portion was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography (SiO_2) using an eluent of DCM:methanol (20:1, v/v) to yield **S1** as an orange solid (67 mg, Y: 49%).

^{1}H NMR (chloroform-*d*, 500 MHz): δ 7.92 (d, *J*=9.0 Hz, 2H), 7.85–7.90 (m, 2H), 7.50 (t, *J*=7.5 Hz, 2H), 7.40–7.47 (m, 1H), 7.04 (d, *J*=9.0 Hz, 2H), 4.17 (t, *J*=5.6 Hz, 2H), 2.81 (t, *J*=5.6 Hz, 2H), 2.39 (s, 6H).

^{13}C NMR (chloroform-*d*, 126 MHz): δ 161.3, 152.8, 147.1, 130.5, 129.2, 124.8, 122.7, 114.9, 66.2, 58.2, 46.0.

HRMS (ESI): m/z calcd for $C_{16}H_{20}N_3O^+$ $[M+H]^+$: 270.1601, found 270.1603.

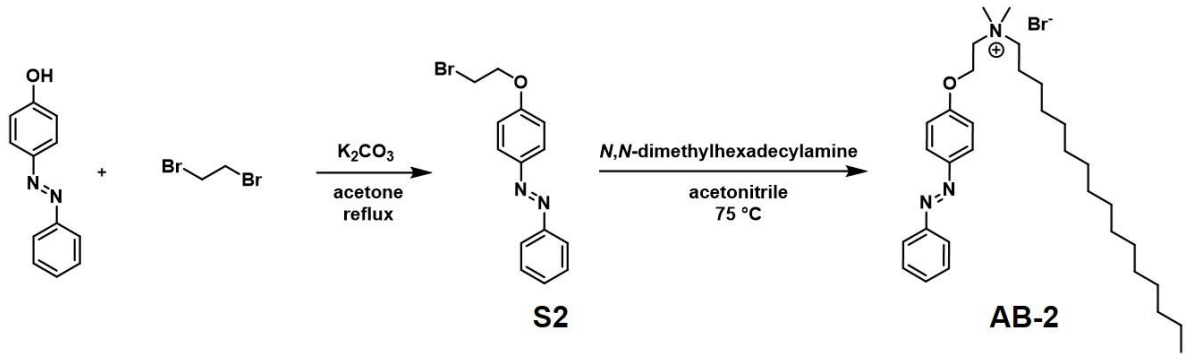
Synthesis of **AB-1**:

To a solution of **S1** (11 mg, 0.041 mmol, 1 equiv.) in chloroform (400 μL) was added iodomethane (25 μL, 0.41 mmol, 10 equiv.). The mixture was stirred at room temperature for 2 h. The mixture was concentrated under reduced pressure. The resulting mixture was washed with hot acetonitrile three times and dried under vacuum to afford the iodide salts of **AB-1** as a yellow solid (12 mg, Y: 71%).

^{1}H NMR (chloroform-*d*, 500 MHz): δ 7.96 (d, *J*=9.0 Hz, 2H), 7.89 (d, *J*=7.1 Hz, 2H), 7.51 (t, *J*=7.3 Hz, 2H), 7.47 (t, *J*=7.1 Hz, 1H), 7.07 (d, *J*=9.0 Hz, 2H), 4.57–4.71 (m, 2H), 4.32–4.46 (m, 2H), 3.58 (s, 9H).

^{13}C NMR (chloroform-*d*, 126 MHz): δ 158.9, 152.6, 148.1, 131.0, 129.3, 125.2, 122.9, 114.8, 65.4, 62.9, 55.4.

HRMS (ESI): m/z calcd for $C_{17}H_{22}N_3O^+$ $[M]^+$: 284.1757, found 284.1756.



Synthesis of **S2**:

To a solution of 4-phenylazophenol (399 mg, 2.01 mmol, 1 equiv.) in acetone (4 mL) were added 1,2-dibromoethane (868 μL, 10.1 mmol, 5 equiv.) and potassium carbonate (1392 mg, 10.1 mmol, 5 equiv.). The mixture was stirred under reflex conditions overnight (>20 h). The resulting mixture was washed with brine and extracted using ethyl acetate (EtOAc). The collected organic portion was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography (SiO_2) using an eluent of EtOAc:hexane (1:10, v/v) to yield **S2** as an orange solid (389.9 mg, Y: 63%).

^{1}H NMR (chloroform-*d*, 500 MHz): δ 7.93 (d, *J*=9.0 Hz, 2H), 7.88 (dd, *J*=8.4, 1.3 Hz, 2H), 7.51 (t, *J*=7.5 Hz, 2H), 7.45 (m, 1H), 7.03 (d, *J*=9.0 Hz, 2H), 4.38 (t, *J*=6.2 Hz, 2H), 3.69 (t, *J*=6.2 Hz, 2H).

^{13}C NMR (chloroform-*d*, 126 MHz): δ 160.5, 152.7, 147.5, 130.7, 129.2, 124.9, 122.7, 115.0, 68.1, 29.0.

HRMS (ESI): m/z calcd for $C_{14}H_{14}BrN_2O^+$ $[M+H]^+$: 305.0284, found 305.0282.

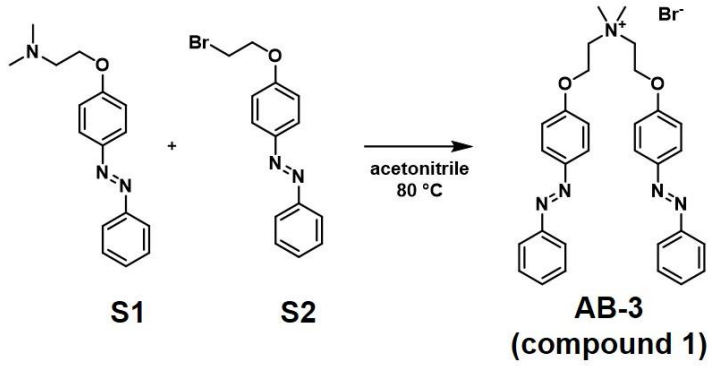
Synthesis of **AB-2**:

To a solution of **S2** (33.8 mg, 0.11 mmol, 1 equiv.) in acetonitrile (1 mL) was added *N*,*N*-dimethylhexadecylamine (372 μL, 1.1 mmol, 10 equiv.). The mixture was stirred at 75 °C overnight (>20 h). After the reaction mixture was condensed under reduced pressure, the crude mixture was washed with ether and a minimal amount of hot acetone (approximately 200 μL). The resulting product was dried under vacuum to obtain the bromide salts of **AB-2** as a yellow solid (35 mg, Y: 55%).

^{1}H NMR (chloroform-*d*, 500 MHz): δ 7.95 (d, *J*=8.8 Hz, 2H), 7.89 (dd, *J*=8.4, 1.1 Hz, 2H), 7.49–7.57 (m, 2H), 7.43–7.49 (m, 1H), 7.04 (d, *J*=9.0 Hz, 2H), 4.62 (m, 2H), 4.38 (m, 2H), 3.57–3.70 (m, 2H), 3.50 (s, 6H), 1.81–1.89 (m, 2H), 1.20–1.45 (m, 26H), 0.87 (t, *J*=7.0 Hz, 3H).

^{13}C NMR (chloroform-*d*, 126 MHz): δ 159.0, 152.6, 148.0, 131.0, 129.2, 125.2, 122.8, 114.7, 66.8*, 62.7, 52.1, 32.1, 29.85*, 29.83, 29.81, 29.80, 29.7, 29.6, 29.5*, 29.4, 26.4, 23.1, 22.8, 14.3. (*multiple peaks overlapped).

HRMS (ESI): m/z calcd for $C_{32}H_{52}N_3O^+$ $[M]^+$: 494.4105, found 494.4103.



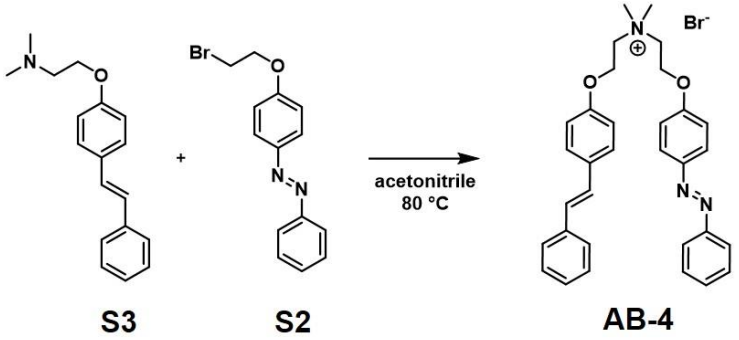
Synthesis of **AB-3** (compound **1**):

An acetonitrile solution (1 mL) containing **S1** (32 mg, 0.12 mmol, 1 equiv.) and **S2** (54 mg, 0.18 mmol, 1.5 equiv.) was stirred at 80 °C overnight (>20 h). After cooling to room temperature, the mixture was subjected to direct purification through flash column chromatography (SiO_2) using an eluent of DCM:methoanol (20:1→10:1, v/v) to yield the bromide salts of **AB-3** as a yellow solid (50.5 mg, Y: 74%).

^{1}H NMR (chloroform-*d*, 500 MHz): δ 7.93 (d, *J*=9.0 Hz, 4H), 7.81–7.90 (m, 4H), 7.39–7.61 (m, 6H), 7.05 (d, *J*=8.8 Hz, 4H), 4.61–4.77 (m, 4H), 4.37–4.55 (m, 4H), 3.66 (s, 6H).

^{13}C NMR (chloroform-*d*, 126 MHz): δ 159.1, 152.5, 147.9, 131.0, 129.2, 125.2, 122.8, 114.9, 64.7, 62.6, 53.2.

HRMS (ESI): m/z calcd for $C_{30}H_{32}N_5O_2^+$ $[M]^+$: 494.2551, found 494.2549.



S3 was synthesized following a reported procedure.[S3]

^{1}H NMR (chloroform-*d*, 500 MHz): δ 7.49 (d, *J*=7.1 Hz, 2H), 7.45 (d, *J*=8.6 Hz, 2H), 7.35 (t, *J*=7.7 Hz, 2H), 7.24 (t, *J*=7.3 Hz, 1H), 7.06 (d, *J*=16.1 Hz, 1H), 6.98 (d, *J*=16.4 Hz, 1H), 6.92 (d, *J*=8.8 Hz, 2H), 4.15 (t, *J*=5.5 Hz, 2H), 2.85 (t, *J*=5.6 Hz, 2H), 2.44 (s, 6H).

^{13}C NMR (chloroform-*d*, 126 MHz): δ 158.3, 137.7, 130.5, 128.8, 128.2, 127.8, 127.4, 126.8, 126.4, 114.8, 65.6, 58.1, 45.7.

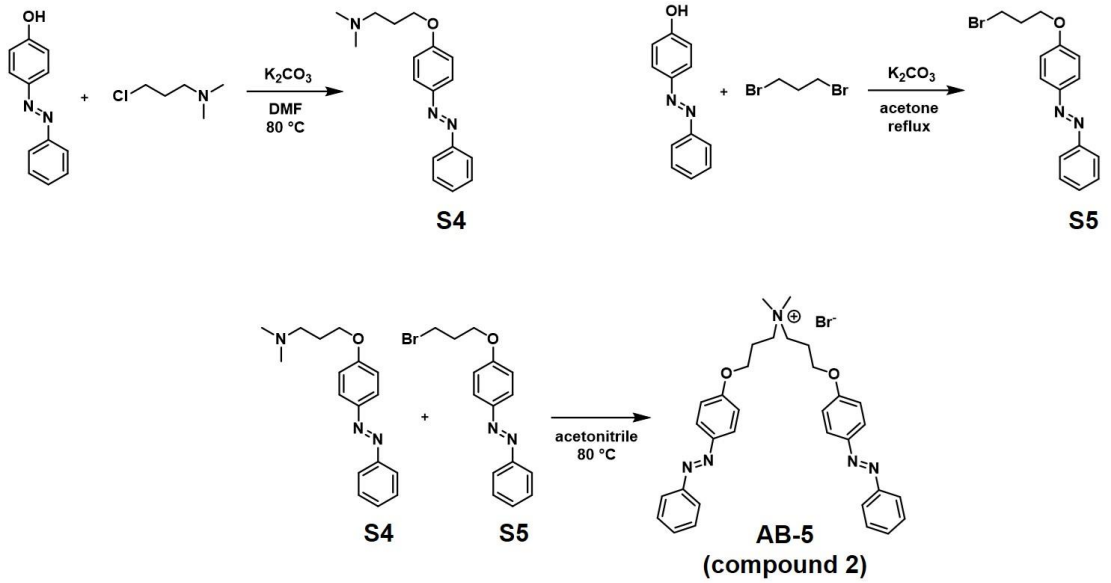
Synthesis of **AB-4**:

An acetonitrile solution (0.5 mL) containing **S3** (13.5 mg, 0.050 mmol, 1 equiv.) and **S2** (23.1 mg, 0.076 mmol, 1.5 equiv.) was stirred at 80 °C overnight (>20 h). After cooling to room temperature, the mixture was subjected to direct purification through flash column chromatography (SiO_2) using an eluent of DCM:methoanol (10:1, v/v) to yield the bromide salts of **AB-4** as a pale yellow solid (18.3 mg, Y: 63%).

^{1}H NMR (chloroform-*d*, 500 MHz): δ 7.96 (d, *J*=8.8 Hz, 2H), 7.83–7.93 (m, 2H), 7.44–7.59 (m, 6H), 7.41 (d, *J*=7.1 Hz, 1H), 7.35 (t, *J*=7.6 Hz, 2H), 7.20 (m, 1H) 7.01–7.10 (m, 3H), 6.98 (m, 1H), 6.91 (d, *J*=8.8 Hz, 2H), 4.63–4.72 (m, 2H), 4.54–4.63 (m, 2H), 4.37–4.54 (m, 4H), 3.54–3.74 (m, 6H).

^{13}C NMR (chloroform-*d*, 126 MHz): δ 158.9, 156.3, 152.6, 148.1, 137.3, 132.1, 131.0, 129.3, 128.8, 128.2, 128.0, 127.7, 127.5, 126.5, 125.2, 122.9, 114.8, 114.7, 64.7, 64.7, 62.6, 62.3, 53.3.

HRMS (ESI): m/z calcd for $C_{32}H_{34}N_3O_2^+$ $[M]^+$: 492.2646, found 492.2650.



Synthesis of **S4**:

To a solution of 4-phenylazophenol (100 mg, 0.50 mmol, 1 equiv.) in DMF (2 mL) were added 3-dimethylamino-1-propyl chloride hydrochloride (159 mg, 1.00 mmol, 2 equiv.) and potassium carbonate (348 mg, 2.52 mmol, 5 equiv.). The mixture was stirred at 80 °C overnight (>20 h). The resulting mixture was washed with brine and extracted using DCM. The collected organic portion was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography (SiO_2) using an eluent of DCM:methanol (20:1→10:1, v/v) to yield **S4** as an orange solid (99 mg, Y: 69%).

^{1}H NMR (chloroform-*d*, 500 MHz): δ 7.91 (d, *J*=9.0 Hz, 2H), 7.84–7.89 (m, 2H), 7.50 (t, *J*=7.5 Hz, 2H), 7.39–7.47 (m, 1H), 7.00 (d, *J*=9.0 Hz, 2H), 4.13 (t, *J*=6.2 Hz, 2H), 2.62–2.77 (m, 2H), 2.43 (s, 6H), 2.13 (quin, *J*=7.2 Hz, 2H).

^{13}C NMR (chloroform-*d*, 126 MHz): δ 161.4, 152.8, 147.1, 130.5, 129.2, 124.9, 122.7, 114.8, 66.2, 56.3, 45.1, 26.8.

HRMS (ESI): m/z calcd for $C_{17}H_{22}N_3O^+$ $[M+H]^+$: 284.1757, found 284.1758.

Synthesis of **S5**:

To a solution of 4-phenylazophenol (100 mg, 0.50 mmol, 1 equiv.) in acetone (2 mL) were added 1,3-dibromopropane (514 μL, 5.0 mmol, 10 equiv.) and potassium carbonate (697 mg, 5.0 mmol, 10 equiv.). The mixture was stirred under reflex conditions overnight (>20 h). The resulting mixture was washed with brine and extracted using EtOAc. The collected organic portion was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography (SiO_2) using an eluent of EtOAc:hexane (1:10, v/v) to yield **S2** as an orange solid (124 mg, Y: 77%).

^{1}H NMR (chloroform-*d*, 500 MHz): δ 7.94 (d, *J*=9.0 Hz, 2H), 7.85–7.91 (m, 2H), 7.51 (t, *J*=7.5 Hz, 2H), 7.45 (d, *J*=7.3 Hz, 1H), 7.02 (d, *J*=9.0 Hz, 2H), 4.20 (t, *J*=5.9 Hz, 2H), 3.64 (t, *J*=6.4 Hz, 2H), 2.37 (quin, *J*=6.1 Hz, 2H).

^{13}C NMR (chloroform-*d*, 126 MHz): δ 161.3, 152.7, 147.1, 130.6, 129.2, 125.0, 122.7, 114.8, 65.7, 32.3, 30.0.

HRMS (ESI): m/z calcd for $C_{15}H_{16}BrN_2O^+$ $[M+H]^+$: 319.0441, found 319.0438.

Synthesis of compound **AB-5** (compound **2)**:

An acetonitrile solution (1 mL) containing **S4** (32 mg, 0.11 mmol, 1 equiv.) and **S5** (54 mg, 0.17 mmol, 1.5 equiv.) was stirred at 80 °C overnight (>20 h). After cooling to room temperature, the mixture was subjected to direct purification through flash column chromatography (SiO_2) using an eluent of DCM:methoanol (20:1→10:1, v/v) to yield the bromide salts of compound **2** as a yellow solid (48.5 mg, Y: 71%).

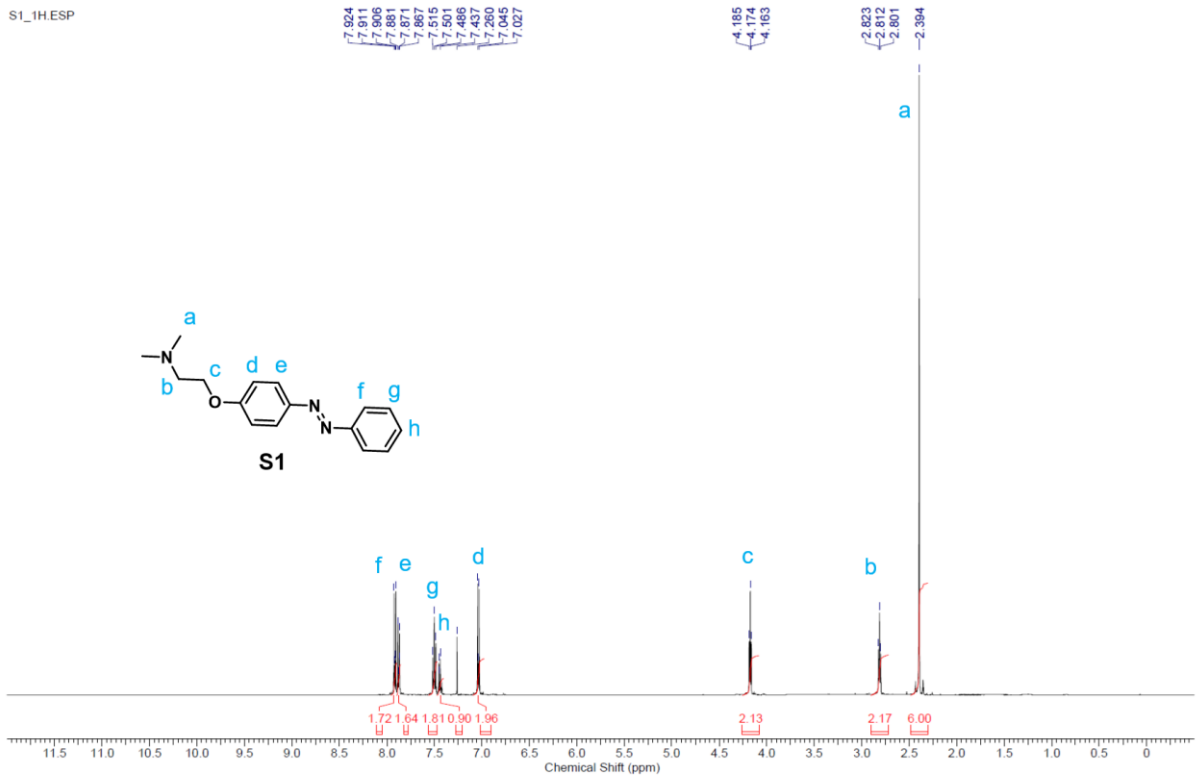
^{1}H NMR (chloroform-*d*, 500 MHz): δ 7.91 (d, *J*=8.8 Hz, 4H), 7.87 (dd, *J*=8.4, 1.3 Hz, 4H), 7.49 (t, *J*=7.2 Hz, 4H), 7.41–7.47 (m, 2H), 7.00 (d, *J*=9.0 Hz, 4H), 4.23 (t, *J*=5.5 Hz, 4H), 3.80–3.97 (m, 4H), 3.47 (s, 6H), 2.28–2.45 (m, 4H).

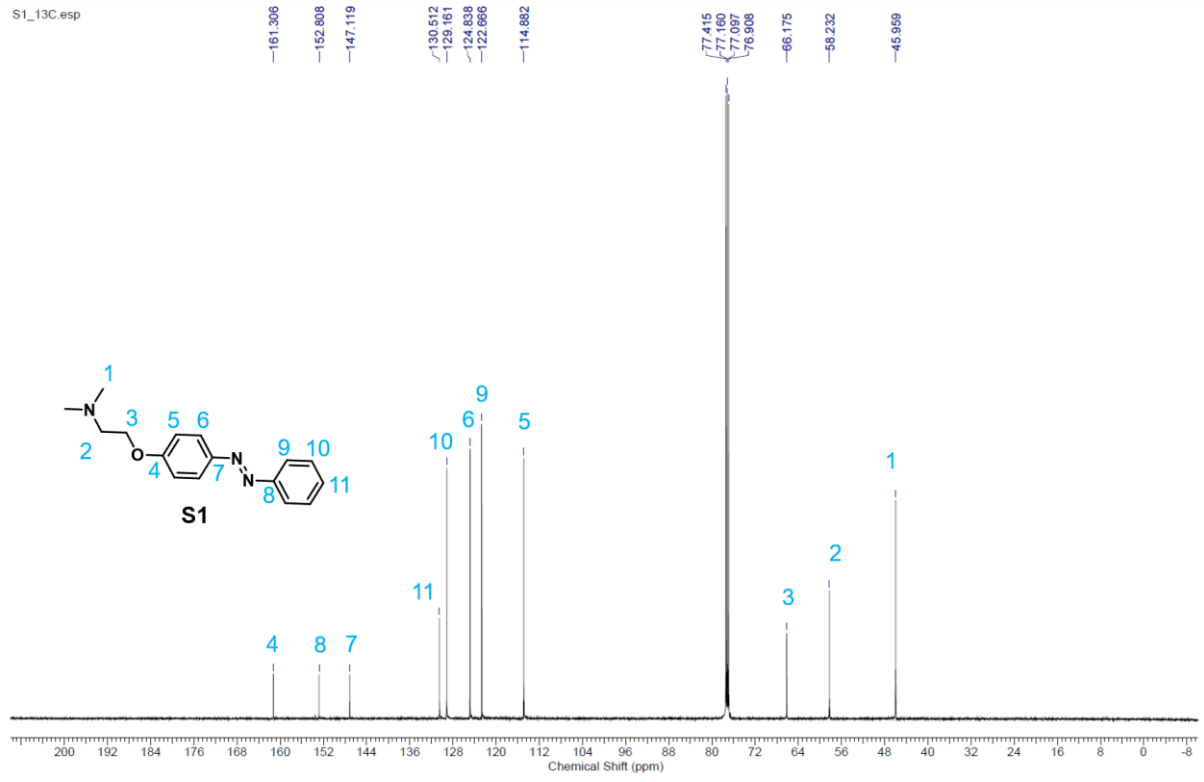
^{13}C NMR (chloroform-*d*, 126 MHz): δ 160.4, 152.6, 147.4, 130.8, 129.2, 125.1, 122.8, 114.9, 64.4, 62.1, 51.8, 23.3.

HRMS (ESI): m/z calcd for $C_{32}H_{36}N_5O_2^+$ $[M]^+$: 522.2864, found 522.2867.

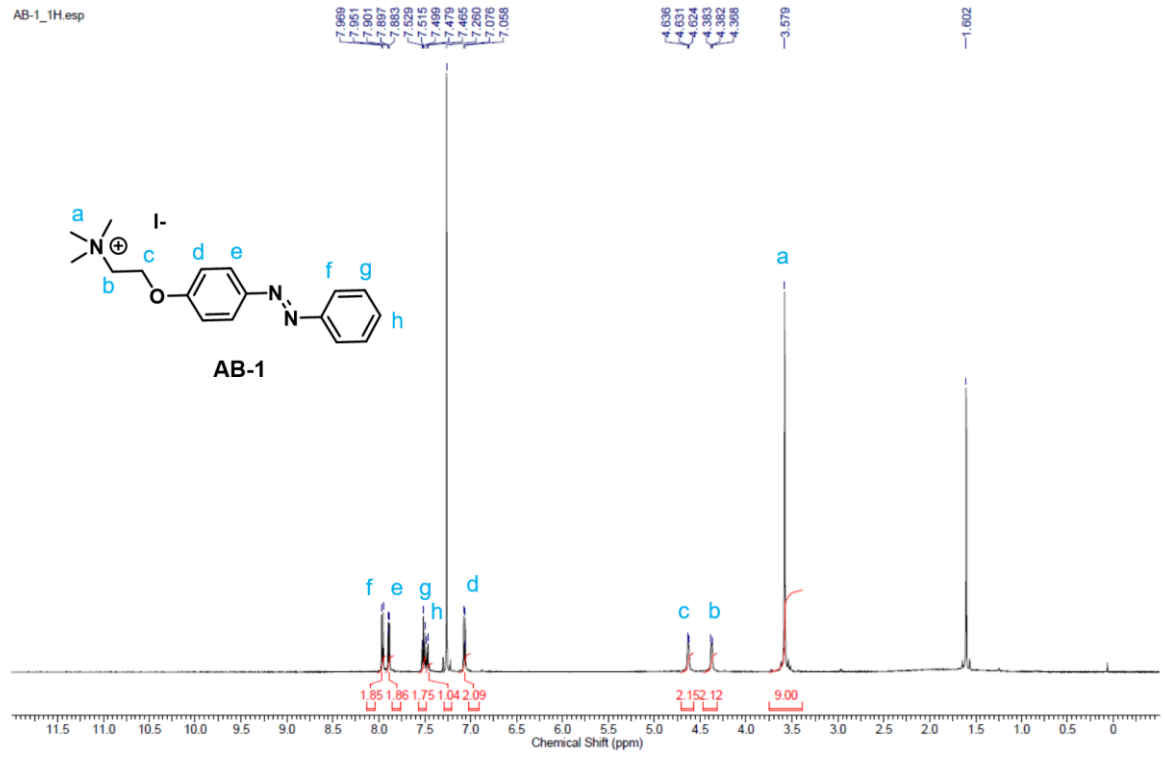
4. Appendix: NMR Spectra

S1





AB-1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b
c
d
e
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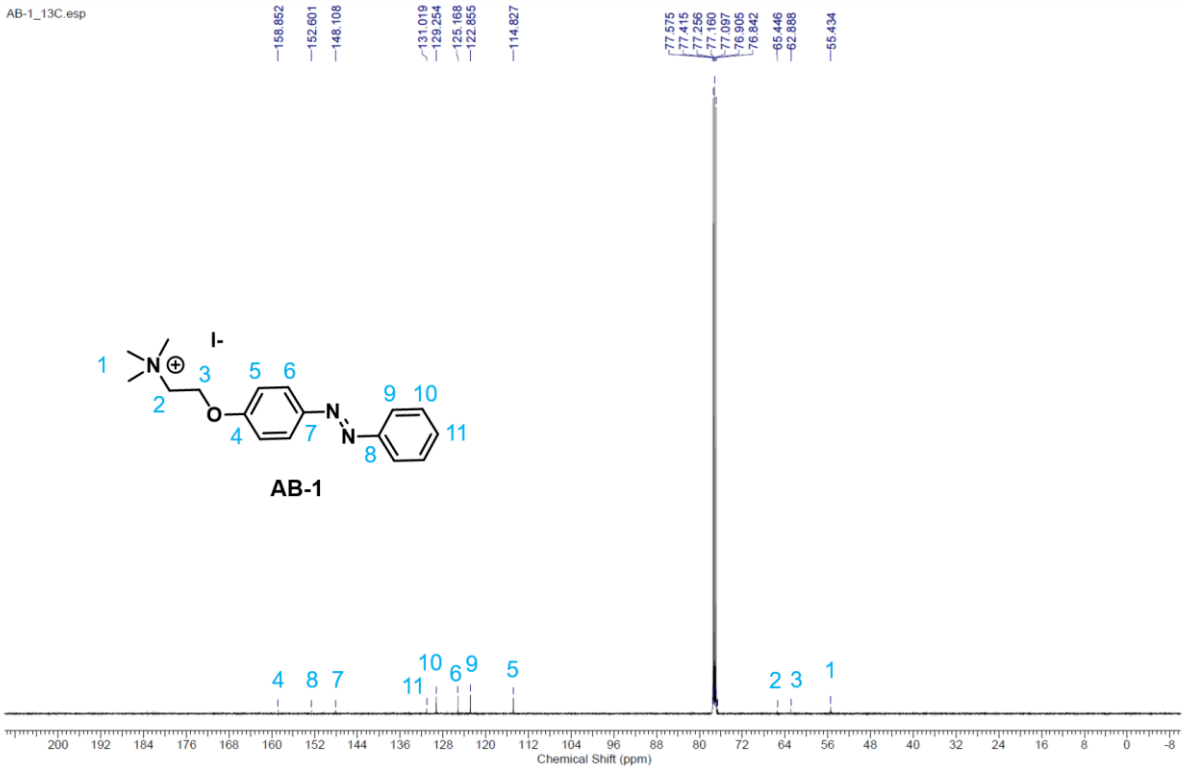
Chemical Shift (ppm)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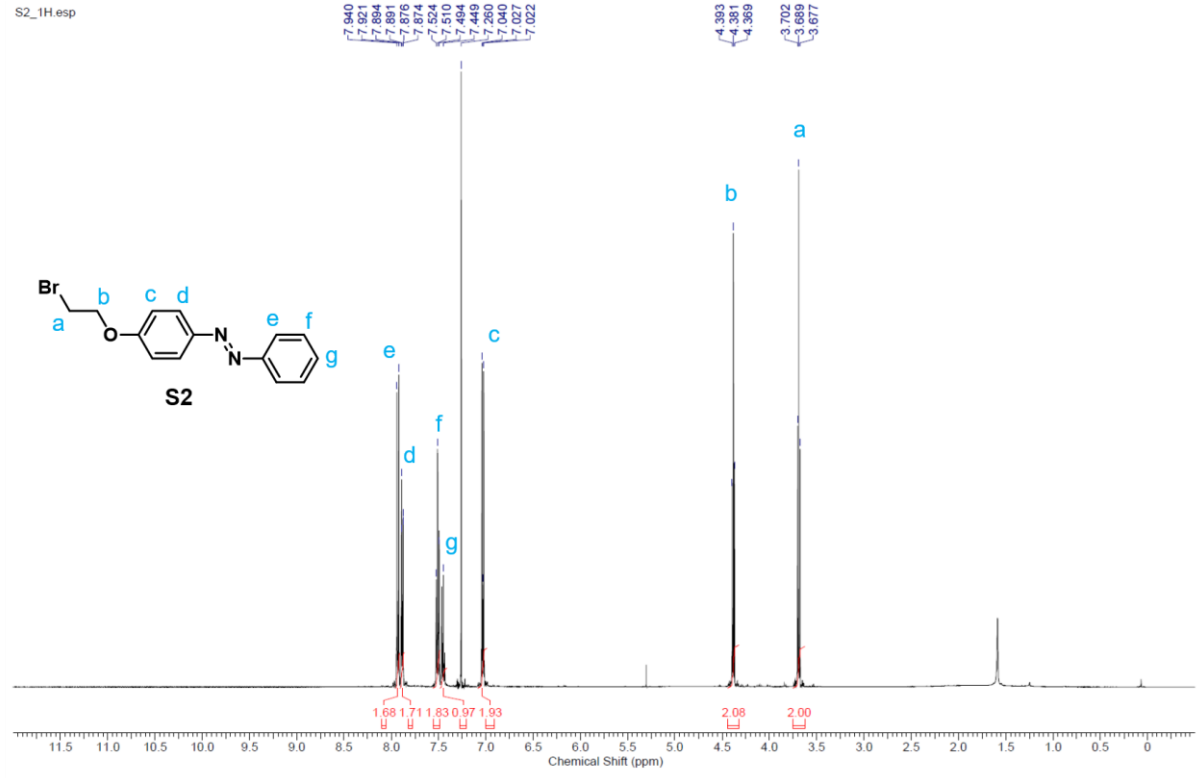
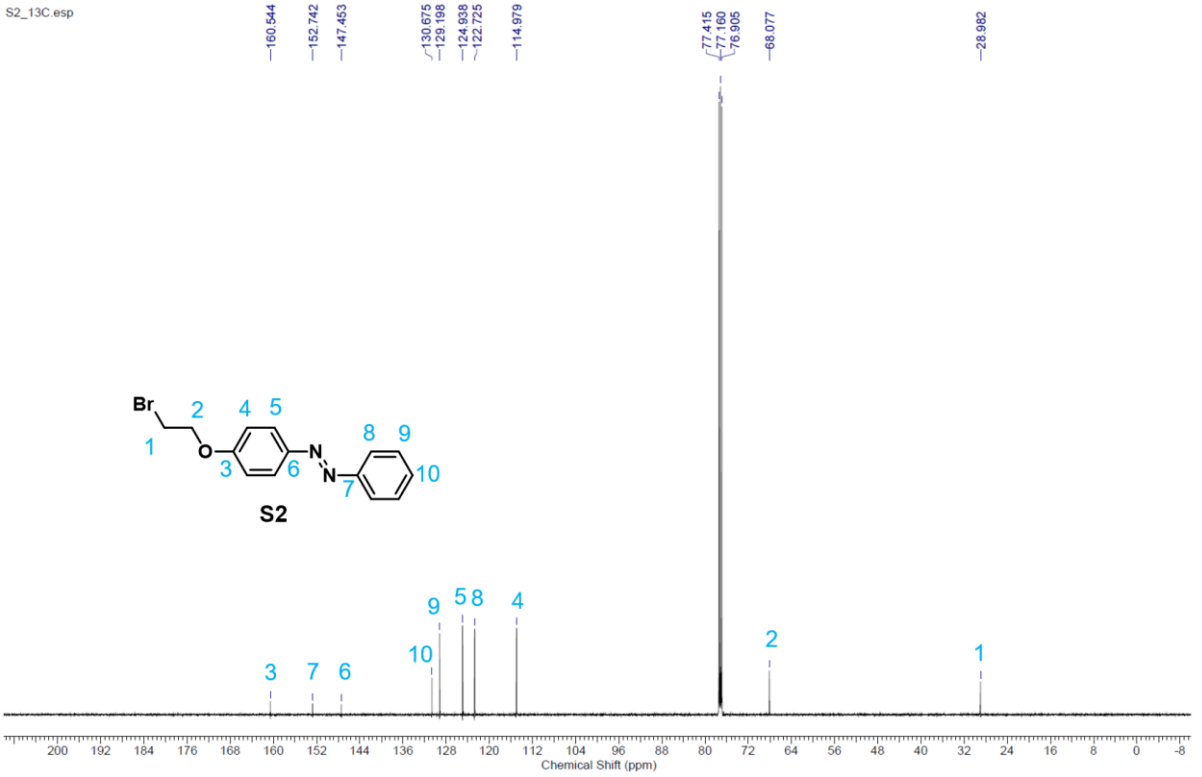
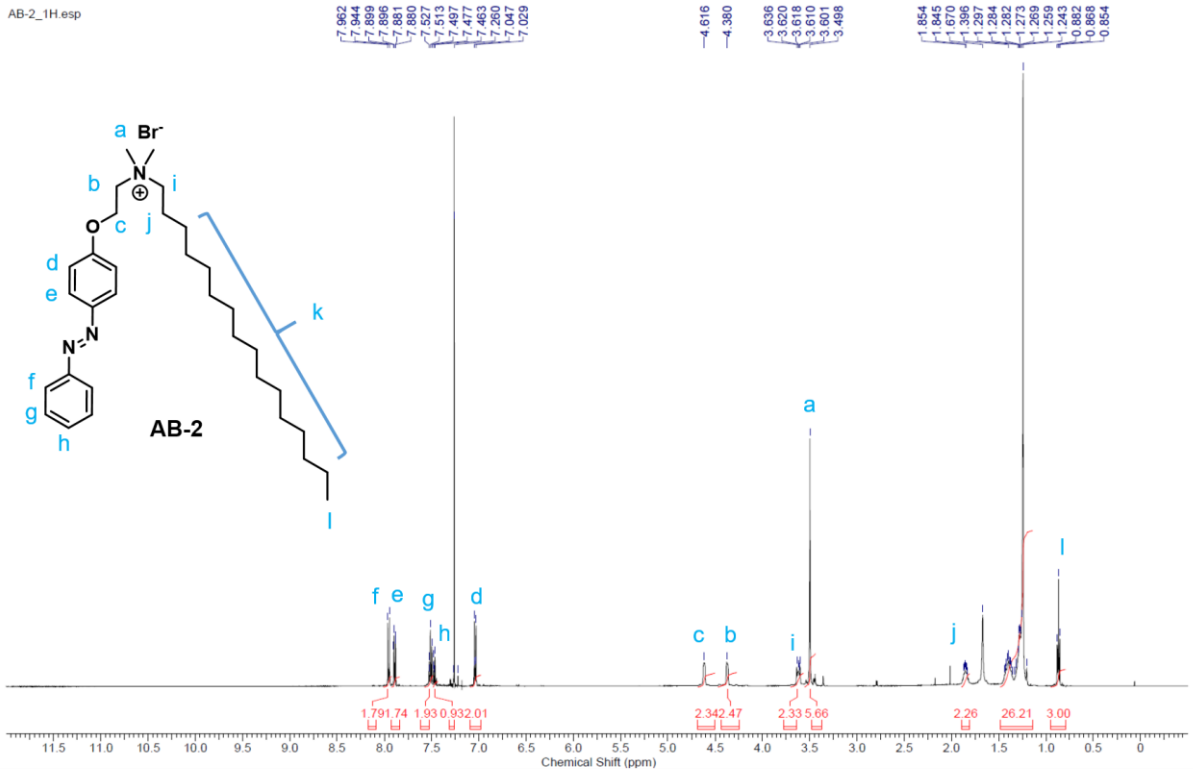
1
2
3
4
5
6
7
8
9
10
11
I-
AB-1
4 8 7 11 10 6 9 5 2 3 1
Chemical Shift (ppm)

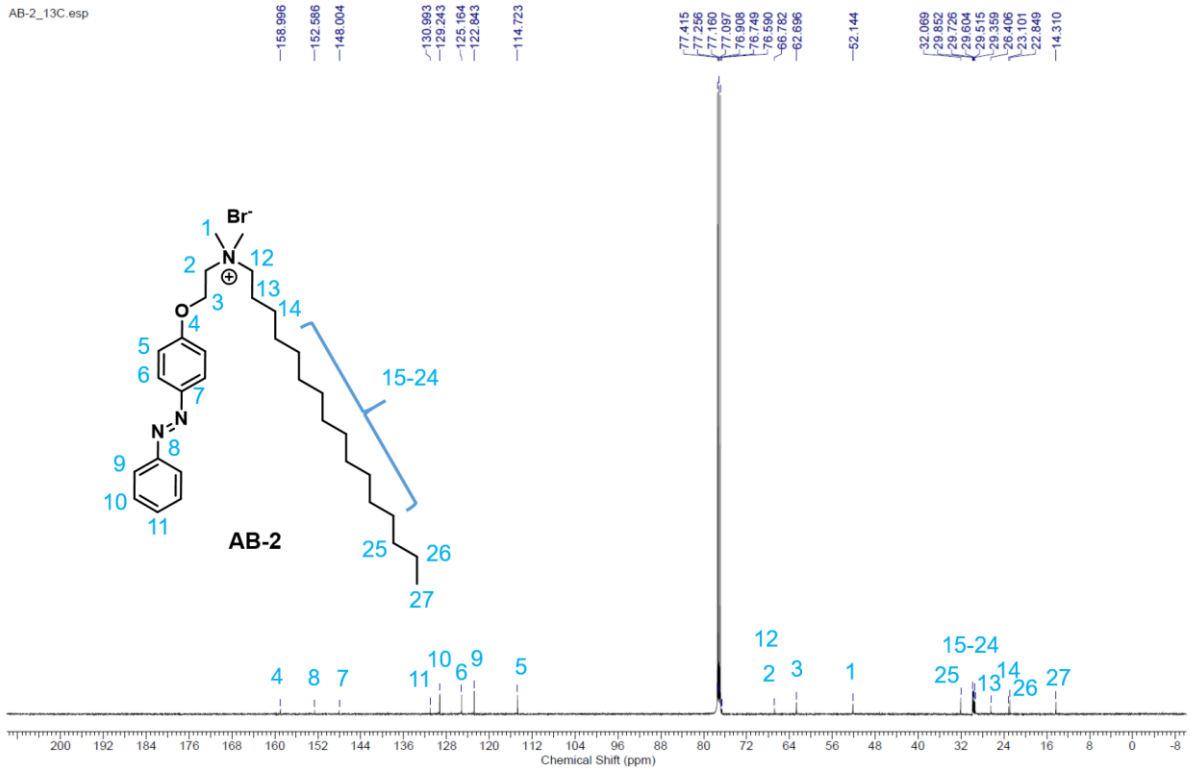
S2


S2_1H.esp
7.940
7.921
7.894
7.891
7.876
7.874
7.524
7.510
7.494
7.449
7.260
7.040
7.027
7.022
4.393
4.381
4.369
3.702
3.689
3.677
a
b
c
d
e
f
g
Br
O
N
N
S2
1.68 1.71 1.83 0.97 1.93
2.08
2.00
Chemical Shift (ppm)

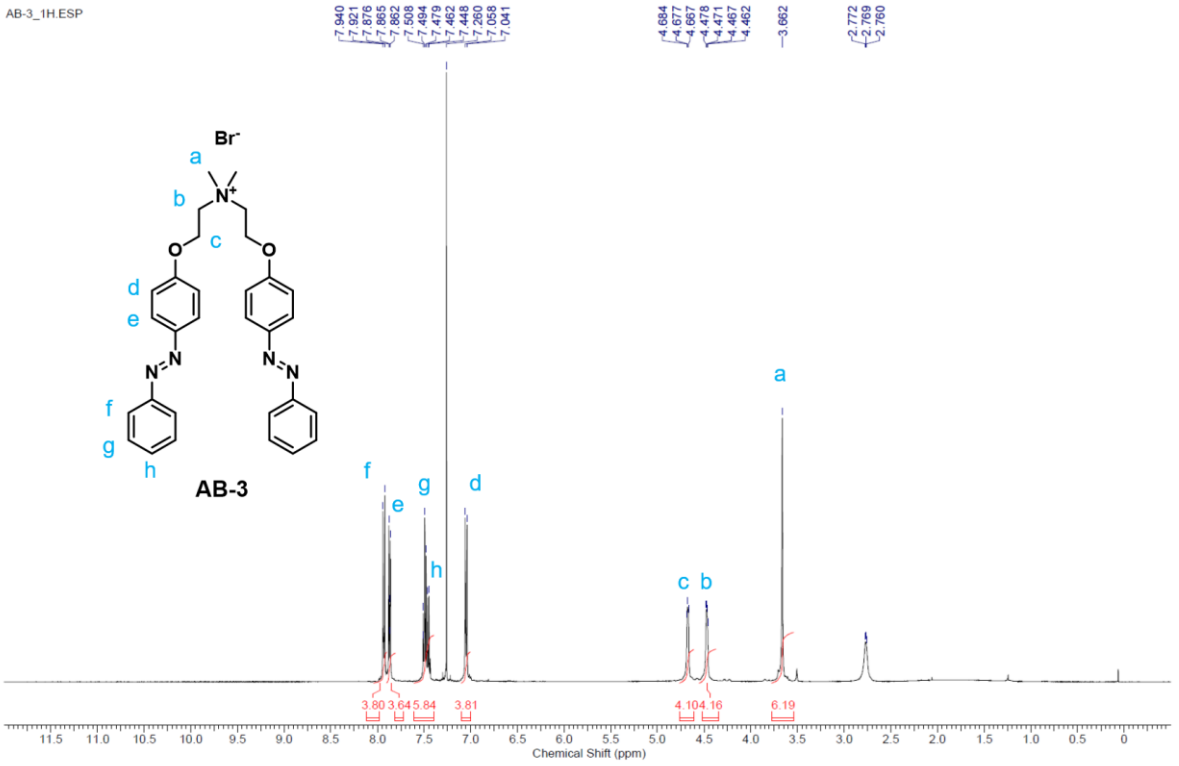

S2_13C.esp
160.544
152.742
147.453
130.675
129.198
124.938
122.725
114.979
77.415
77.160
76.905
68.077
28.982
Br
O
N
N
S2
1
2
3
4
5
6
7
8
9
10
Chemical Shift (ppm)

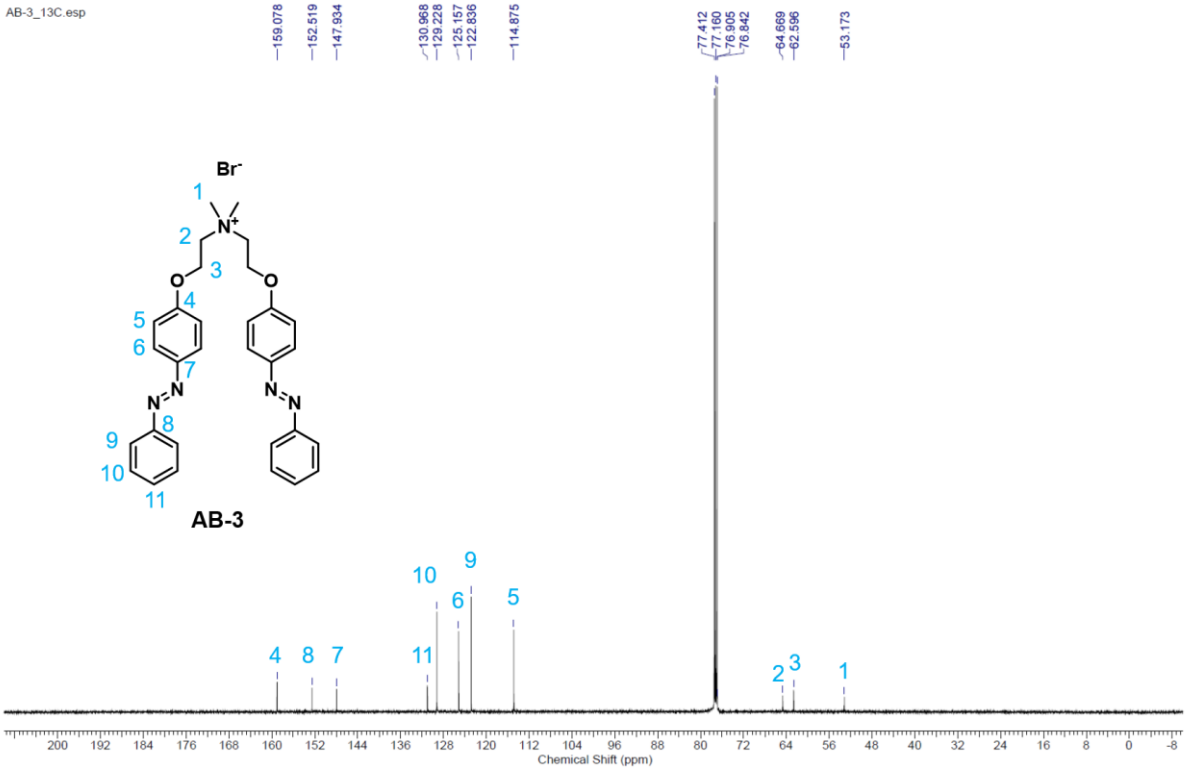
AB-2


AB-2_1H.esp
AB-2
Chemical Shift (ppm)

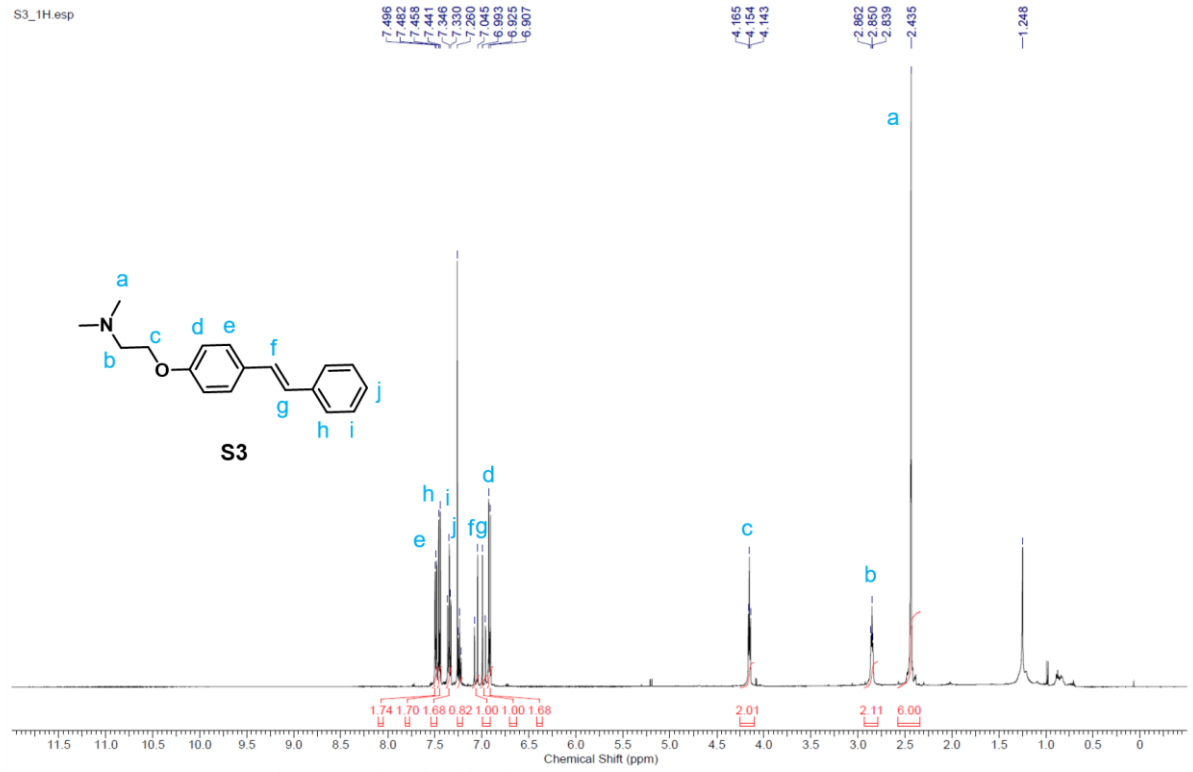

AB-2_13C.esp
AB-2
Chemical Shift (ppm)

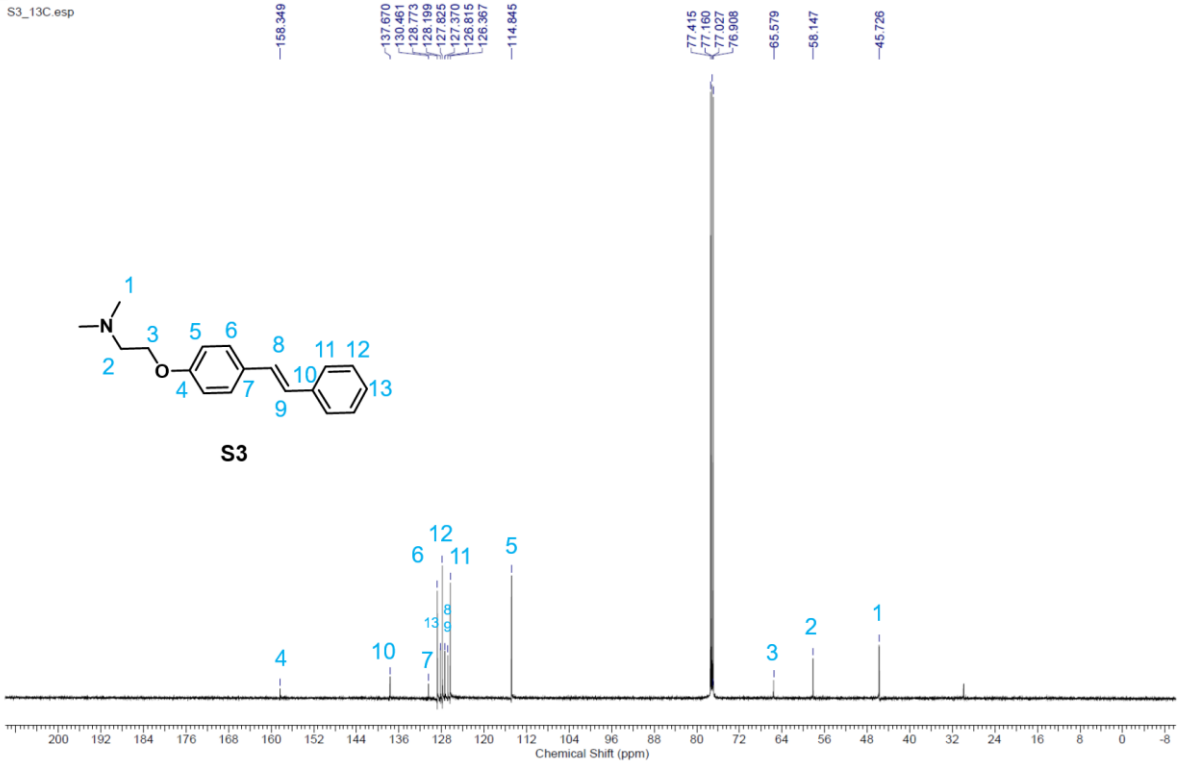
AB-3


AB-3_1H.ESP
7.940
7.921
7.876
7.865
7.662
7.508
7.484
7.479
7.462
7.448
7.260
7.058
7.041
4.684
4.677
4.667
4.478
4.471
4.467
4.462
3.662
2.772
2.769
2.760
Br-
a
b
c
d
e
f
g
h
AB-3
f
e
g
h
d
c
b
a
3.80
3.64
5.84
3.81
4.10
4.16
6.19
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Chemical Shift (ppm)

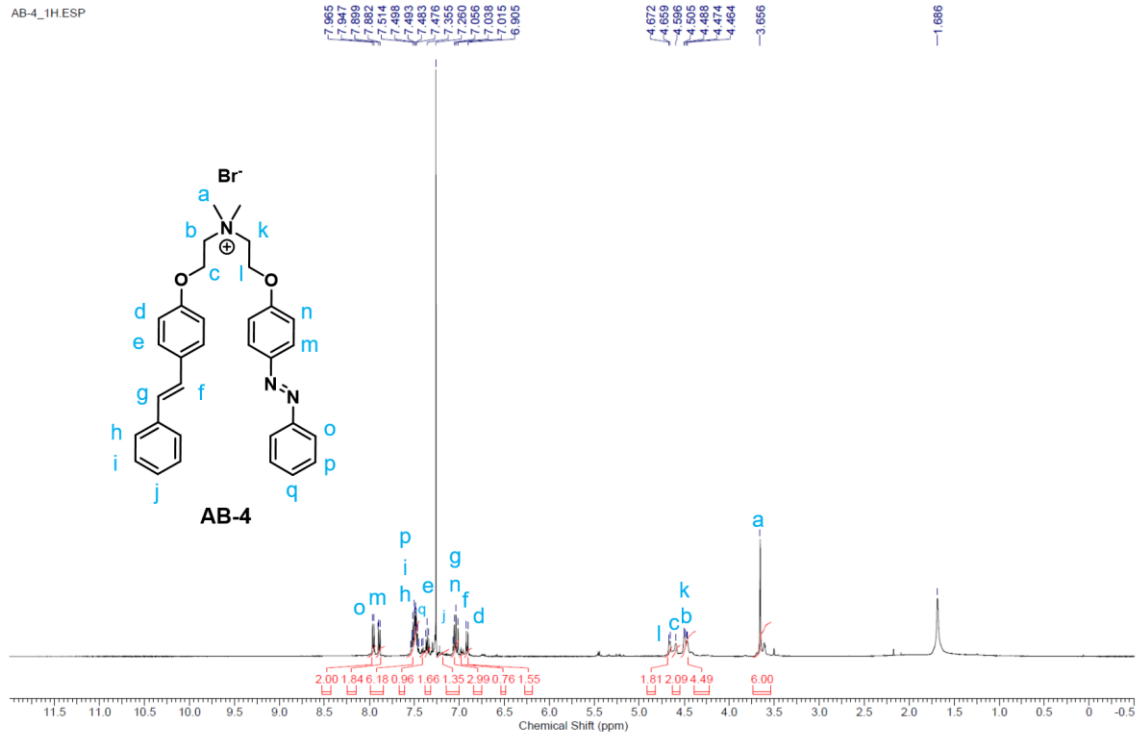

AB-3_13C.esp
159.078
152.519
147.934
130.968
129.228
125.157
122.836
114.875
77.412
77.160
76.905
76.842
64.669
62.596
53.173
Br-
1
2
3
4
5
6
7
8
9
10
11
AB-3
10
9
6
5
4
8
7
11
2
3
1
200
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0
-8
Chemical Shift (ppm)

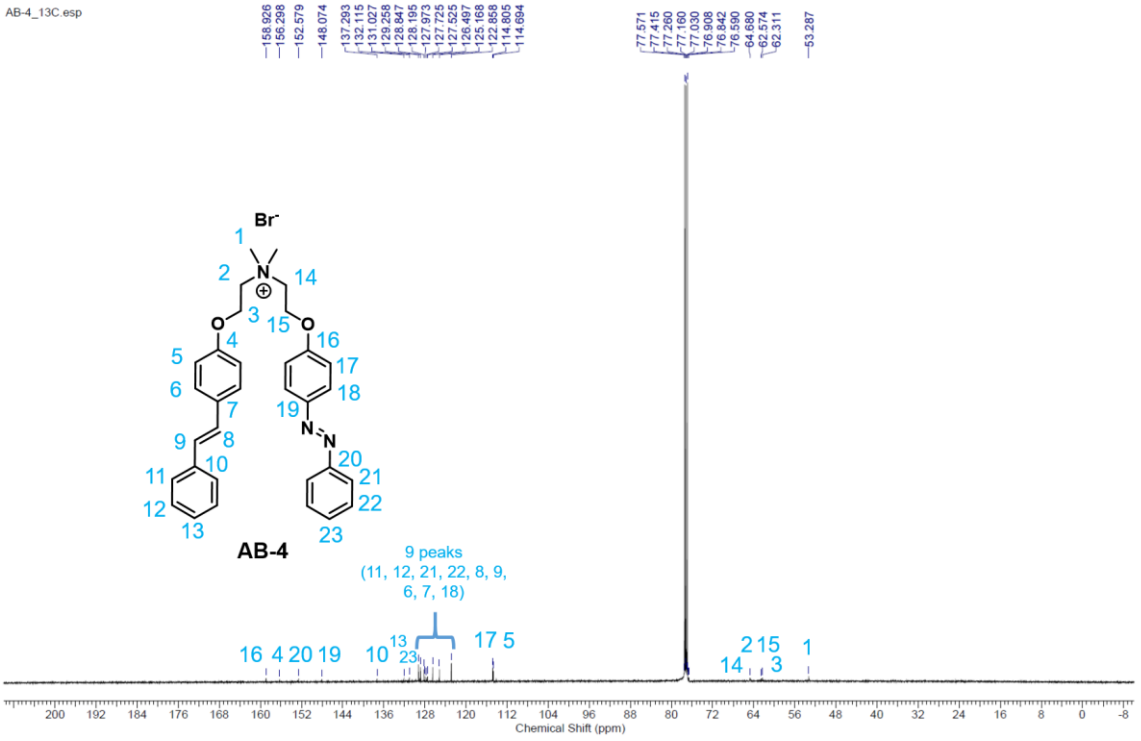
S3


S3_1H.esp
7.496
7.482
7.458
7.441
7.346
7.330
7.260
7.045
6.993
6.925
6.907
4.165
4.154
4.143
2.862
2.850
2.839
2.435
1.248
a
b
c
d e
f
g
h i
j
S3
h i
e
j
fg
d
c
b
1.74 1.70 1.68 0.82 1.00 1.00 1.68
2.01
2.11
6.00
Chemical Shift (ppm)

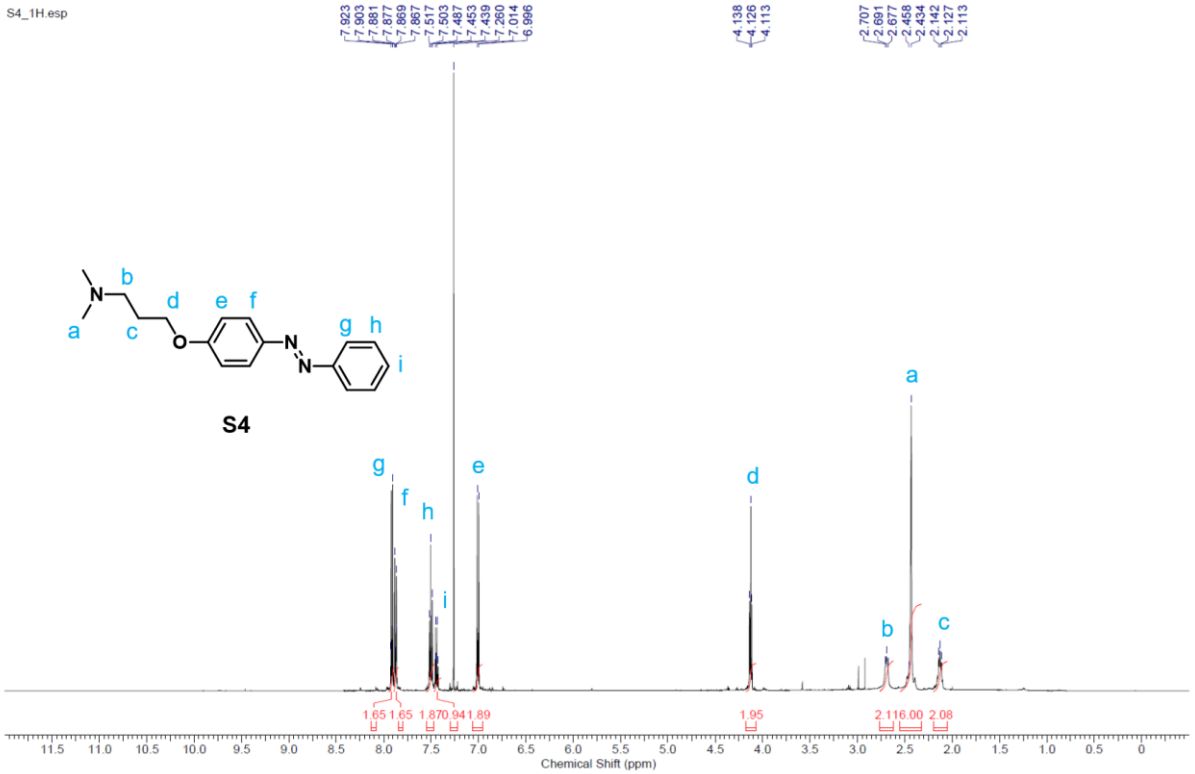

S3_13C.esp
158.349
137.670
130.461
128.773
128.199
127.825
127.370
126.815
126.367
114.845
77.415
77.160
77.027
76.908
65.579
58.147
45.726
1
2
3
4
5
6
7
8
9
10
11
12
13
S3
12
6
11
5
13
8
9
4
10
7
3
2
1
Chemical Shift (ppm)

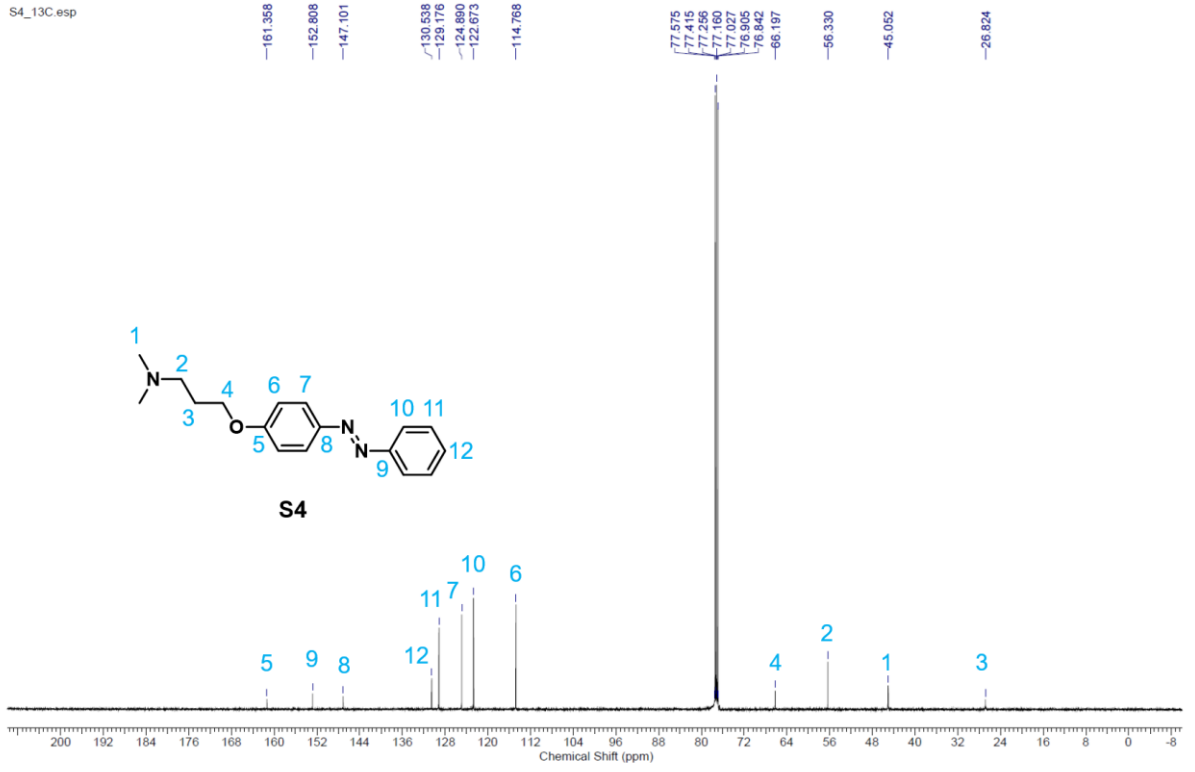
AB-4


AB-4_1H.ESP
7.965
7.947
7.899
7.882
7.514
7.498
7.493
7.483
7.476
7.355
7.280
7.066
7.058
7.015
6.905
4.672
4.659
4.596
4.505
4.488
4.474
4.464
3.656
1.686
Br-
AB-4
Chemical Shift (ppm)

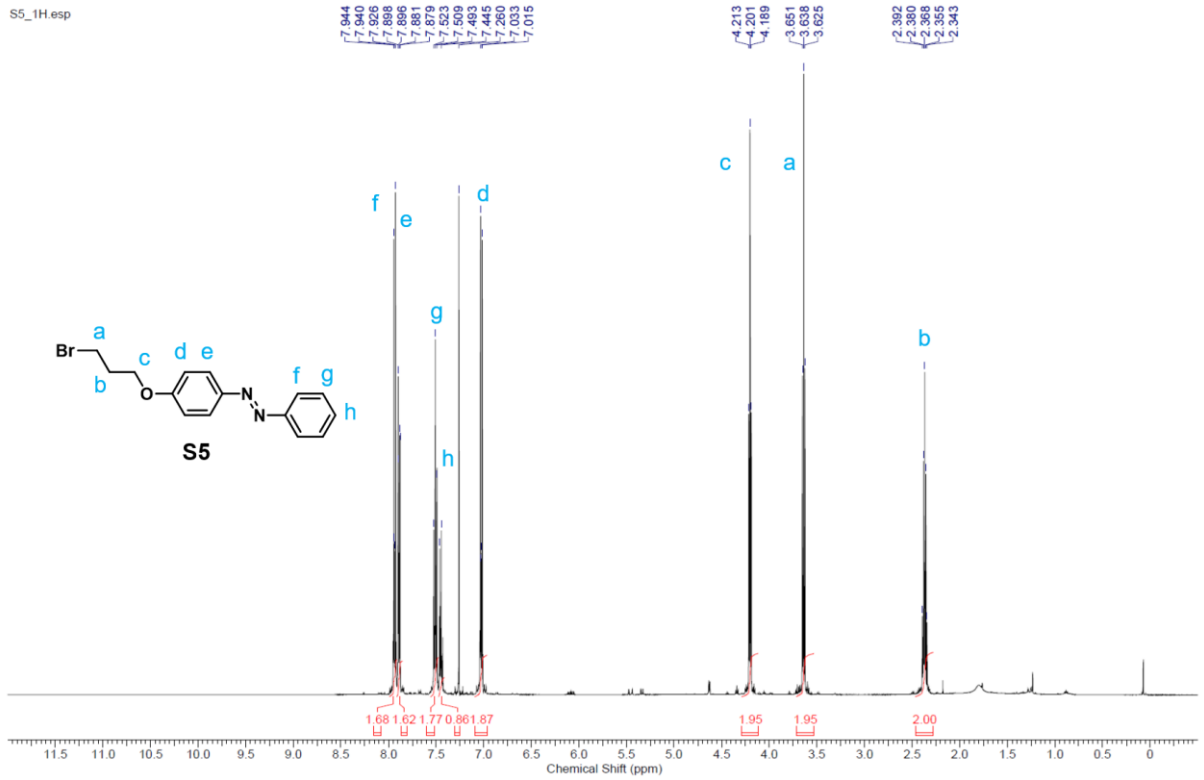

AB-4_13C.esp
158.926
156.298
152.579
148.074
137.293
132.115
131.027
129.258
128.847
128.195
127.973
127.725
127.525
126.497
125.168
122.858
114.805
114.694
77.571
77.415
77.260
77.160
77.030
76.908
76.842
76.590
64.680
62.574
62.311
53.287
Br-
AB-4
9 peaks
(11, 12, 21, 22, 8, 9, 6, 7, 18)
Chemical Shift (ppm)

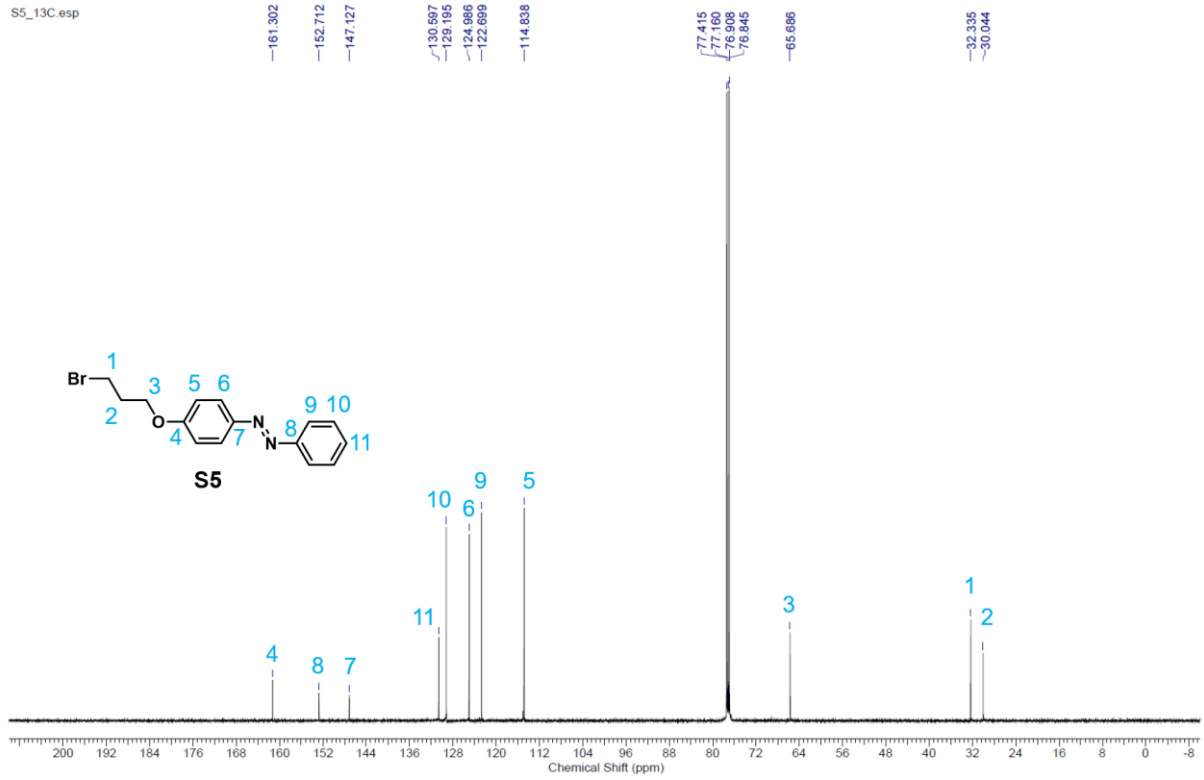
S4


S4_1H.esp
7.923
7.903
7.881
7.877
7.869
7.867
7.517
7.503
7.487
7.453
7.439
7.280
7.014
6.996
4.138
4.126
4.113
2.707
2.691
2.677
2.458
2.434
2.142
2.127
2.113
a
b
c
d
e
f
g
h
i
S4
1.65 1.65 1.87 0.94 1.89
1.95
2.11 6.00 2.08
Chemical Shift (ppm)

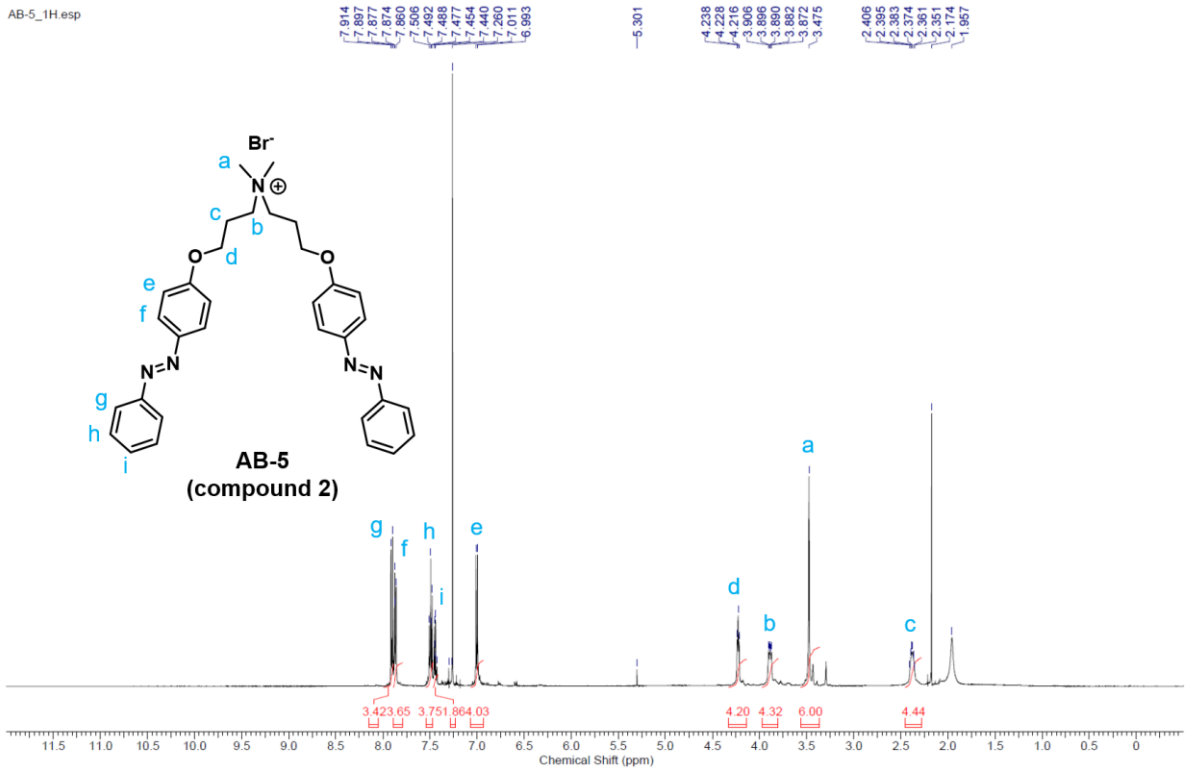

S4_13C.esp
161.358
152.808
147.101
130.538
129.176
124.890
122.673
114.768
77.575
77.415
77.256
77.160
77.027
76.905
76.842
66.197
56.330
45.052
26.824
1
2
3
4
5
6
7
8
9
10
11
12
S4
Chemical Shift (ppm)

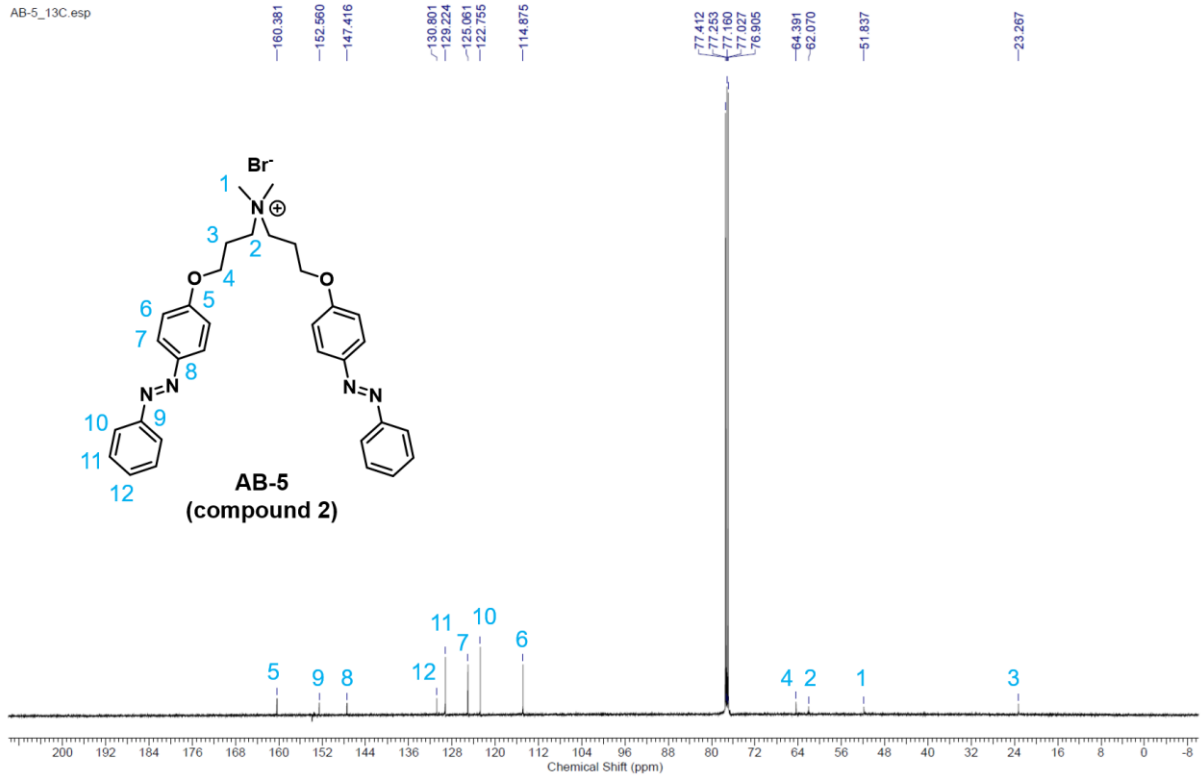
S5


S5_1H.esp
7.944
7.940
7.926
7.888
7.886
7.881
7.879
7.523
7.509
7.493
7.445
7.260
7.033
7.015
4.213
4.201
4.189
3.651
3.638
3.625
2.392
2.380
2.368
2.355
2.343
a
b
c
d e
f g
h
S5
1.68 1.62 1.77 0.86 1.87
1.95
1.95
2.00
Chemical Shift (ppm)


S5_13C.esp
161.302
152.712
147.127
130.597
129.195
124.986
122.699
114.838
77.415
77.160
76.908
76.845
65.686
32.335
30.044
1
2
3
5 6
9 10
4
7
8
11
S5
Chemical Shift (ppm)

AB-5


AB-5_1H.esp
7.914
7.897
7.877
7.874
7.860
7.506
7.492
7.488
7.477
7.454
7.440
7.280
7.011
6.993
5.301
4.238
4.228
4.216
3.906
3.896
3.890
3.882
3.872
3.475
2.406
2.395
2.383
2.374
2.361
2.351
2.174
1.957
Br-
a
N⊕
c
b
d
O
O
e
f
N=N
N=N
g
h
i
AB-5
(compound 2)
g
f
h
i
e
a
d
b
c
3.42
3.65
3.75
1.86
4.03
4.20
4.32
6.00
4.44
Chemical Shift (ppm)


AB-5_13C.esp
160.381
152.560
147.416
130.801
129.224
125.061
122.755
114.875
77.412
77.253
77.160
77.027
76.905
64.391
62.070
51.837
23.267
Br-
1
N⊕
3
2
4
O
5
6
7
8
N=N
N=N
10
9
11
12
AB-5
(compound 2)
11
10
7
6
5
9
8
12
4
2
1
3
Chemical Shift (ppm)

5. Supporting Figures and Tables

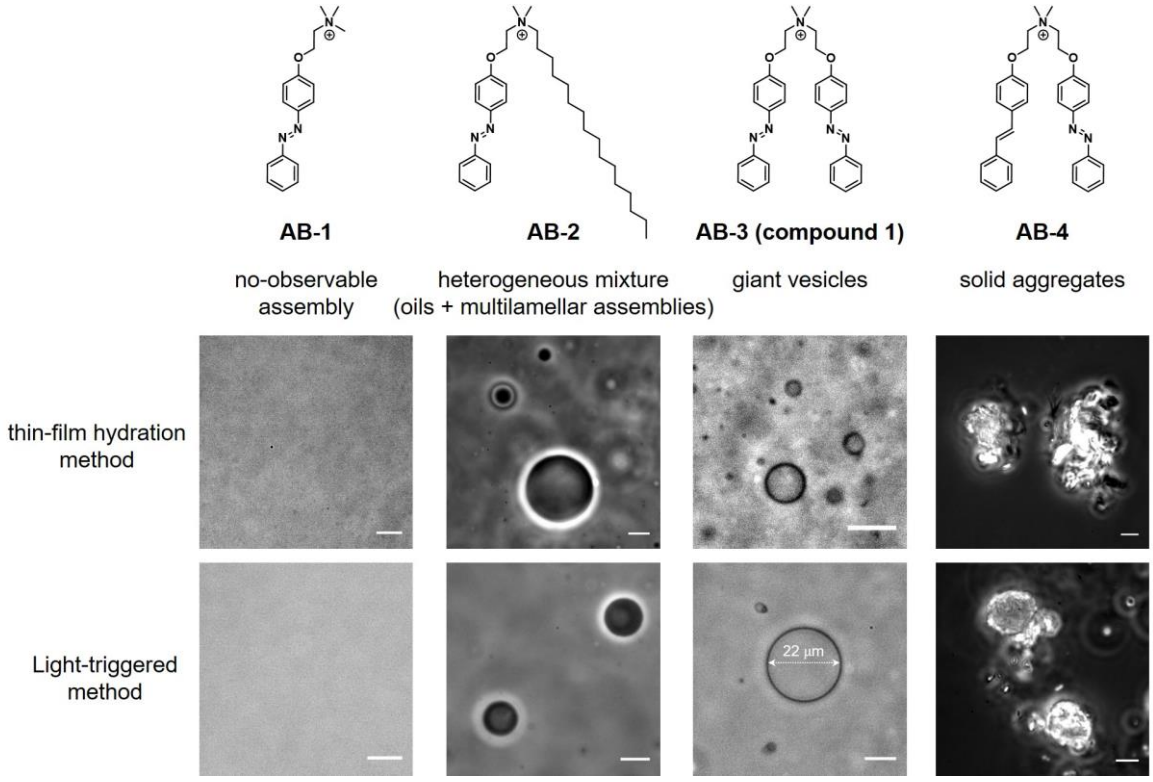


Figure S1. Phase-contrast microscope images illustrating the self-assembly products of **AB-1** to **AB-4** using the thin-film hydration method (top) or the light-triggered method in an aqueous solution with 50 mM NaCl. The concentration of **AB-1** to **AB-4** were 1 mM. Notably, **AB-1** did not yield any observable product even at 10 mM. Scale bars, 10 µm. The thin-film hydration method involves the evaporation of lipid-containing organic solvents followed by hydration and tumbling for a minimum of 1–2 h. The light-triggered method involves the preparation of water-soluble *cis*-AB stocks under UV, followed by mixing with a saline solution and subsequent visible light irradiation. For more details, please refer to the Supporting Information (Experimental section 2.1).

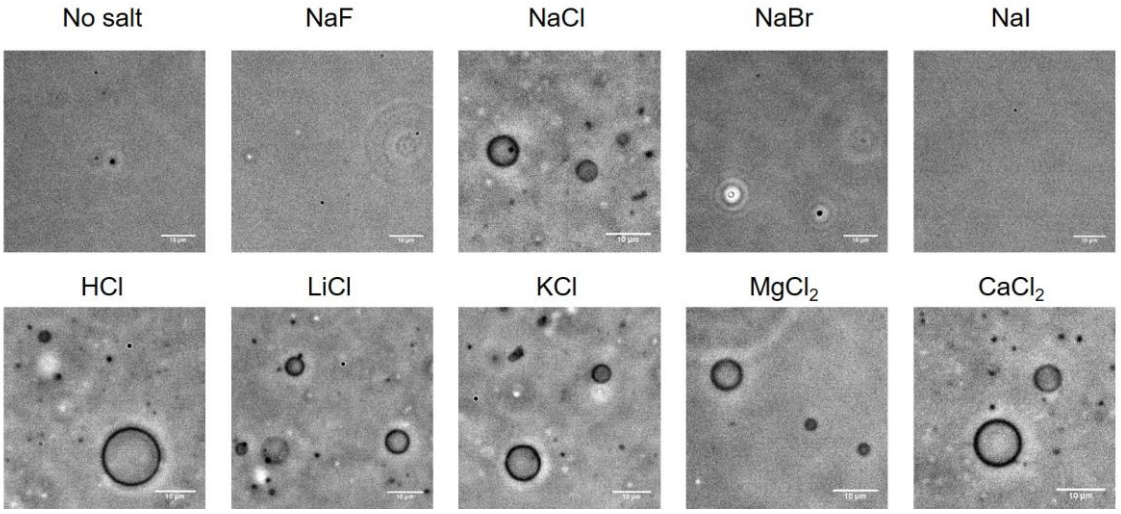


Figure S2. Phase-contrast microscope images depicting the self-assembly products of compound **1** in aqueous solutions with various anionic and cationic salts. Scale bars, 10 μm. [salts]: 50 mM, [**1**]: 0.5 mM.

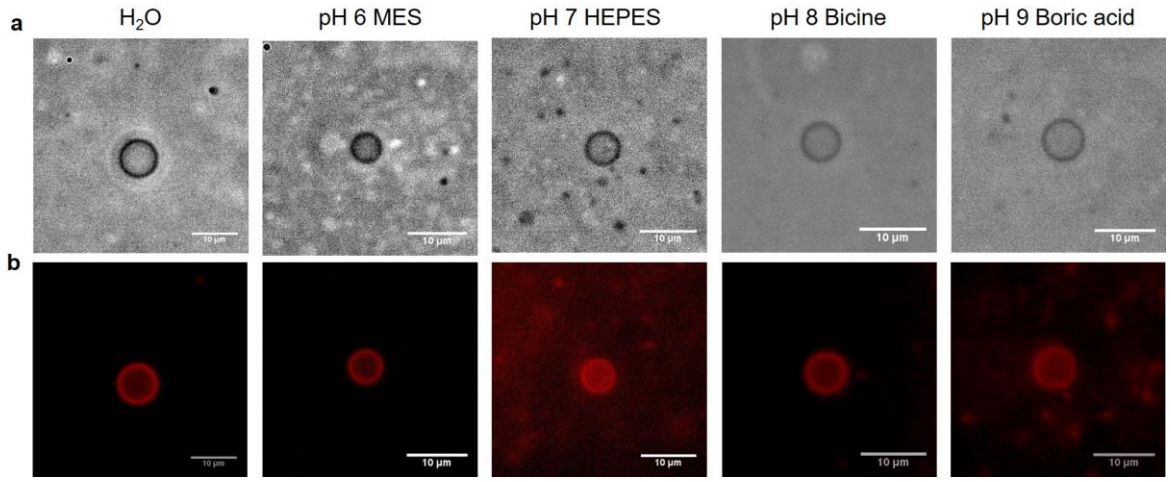
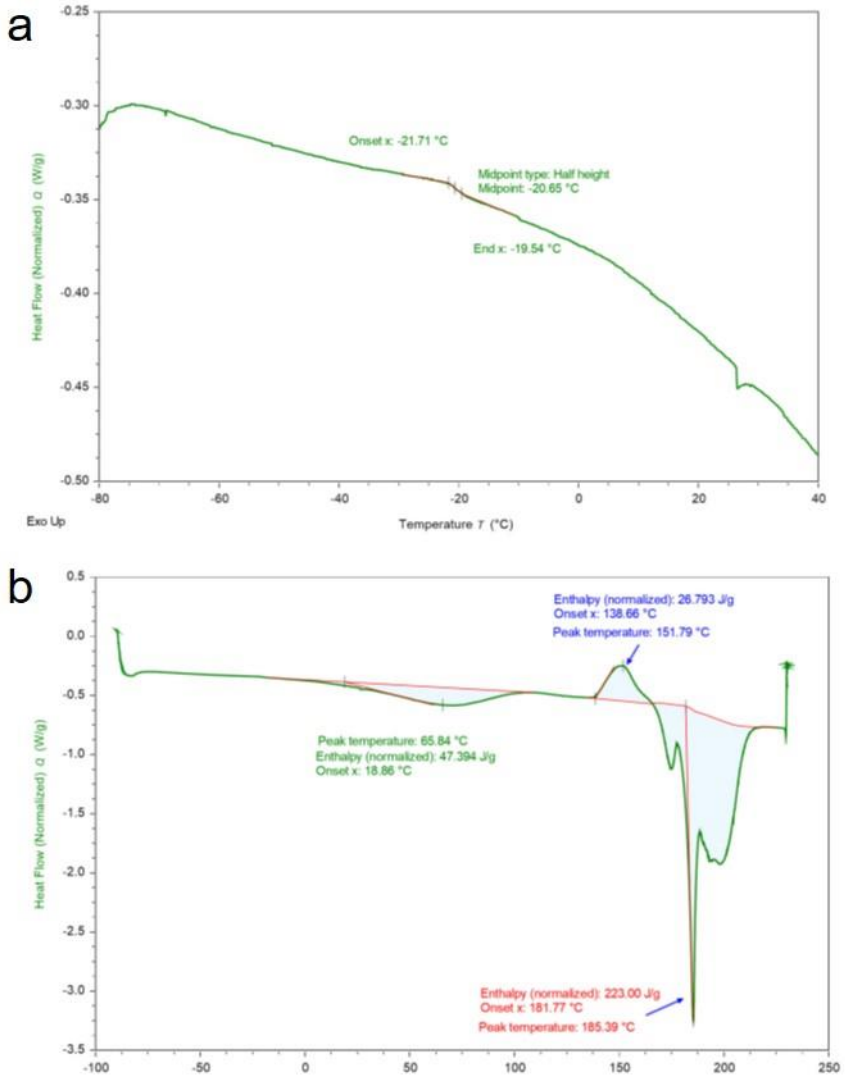


Figure S3. Microscope images depicting the vesicles of compound **1** in different buffers (25 mM) with the addition of 50 mM NaCl. (a) Phase-contrast micrographs, (b) Fluorescence micrographs stained with 1 mol% Nile-Red. Scale bars, 10 µm. [**1**]: 0.5 mM.



	Phase transition temperature T (°C)	**Melting/decomposition T (°C)**
Compound **1**	-20.7	185.4

Figure S4. Differential scanning calorimetry (DSC) thermograms for compound **1** and the corresponding determined values. (A: zoomed-in for phase transition temperature, B: overall thermogram)

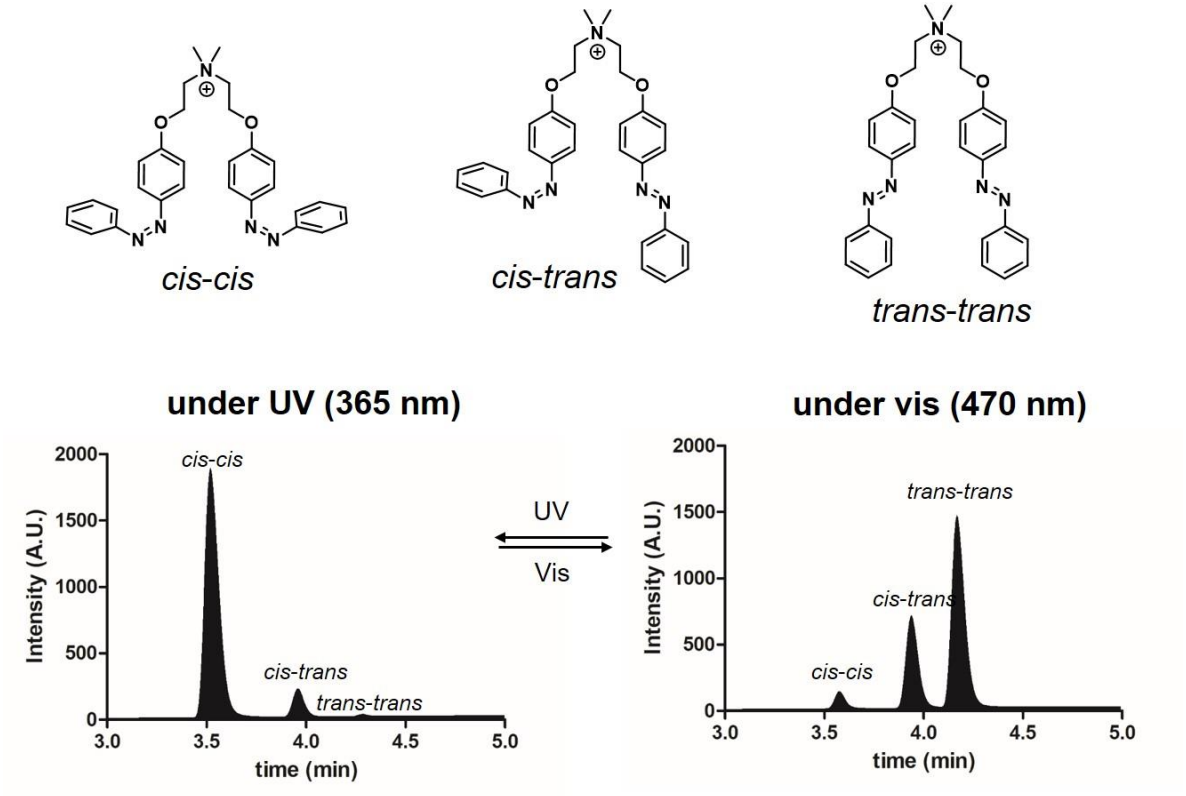


Figure S5. Chemical structures (top) and representative chromatograms (bottom) of isomers of compound **1** obtained through an LC-DAD method (at 242 nm) under illumination with 365 nm UV light (left) or 470 nm visible light (right).

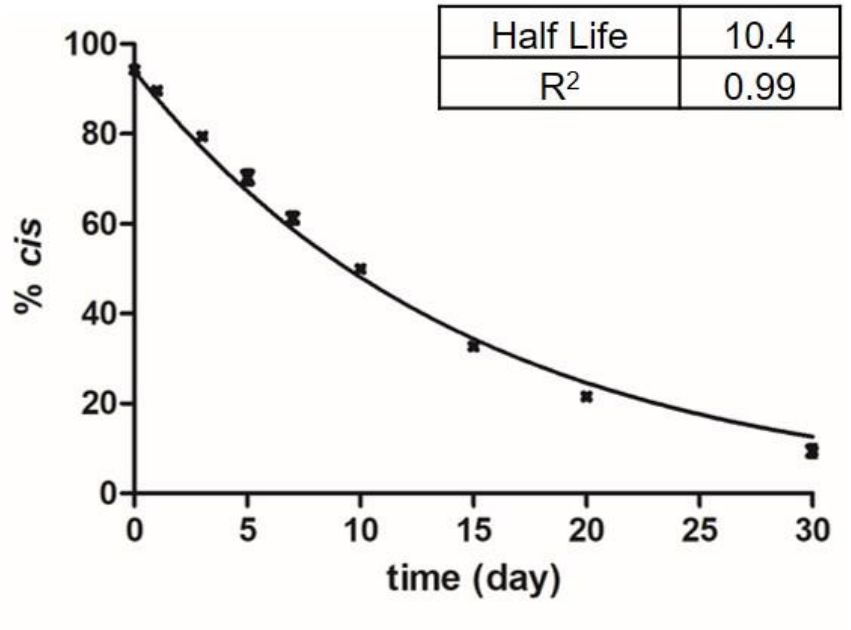
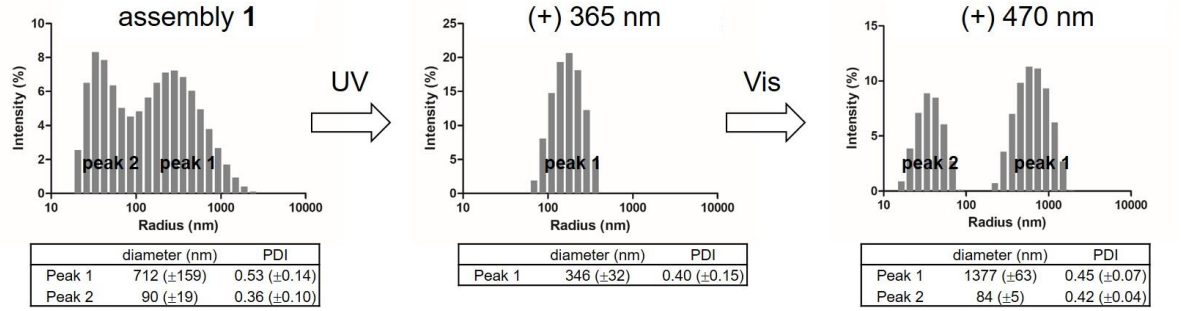


Figure S6. Kinetic profile illustrating the proportion of *cis*-**1** isomers in the dark over time after discontinuing 365 nm illumination. The graph was fitted using a one-phase exponential decay model with the equation: $Y = (Y_0 - \text{plateau}) * \exp(-K * X) + \text{plateau}$, where Y_0=94, plateau=0, K=0.067. Each data point at 0, 1, 3, 5, 7, 10, 15, 20 and 30 days was obtained from isomer analysis using LC-MS in triplicate. Error bars represent the standard deviation.



	diameter (nm)	PDI
Peak 1	712 (±159)	0.53 (±0.14)
Peak 2	90 (±19)	0.36 (±0.10)

	diameter (nm)	PDI
Peak 1	346 (±32)	0.40 (±0.15)

	diameter (nm)	PDI
Peak 1	1377 (±63)	0.45 (±0.07)
Peak 2	84 (±5)	0.42 (±0.04)

Figure S7. Representative histograms depicting in situ assemblies of compound **1** obtained from dynamic light scattering (DLS) analysis before (left) and after exposure to 365 nm light (middle), followed by successive 470 nm irradiation (right). The histogram under 470 nm light was measured after a 2 h waiting time to allow for the self-assembly process of **1**. The tables present the average size of each peak and its polydispersity index (n=3).

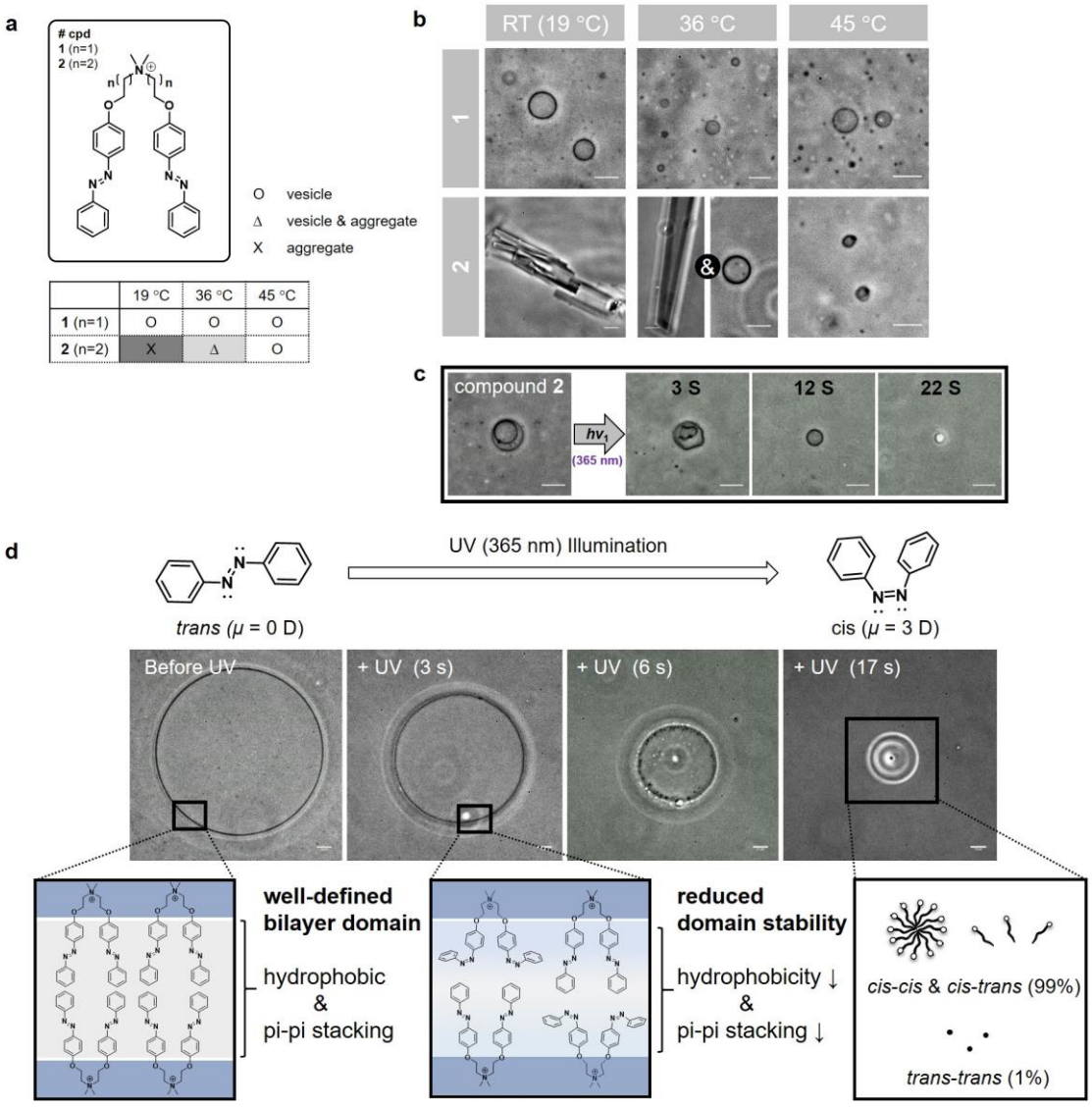


Figure S8. (a) Chemical structures of compound **1** and **2** together with a table displaying their morphological changes at different temperatures. (b) Phase-contrast images of self-assembled products of **1** and **2** at 19, 36, and 45 °C. (c) Phase-contrast images of compound **2** at 50 °C after irradiation with 365 nm light. Scale bars, 10 μm. (d) Time-series images of the loss of the bilayer domain of a GV of **1** under UV irradiation (top) and proposed transformation of lipid assemblies after UV illumination (bottom). Scale bars, 10 μm.

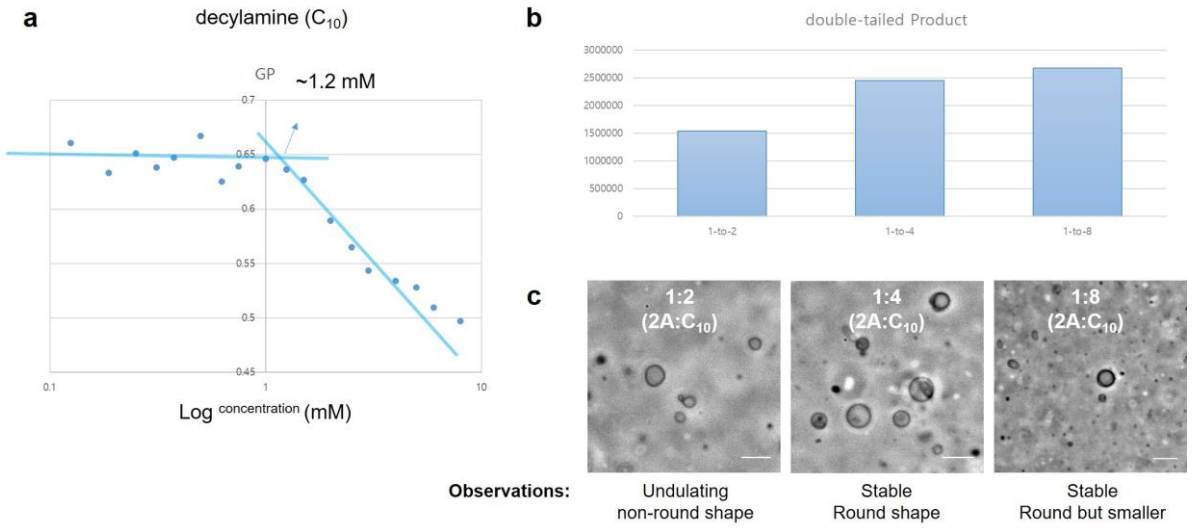


Figure S9. A series of optimization processes were conducted for the in situ **2A-C_{10}** reaction under pH 7.5 HEPES (25 mM) buffered saline (50 mM) solution. (a) The generalized polarization (GP) was measured using laurdan dye[S4] with varying concentrations of decylamine. The estimated critical micelle concentration (CMC) value of decylamine was found to be 1.2 mM, which aligned well with previously reported values.[S5] Consequently, the concentration of decylamine was maintained above 1.2 mM in all subsequent reactions. (b) The relative mass intensity of reduced **2A-C_{10}** products was assessed after 24 hour reaction between 1 equiv. of **2A** aldehyde precursor and 2, 4, and 8-fold excesses of amines. The mass was analyzed after the post treatment of samples with sodium borohydride solution (100 equiv.) in methanol. It was observed that excess amines beyond 4 equiv. did not significantly affect the increase in products. (c) Microscopy images were captured after 2, 4, and 8-fold excesses of amines reacted with 1 equiv. of **2A** aldehyde precursor (1 mM). Notably, the use of 8 equiv. of decylamine resulted in smaller vesicles in general, presumably due to the surfactant effect of unreacted excess decylamine. Based on these analyses, the reaction condition of 1:4 (**2A**:decylamine) was selected to generate stable in situ **2A-C_{10}** vesicles. Scale bars, 10 μm.

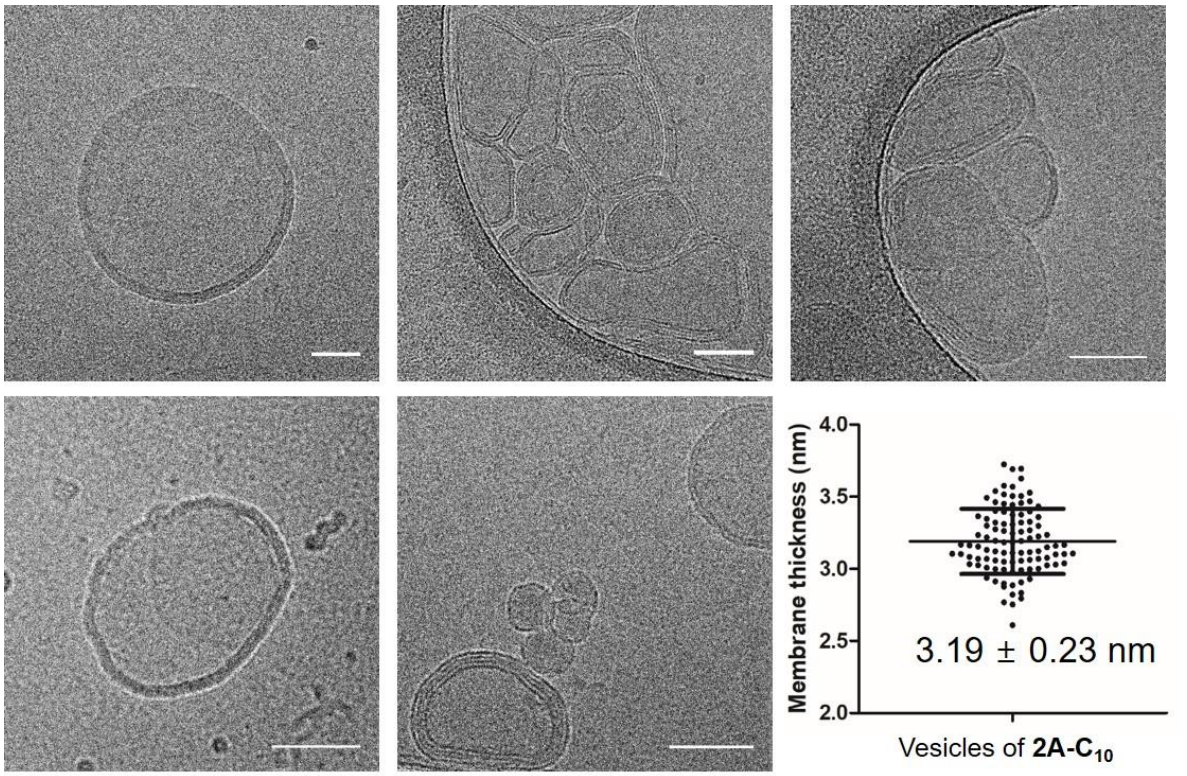


Figure S10. Cryo-EM images showing the bilayer structures of the **2A-C_{10}** assemblies, accompanied by a scatter plot of measured bilayer thickness from ten images (n=100 vesicles). Scale bars, 50 nm.

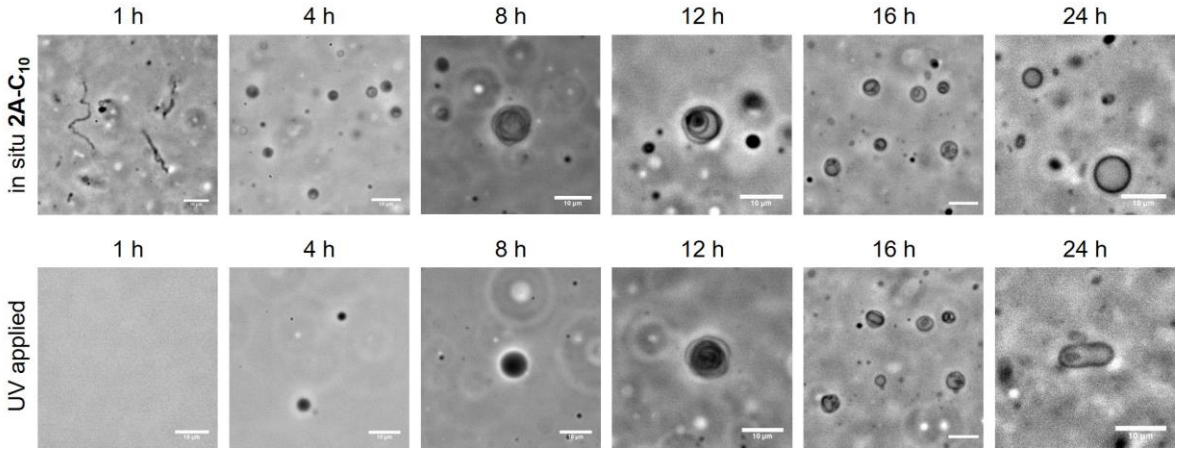


Figure S11. Time-series micrographs illustrating the morphological changes during an in situ process for forming **2A-C_{10}** products. In situ conditions: [**2A**]: 0.5 mM; [decylamine (C_{10})]: 2 mM; [HEPES buffer]: 25 mM; [NaCl]: 50 mM at pH 7.5 under room temperature (20 °C). Scale bars, 10 μm.

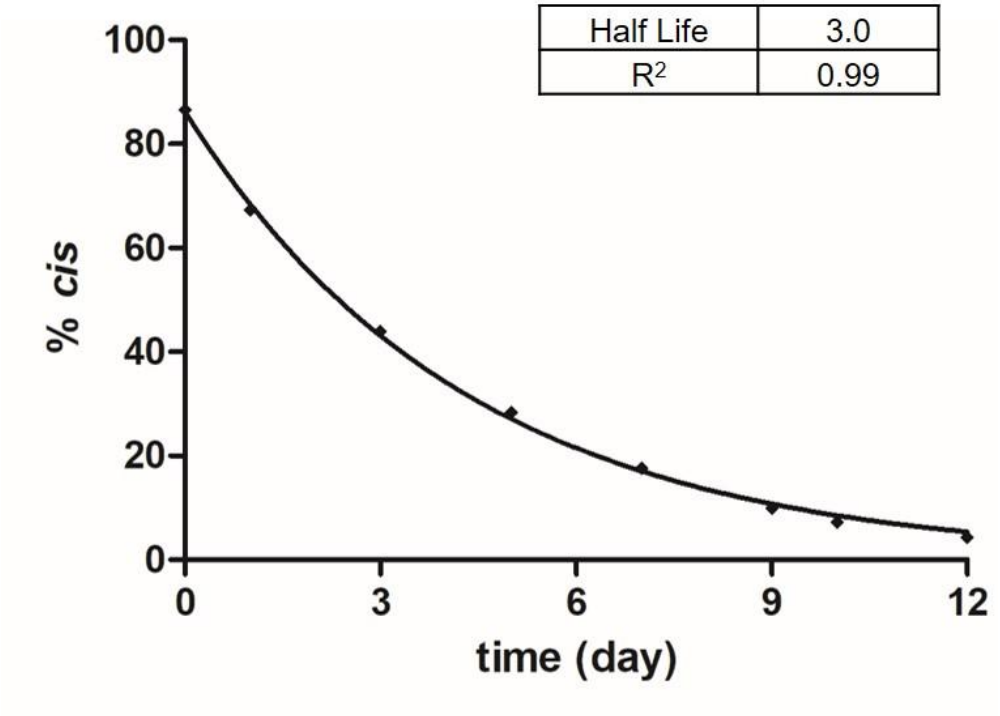


Figure S12. Kinetic profile illustrating the proportion of *cis*-**2A-C$_{10}$** isomers in the dark as a function of time after discontinuing 365 nm illumination. The graph was fitted using a one-phase exponential decay model with the equation Y = (Y_0 - plateau) * exp(-K * X) + plateau, where Y_0=86, plateau=0, K=0.23. Each value at day 0, 1, 3, 5, 7, 9, 10, and 12 was obtained from isomer analysis using LC-MS in triplicate. Error bars represent the standard deviation.

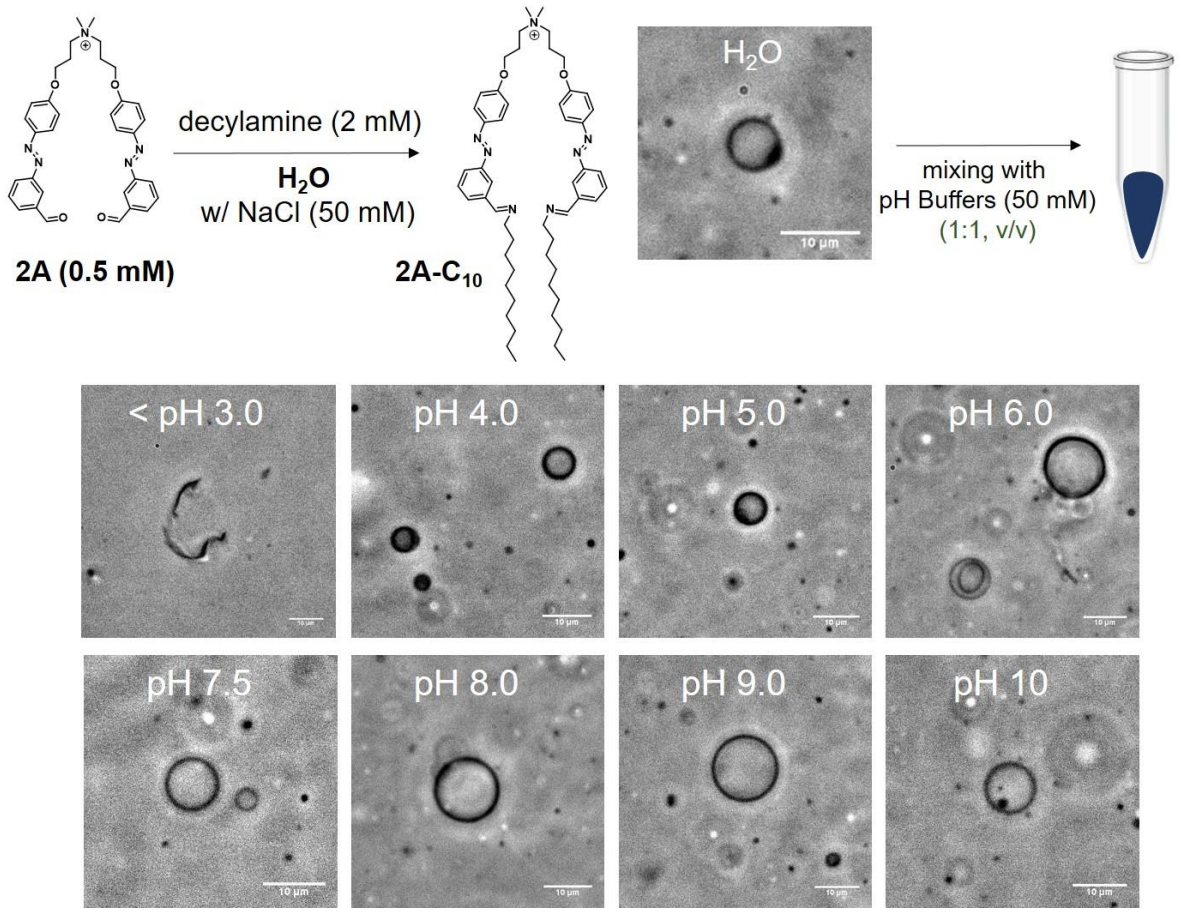


Figure S13. Phase-contrast microscope images displaying vesicles of **2A-C$_{10}$** prepared in H_2O, after mixing with various pH buffers ranging from pH 3 to pH 10. The images were captured after 0.5 h of mixing. Scale bars, 10 μm.

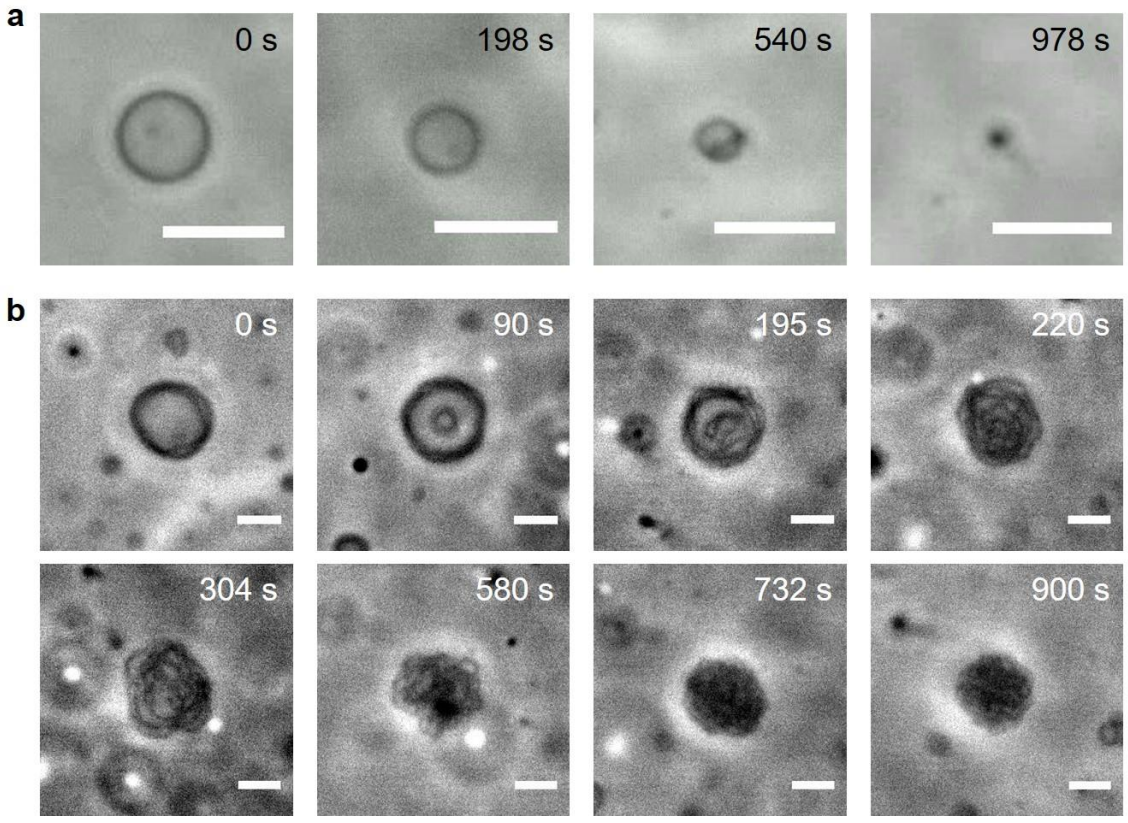


Figure S14. Time-series micrographs illustrating the morphological changes of **2A-C$_{10}$** vesicles induced by acidification through a merocyanine-based photoacid and visible light (470 nm) irradiation. (a) Images displaying a shrinking process. Scale bars, 10 μm. (b) Images showing the development of highly multilamellar assemblies. Scale bars, 5 μm. [photoacid]= ~7.5 mM; [**2A-C$_{10}$**]: 1 mM; [NaCl]: 50 mM.

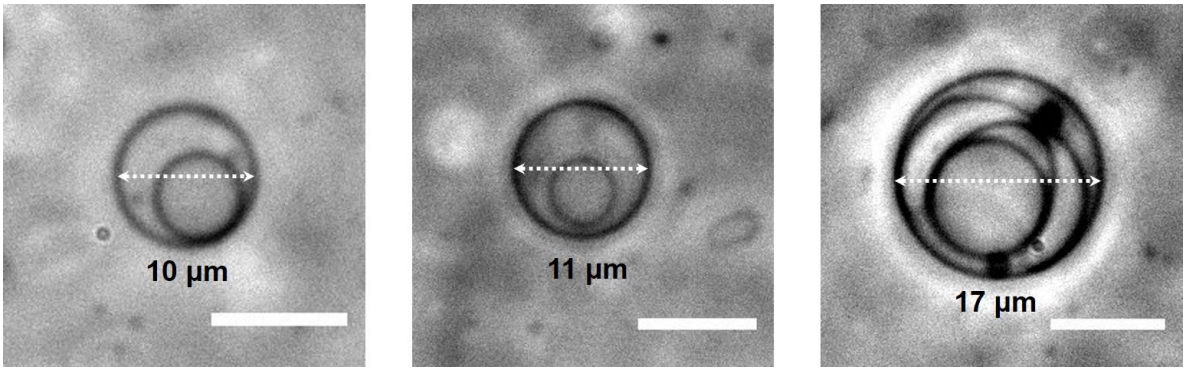


Figure S15. Representative phase-contrast micrographs of enlarged **2A-C$_{10}$** vesicles formed by UV-activation followed by 2 h of tumbling in the dark. In situ reaction condition: [**2A**] = 0.5 mM; [decylamine] = 2 mM; [sodium oleate] = 0.05 mM; [HEPES] = 25 mM; [NaCl] = 50 mM; at pH 7.5 at room temperature (20 °C).

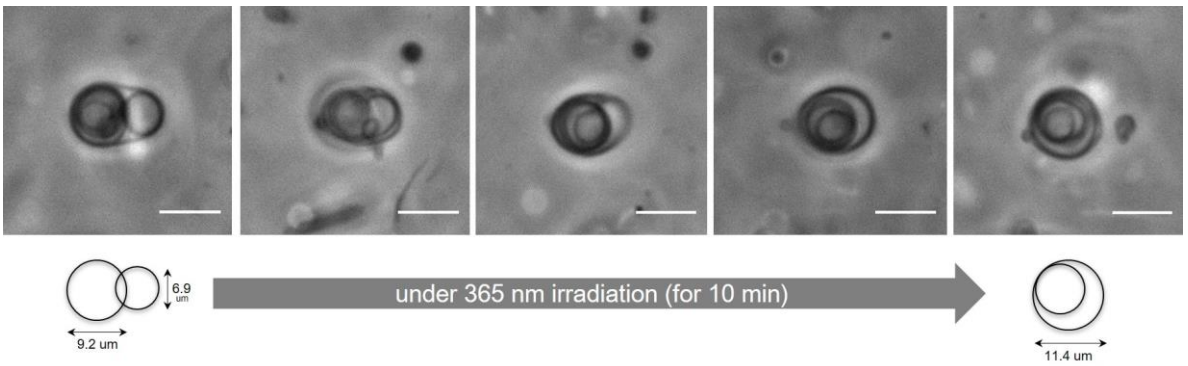


Figure S16. Micrographs illustrating the light-induced merging of **2A-C$_{10}$** giant vesicles (GVs) under the illumination of UV light (365 nm). The images were captured during a 10-min process. Scale bars, 10 μm.

Table S1. Isomer distribution of compound **1** under 365 nm light irradiation (n=3)

	cis-cis (%)	c*is-trans* (%)	*trans-trans* (%)
Sample 1	89.4	9.5	1.0
Sample 2	89.4	9.6	1.0
Sample 3	89.8	9.3	0.9
average	89.6	9.5	1.0
SD	0.2	0.1	
% *trans*	5.7		

Table S2. Isomer distribution of compound **1** under 470 nm light irradiation (n=3)

	cis-cis (%)	c*is-trans* (%)	*trans-trans* (%)
Sample 1	5.2	29.6	65.3
Sample 2	4.9	29.6	65.6
Sample 3	5.1	29.9	65.0
average	5.0	29.7	65.3
SD	0.1	0.2	0.2
% *trans*	80.1		

Table S3. Time-dependent changes in the proportion of *cis*-**1** isomers in the dark after discontinuing 365 nm illumination. (n=3)

***cis*-1**		
time (day)	**average (%)**	**SD (%)**
0	94.3	0.1
1	89.6	0.2
3	79.5	0.3
5	70.3	1.1
7	61.3	0.9
10	49.9	0.7
15	32.7	0.7
20	21.5	0.5
30	9.4	0.9

Table S4. Time-dependent changes in absorbance at 242 nm for in situ **2A-C_{10}** samples (9.8 μM) in methanol solution. The absorbance of GVs assembled from isolated **2A-C_{10}** was estimated to be 100%.

in situ (time)	average absorbance (at 242 nm, a.u.)	SD	estimated % imine
0 h	0.244	0.018	0
1 h	0.277	0.005	21
2 h	0.280	0.003	23
4 h	0.290	0.002	29
8 h	0.315	0.005	44
12 h	0.319	0.008	47
16 h	0.334	0.004	56
20 h	0.345	0.005	63
24 h	0.349	0.005	65
36 h	0.351	0.008	67
2A-C_{10} GVs	0.405	0.007	100

Table S5. Time-dependent changes in the proportion of *cis*-**2A-C_{10}** isomers in the dark after discontinuing 365 nm illumination. (n=3)

***cis*-2A-C_{10}**		
time (day)	**average (%)**	**SD (%)**
0	86.5	0.1
1	67.3	0.1
3	44.0	0.4
5	28.4	0.6
7	17.6	0.1
9	10.0	0.1
10	7.2	0.4
12	4.3	0.0

Table S6. The distribution of **2A-C$_{10}$** vesicles based on their diameter (μm) after tumbling for 2 h without light illumination. To mitigate errors caused by light microscopy resolution limits, only clearly observable vesicles with a diameter exceeding 5 μm were selected from the randomly counted 275 vesicles observed across 43 images.

size	# of vesicles	%
5-6 μm	72	56.7
6-7 μm	49	38.6
7-8 μm	5	3.9
8-9 μm	1	0.8
9-10 μm	0	0
over 10 μm	0	0
total	127	100

Table S7. The distribution of **2A-C$_{10}$** vesicles based on their diameter (μm) after 365 nm illumination (for 3 min) followed by tumbling for 2 h in the dark. To mitigate errors caused by light microscopy resolution limits, only clearly observable vesicles with a diameter exceeding 5 μm were selected from the randomly counted 273 vesicles observed across 50 images.

size	# of vesicles	%
5-6 μm	72	52.2
6-7 μm	45	32.6
7-8 μm	13	9.4
8-9 μm	2	1.4
9-10 μm	4	2.9
over 10 μm	2	1.5
total	138	100

6. Description for Movies S1 to S4

Movie S1.

UV-triggered vesicle disassembly

Time-lapse video (1 min) showing the disassembly of GVs of compound **1** under exposure to 365 nm LED light. Conditions: 1 mM compound **1** in a 50 mM NaCl solution.

Movie S2.

Generation of multilamellar assemblies using photoacid

Time-lapse video (12 min) illustrating the development of multilamellar assemblies in **2A-C_{10}** membranes, facilitated by visible-light-induced acidification through a photoacid. Conditions: Isolated **2A-C_{10}** film at 1 mM in a 7.5 mM photoacid solution with 50 mM NaCl, pH 5.5. The recording was conducted under intense visible light illumination using a tungsten halogen lamp in a microscopy setting.

Movie S3.

UV-induced vesicle merging

Time-lapse video illustrating the merging of two giant vesicles under illumination of a 365 nm LED light for 6.5 min. In situ conditions: [**2A**]=0.5 mM; [decylamine]=2 mM; [sodium oleate]=0.2 mM; in pH 7.5 HEPES buffered saline solution (HEPES 25 mM, NaCl 50 mM).

Movie S4.

Visible light-induced vesicle release

Time-lapse video featuring two samples (0.5 min and 4 min, respectively) demonstrating visible light-induced vesicle release of previously enlarged **2A-C_{10}** vesicles. The recording was conducted under intense visible light illumination using a tungsten halogen lamp in a microscopy setting.

7. References

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