Supporting Information

Light-controlled artificial transmembrane signal transduction for 'ON/OFF'-switchable transphosphorylation of RNA model substrate.

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Contents

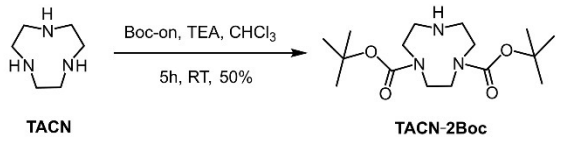
1. Materials

All starting reagents and solvents were procured from Energy Chemical and Aladdin and were used without further purification. 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC) and 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) were purchased from Tokyo Chemical Industry. All the reagents and solvents were used without further purification.

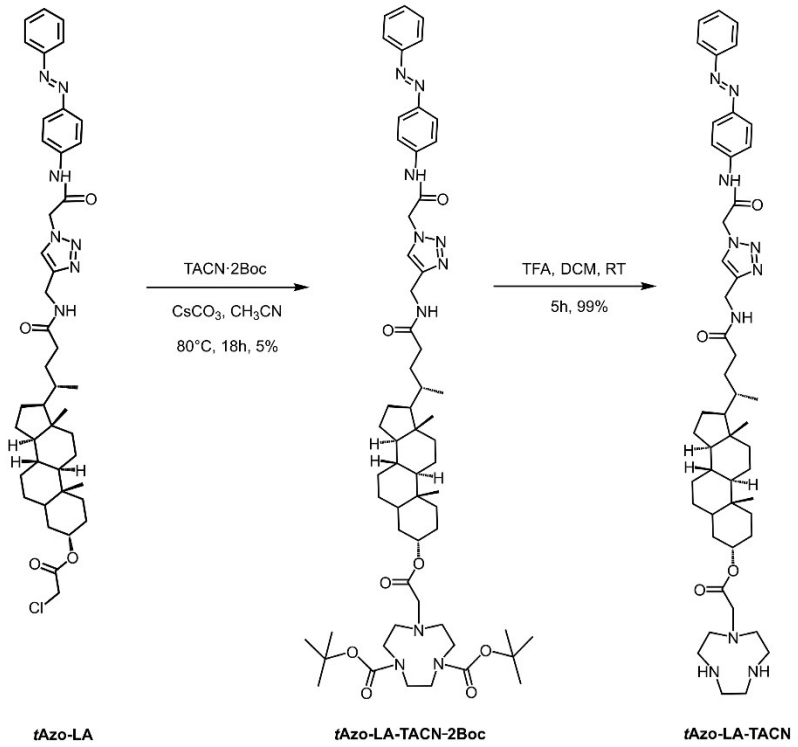
2. General characterizations

^{1}H NMR and ^{13}C NMR spectra were reported at 25 ºC using Bruker AVANCEIII instruments at 500 and 400 MHz. ^{1}H NMR and ^{13}C NMR signal were outputted as chemical shifts (δ) in ppm. Mass spectra (ESI-MS) were obtained on Bruker Agilent1290-micrOTOF Q II. Fluorescence experiments were carried out on Shimadzu Fluorescence Spectrometers (5301PC). Confocal laser scanning microscopy (CLSM) assays were performed on Nikon AX R confocal microscope. Differential scanning calorimetry (DSC) analysis were tested on TA INSTRUMENTS DSC Q20. Dynamic light scattering (DLS) experiments were performed on DLS Zetasizer Nano Series from Malvern Company. UV-visible spectra were obtained on UV-vis Absorption Spectrometry UV-2450 from Shimadzu Company.

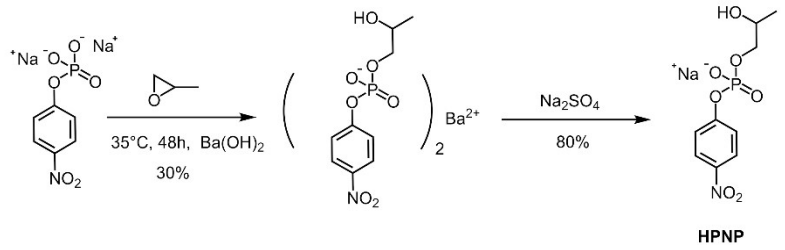
3. Synthetic procedures



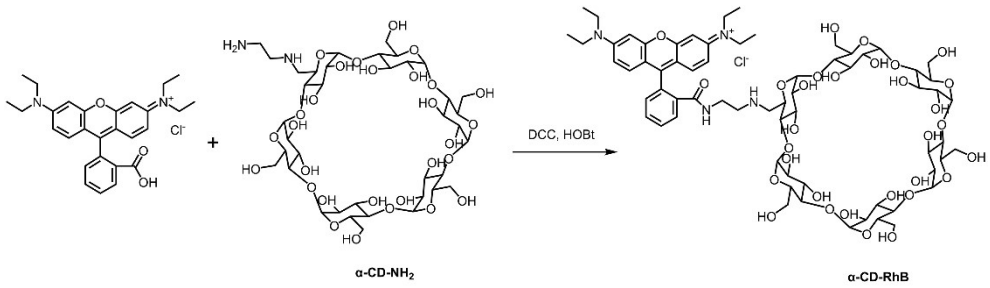
Scheme S1. Synthetic routes of TACN-2Boc



Scheme S2. Synthetic routes of *t*Azo-LA-TACN



Scheme S3. Synthetic routes of 2-hydroxypropyl-4-nitrophenylphosphate (HPNP)



Scheme S4. Synthetic routes of α-CD-RhB

Synthesis of **TACN-2Boc**

At 0 ºC, 1,4,7-triazacyclononane (TACN, 0.50 g, 3.90 mmol) was added to a solution of triethylamine (TEA, 1.60 mL) and chloroform (15 mL). [2-(Tert-butoxycarbonyloxyimino)-2-phenylacetonitrile] (Boc-on, 1.69 g, 7.75 mmol) in dry chloroform (10 mL) was added dropwise over 5 h. Then, the resulting solution was stirred at rt for another 2 h before the solvents were removed in vacuo. The obtained yellow oil was extracted with 10% aqueous solution of sodium carbonate (5 mL) and diethyl ether (15 mL). The organic phase was washed with water (3 x 10 mL) and then was evaporated to obtain colorless oil. The colorless oil was further extracted with 10% aqueous solution of citric acid (20 mL), and then the acidic aqueous phase was alkalized to pH 11 with 1 M sodium hydroxide and then extracted with chloroform. The organic phase was evaporated to obtain colorless oil as the product **TACN-2Boc** (642 mg, 50%). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 3.53 – 3.42 (m, 4H), 3.27 (dd, J = 28.4, 3.6 Hz, 4H), 2.98 – 2.89 (m, 4H), 1.50 (s, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 155.72, 155.47, 79.60 – 79.16, 77.85 – 77.21, 76.99, 52.85, 52.23, 51.45, 50.19, 49.57, 49.26, 48.01, 47.55, 47.15, 28.33. HRMS (ESI): *m/z* calcd for $C_{16}H_{31}N_3O_4H^+$: 330.2393, Found: 330.2442.

Synthesis of ***t*Azo-LA-TACN**

***t*Azo-LA** was synthesized according to the previous reference.[1] To a solution of ***t*Azo-LA** (71.06 mg, 0.10 mmol) in CH_3CN (10 mL), **TACN-2Boc** (49.20 mg, 0.15 mmol) and $CsCO_3$ (97.50 mg. 0.30 mmol) was added. The mixture solution was refluxed at 80 ºC for 18 h. The crude product was obtained after removing the solvent, and then was further extracted with DCM (20 mL) and water (10 mL). The yellow product ***t*Azo-LA-TACN-2Boc** was obtained via column chromatography (5.32 mg, 5%). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 9.24 (s, 1H), 7.86 (dd, *J* = 26.3, 17.7 Hz, 4H), 7.81 (s, 1H), 7.69 (d, *J* = 8.6 Hz, 2H), 7.47 (dt, *J* = 20.9, 7.0 Hz, 3H), 6.48 (t, *J* = 5.5 Hz, 1H), 5.17 (s, 2H), 4.82 – 4.65 (m, 1H), 4.54 (d, *J* = 5.6 Hz, 2H), 3.58 – 3.41 (m, 4H), 3.39 (s, 2H), 3.23 (d, *J* = 24.4 Hz, 4H), 2.80 (d, *J* = 35.3 Hz, 4H), 2.36 – 2.19 (m, 2H), 1.86 – 1.00 (m, 53H). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 174.23, 171.62, 163.31, 155.61, 152.61, 149.45,

145.47, 139.57, 130.91, 129.07, 124.26, 123.94, 122.79, 120.22, 79.55, 79.43, 74.60, 56.74, 56.65, 56.48, 56.40, 56.00, 54.39, 53.84, 53.76, 53.68, 53.44, 51.05, 50.76, 50.55, 49.80, 49.64, 49.44, 42.74, 41.94, 40.50, 40.43, 40.08, 35.77, 35.48, 35.01, 34.58, 33.49, 32.44, 31.69, 29.69, 28.57, 28.23, 26.99, 26.84, 26.29 24.14, 23.32, 20.83, 18.38, 12.03. HRMS (ESI): *m/z* calcd for $C_{59}H_{86}N_{10}O_8H^+$:1063.6708, Found: 1063.1741.

Synthesis of compound ***t*Azo-LA-TACN**

At 0 ºC, ***t*Azo-LA-TACN-2Boc** (5 mg, 0.005 mmol) was dissolved in the 1 mL dichloromethane/trifluoroacetic acid (DCM/TFA, 1/1, V/V) solution. The mixture solution was stirred at rt for 5 h before the DCM and TFA was removed. The crude was washed with diethyl ether (50 mL) to obtain the yellow product ***t*Azo-LA-TACN** (4.27 mg, 99%). ^{1}H NMR (500 MHz, MeOD) δ = 7.95 – 7.89 (m, 3H), 7.87 (d, *J* = 7.3 Hz, 2H), 7.79 (d, *J* = 8.8 Hz, 2H), 7.57 – 7.45 (m, 3H), 5.36 (s, 2H), 4.78 – 4.72 (m, 1H), 4.68 (d, *J* = 6.5 Hz, 2H), 4.46 (q, *J* = 15.2 Hz, 2H), 3.72 – 3.50 (m, 5H), 3.20 (d, *J* = 19.8 Hz, 2H), 3.19 – 3.04 (m, 5H), 2.26 (dd, *J* = 8.9, 4.9 Hz, 1H), 2.20 – 2.09 (m, 1H), 1.98 (d, *J* = 11.6 Hz, 1H), 1.93 – 1.74 (m, 6H), 1.67 (d, *J* = 11.8 Hz, 1H), 1.54 – 0.91 (m, 27H). ^{13}C NMR (126 MHz, MeOD) δ = 175.27, 172.05, 168.65, 164.60, 155.53, 152.64, 148.98, 145.09, 140.84, 130.67, 128.85, 124.58, 123.45, 122.29, 119.71, 75.83, 61.58, 56.46, 56.16, 55.03, 52.22, 51.00, 50.44, 48.96, 46.62, 45.70, 45.56, 45.38, 43.98, 42.85, 42.50, 41.93, 40.42, 40.04, 35.73, 35.39, 34.59, 34.23, 34.19, 32.62, 31.83, 31.79 27.88, 26.71, 26.12, 23.83, 22.39, 20.52, 17.38, 11.07. HRMS (ESI): *m/z* calcd for $C_{49}H_{70}N_{10}O_4H^+$: 863.5660, Found: 863.5512.

Synthesis of **HPNP**

4-Nitrophenyl phosphate disodium salt hexahydrate (1.8557 g, 5 mmol) was dissolved in 10 ml water, and then the solution was acidized by an IR-120 (H^+) resin column. The obtained acid solution was neutralized to pH 7.0 using aqueous ammonia. 1,2-Epoxypropane (20 ml) was added to the solution and stirred at 35 ºC for 48 h. The solution was acidized by an IR-120 (H^+) resin column again after the unreacted 1,2-

Epoxypropane was removed. The pH of the acid solution was neutralized to no higher than 7.0 using $Ba(OH)_2$ solution. The obtained solution was concentrated to about 10 ml at rt and then 40 ml ethanol was added to form the white precipitate. The precipitate was filtered off and the filtrate was concentrated to a small volume. Under vigorous stirring, the concentrated filtrate was added to 10% ethanol in acetone and the white precipitate was collected. Subsequently, the precipitate (450 mg) was dissolved in 90 mL water and 4.68 mL of Na_2SO_4 (0.14 M) was added. The obtained solution was centrifuged for 1.5 h. The supernatant was collected and lyophilized to obtain the **HPNP** (360 mg, 24%). ^{1}H NMR (400 MHz, D_2O) δ = 8.16 (d, *J* = 9.1 Hz, 2H), 7.25 (d, *J* = 9.0 Hz, 2H), 3.87 (dddd, *J* = 14.1, 9.3, 6.1, 3.4 Hz, 2H), 3.70 (dt, *J* = 10.7, 6.6 Hz, 1H), 1.05 (t, *J* = 6.3 Hz, 3H). ^{13}C NMR (126 MHz, D_2O) δ = 157.38, 143.44, 125.81, 120.43, 70.99, 66.46, 17.64. HRMS (ESI): *m/z* calcd for $C_9H_{11}NO_7P^-$: 276.0278, Found: 276.0939.

Synthesis of **α-CD-RhB**

α-CD-NH$_2$ was synthesized according to the previous reference. Under N_2, Rhodamine B (RhB, 240 mg, 0.5 mmol), N,N'-Dicyclohexylcarbodiimide (DCC, 110 mg, 0.53 mmol) and 1-Hydroxybenzotriazole (HOBt, 75 mg, 0.56 mmol) were dissolved in DMF (10 mL), and then the solution was stirred for 1 h at 0 ºC. A solution of **α-CD-NH$_2$** (507 mg, 0.5 mmol) in DMF (10 mL) was added to the above solution and then the resulting mixture was stirred for 2 h at 0 ºC before recovered to rt for 12 h. The solvent was removed and the crude product was purified by Sephadex G-50 to obtained red product **α-CD-RhB.** (59 mg, 8%). ^{1}H NMR (500 MHz, D_2O) δ = 7.73 (s, 2H), 7.67 (s, 3H), 7.43 (d, *J* = 6.4 Hz, 5H), 4.98 (d, *J* = 3.4 Hz, 6H), 3.85 (ddd, *J* = 25.8, 16.6, 9.8 Hz, 18H), 3.64 – 3.45 (m, 16H), 3.30 (s, 6H), 3.12 (q, *J* = 7.3 Hz, 8H), 1.20 (t, *J* = 7.3 Hz, 12H). ^{13}C NMR (126 MHz, D_2O) δ = 165.30, 139.65, 127.69, 127.29, 125.75, 115.88, 111.94, 101.41, 81.21, 73.29, 72.00, 71.65, 60.35, 46.65, 39.04, 36.44, 32.18, 24.48, 8.20. HRMS (ESI): *m/z* calcd for $C_{66}H_{95}N_4O_{31}H_2^{2+}$: 1441.6132, Found: 1441.5233.

4. Interactions between *t*Azo-LA-TACN/α-CD and lipid bilayers.

4.1 Confocal fluorescence imaging.

0.8 mg DOPC/DOPE lipids (molar ratio at 4:1) containing 2.5 mol% DiO was dissolved in 500 μl of dry chloroform and dried under high vacuum for 3 h to form a thin film. 530 μl of sucrose solution (0.2 M) containing *t*Azo-LA-TACN/α-CD-RhB (1.5 mol% relative to lipid) was added to hydrate the obtained film at 37 ºC overnight. To remove the excess dye and *t*Azo-LA-TACN/α-CD-RhB, 30 μl of GUVs suspension was gently added to the top of 1.0 mL glucose solution (0.2 M) in a 1.5 mL microcentrifuge tube and allowed to sediment in the dark. The GUVs containing sucrose solution will deposit on the bottom of microcentrifuge tube due to that sucrose solution has a higher density than glucose solution. After sedimented for 1 h, the top 1 mL solution was pipetted and replaced by another 1.0 mL of glucose solution (0.2 M) to sediment in the dark for 1 h again. The 30 μl solution was collected from the bottom of the microcentrifuge tube for microscopy investigations. The fluorescence imaging was taken on a Nikon AX R confocal microscope at 488 nm and 594 nm for DiO and RhB, respectively.

4.2 DSC measurement.

DSC thermograms recording the lipid phase transition were obtained using a TA INSTRUMENTS DSC Q20. The samples were equilibrated at -40 °C for 2 min and heated from -40 °C to 40 °C at a rate of 5 °C min^{-1}. The heating and cooling process was repeated for several cycles. The full-width at half-maximum of the peak was obtained with baseline correction using the Origin software.

4.3 Calcein release experiment

DOPC/DOPE lipids (molar ratio, 4:1, 6.9 mg/1.6 mg) and 3 ml of dry chloroform was added to a glass bottle, and then the mixture solution was placed under high vacuum for 3 h to remove chloroform for obtaining a thin film. A buffer solution of 1 ml (20 mM HEPES, pH=8.0) containing 100 mM calcein was added to the bottle to hydrate the lipid. The resulting solution was sonicated for 1 min before 10 times freeze-thaw

cycles. The obtained suspension was extruded 19 times through 200 nm polycarbonate membrane, and then purified by Sephadex G-50 using the buffer solution (20 mM HEPES, pH=8.0) to remove the calcein outside the vesicles. The control LUVs, the LUVs containing *t*Azo-LA-TACN/α-CD (7.5 μM *t*Azo-LA-TACN and 0.5 mM α-CD), the LUVs containing *t*Azo-LA-TACN/α-CD (7.5 μM *t*Azo-LA-TACN and 0.5 mM α-CD) and subsequent UV light were used for time dependent fluorescence experiments. Fluorescence intensity after addition of Triton X-100 at t = 400 s was set to 1.0. Fluorescence experiments were recorded using the following parameters: λ_{ex} = 497 nm, λ_{em} = 518 nm. The emission and excitation slits were set at 3.0 nm and 1.5 nm.

4.4 DLS experiments.

The (Zn^{2+}/HPNP)-loaded LUVs were prepared according to the method of calcein-contained LUVs except that 25 mM HPNP and 0.25 mM Zn^{2+} was used to replace calcein. To a quartz cuvette, the resulting LUVs containing *t*Azo-LA-TACN/α-CD (7.5 μM *t*Azo-LA-TACN and 0.5 mM α-CD), the LUVs containing *t*Azo-LA-TACN/α-CD (7.5 μM *t*Azo-LA-TACN and 0.5 mM α-CD) and subsequent UV light, and the control LUVs were used for DLS analysis.

4.5 Photoisomerization of *t*Azo-LA-TACN/α-CD in the membrane.

*t*Azo-LA-TACN in ethanol and α-CD in water (1.8 mol% relative to lipid) were added to DOPC/DOPE lipids (molar ratio, 4:1, 6.9 mg/1.6 mg) in 3 ml of dry chloroform, and then the mixture solution was placed under high vacuum for 3 h to remove all the solution for obtaining a thin film. A buffer solution of 1 ml (20 mM HEPES, pH=8.0) containing 25 mM HPNP and 0.25 mM zinc nitrate was added to the bottle to hydrate the lipid. The resulting solution was sonicated for 1 min before 10 times freeze-thaw cycles. The obtained suspension was extruded 19 times through 200 nm polycarbonate membrane, and then purified by Sephadex G-50 using the buffer solution (20 mM HEPES, pH=8.0) to remove the HPNP, zinc nitrate, *t*Azo-LA-TACN/α-CD (7.5 μM *t*Azo-LA-TACN and 0.5 mM α-CD) outside the vesicles. The obtained LUVs containing was used for photoisomerization assays under UV and visible light.

5. In situ light-responsive signal transduction based on LUVs.

5.1 Preparation of (Zn^{2+}/HPNP)-contained LUVs.

DOPC/DOPE lipids (molar ratio, 4:1, 6.9 mg/1.6 mg) and 3 ml of dry chloroform was added to a glass bottle, and then the mixture solution was placed under high vacuum for 3 h to remove chloroform for obtaining a thin film. A buffer solution of 1 ml (20 mM HEPES, pH=8.0) containing 25 mM HPNP and 0.25 mM zinc nitrate was added to the bottle to hydrate the lipid. The resulting solution was sonicated for 1 min before 10 times freeze-thaw cycles. The obtained suspension was extruded 19 times through 200 nm polycarbonate membrane, and then purified by Sephadex G-50 using the buffer solution (20 mM HEPES, pH=8.0) to remove the excess molecules HPNP and zinc nitrate outside the vesicles.

5.2 Light-triggered transmembrane signal transduction assays.

A 50 μl of HEPES buffer (20 mM HEPES, pH=8.0) containing *t*Azo-LA-TACN/α-CD (7.5 μM *t*Azo-LA-TACN and 0.5 mM α-CD) was prepared as the stock solution of *t*Azo-LA-TACN/α-CD. To 250 μl of above prepared (Zn^{2+}/HPNP)-contained LUVs in a cuvette, 50 ul stock solution of *t*Azo-LA-TACN/α-CD *t*Azo-LA-TACN/α-CD (7.5 μM *t*Azo-LA-TACN and 0.5 mM α-CD) was added to form the 'OFF' state for 50 s. A UV lamp (365 nm, 5.0 $mW \cdot cm^{-2}$) was used for in situ light irradiation for 120 s to switch 'ON' signal transduction for 430 s. The time dependent increase in absorbance intensity was monitored at λ_{abs} = 405 nm. The total testing time was 600 s, and the testing temperature was set at 25 ℃.

5.3 Light signal transduction with varied concentration of Zn^{2+}

A 50 μl of HEPES buffer (20 mM HEPES, pH=8.0) containing *t*Azo-LA-TACN/α-CD (7.5 μM *t*Azo-LA-TACN and 0.5 mM α-CD) was prepared as the stock solution of *t*Azo-LA-TACN/α-CD. To 250 μl of above prepared (Zn^{2+}/HPNP)-contained LUVs (loaded with varied concentration of Zn^{2+} at 0 mM, 0.05 mM, 0.15 mM, 0.20 mM and 0.25 mM respectively) in a cuvette, 50 ul stock solution of *t*Azo-LA-TACN/α-CD *t*Azo-LA-TACN/α-CD (7.5 μM *t*Azo-LA-TACN and 0.5 mM α-CD) was added to form the 'OFF' state for 50 s. A UV lamp (365 nm, 5.0 $mW \cdot cm^{-2}$) was used for in situ light

irradiation for 120 s to switch 'ON' signal transduction for 550 s. The time dependent increase in absorbance intensity was monitored at λ_{abs} = 405 nm. The testing temperature was set at 25 ℃. For easier observation and comparison, we further processed the data:

The time axis was normalized according to eq. S1:

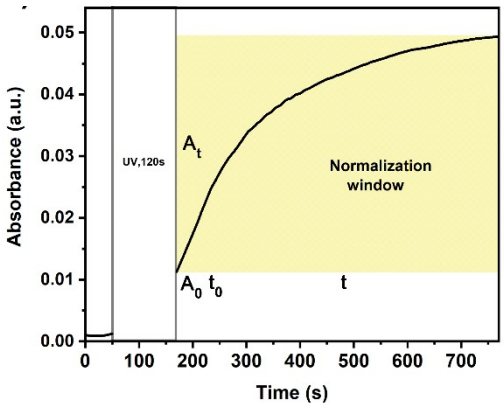
$$t = t\text{-}170 \qquad \text{eq. S1}$$

so that, the time of UV light stop gets normalized to t = 0 s and time of reaction end gets normalized to t = 430 s.

Absorbance intensities (A_t) were normalized to relative intensity A_r using eq. S2:

$$A_r = A_t - A_0 \qquad \text{eq. S2}$$

where, A_0 = Absorbance just when the UV light stop. A_t = Absorbance intensity at the time t.



Representative UV-vis kinetics experiment of light-triggered transmembrane signal transduction.

5.4 ^{31}P NMR spectroscopy measurements.

The above LUVs containing *t*Azo-LA-TACN/α-CD (7.5 μM *t*Azo-LA-TACN and 0.5 mM α-CD) ('OFF'), the LUVs containing *t*Azo-LA-TACN/α-CD (7.5 μM *t*Azo-LA-TACN and 0.5 mM α-CD) and subsequent UV light ('ON'), and the control LUVs were lyophilized at -50 ℃ at t = 600 s, respectively. Then the obtained solid was dissolved in 500 μl DMSO-d_6 for ^{31}P NMR investigation on a Bruker AVANCEIII instruments at 500 MHz.

5.5 ESI-MS analysis

A 10 μl of the LUVs containing *t*Azo-LA-TACN/α-CD (7.5 μM *t*Azo-LA-TACN and 0.5 mM α-CD) and subsequent UV light ('ON') was dissolved in 1.0 mL CH_3CN for ESI-MS test on a Bruker Agilent1290-micrOTOF Q II.

6 In situ 'ON/OFF' switches of signal transduction.

A 50 μl of HEPES buffer (20 mM HEPES, pH=8.0) containing *t*Azo-LA-TACN/α-CD (7.5 μM *t*Azo-LA-TACN and 0.5 mM α-CD) was prepared as the stock solution of *t*Azo-LA-TACN/α-CD. To 250 μl of above prepared (Zn^{2+}/HPNP)-contained LUVs in a cuvette, 50 ul stock solution of *t*Azo-LA-TACN/α-CD (7.5 μM *t*Azo-LA-TACN and 0.5 mM α-CD) was added to form the 'OFF' state for 50 s. A UV lamp (365 nm, 5.0 $mW \cdot cm^{-2}$) was used for in situ light irradiation for 120 s to switch 'ON' signal transduction for 50 s. Then a visible light lamp (530 nm, 5.0 $mW \cdot cm^{-2}$) was used for in situ light irradiation for 30 s to switch 'OFF' signal transduction for 50 s. Finally, the UV lamp ((365 nm, 5.0 $mW \cdot cm^{-2}$)) was used for in situ light irradiation for 240 s to switch 'ON' signal transduction for 50 s again. The time dependent increase in absorbance intensity was monitored at λ_{abs} = 405 nm. The total testing time was 600 s, and the testing temperature was set at 25 ℃

7 Supplementary Figures.

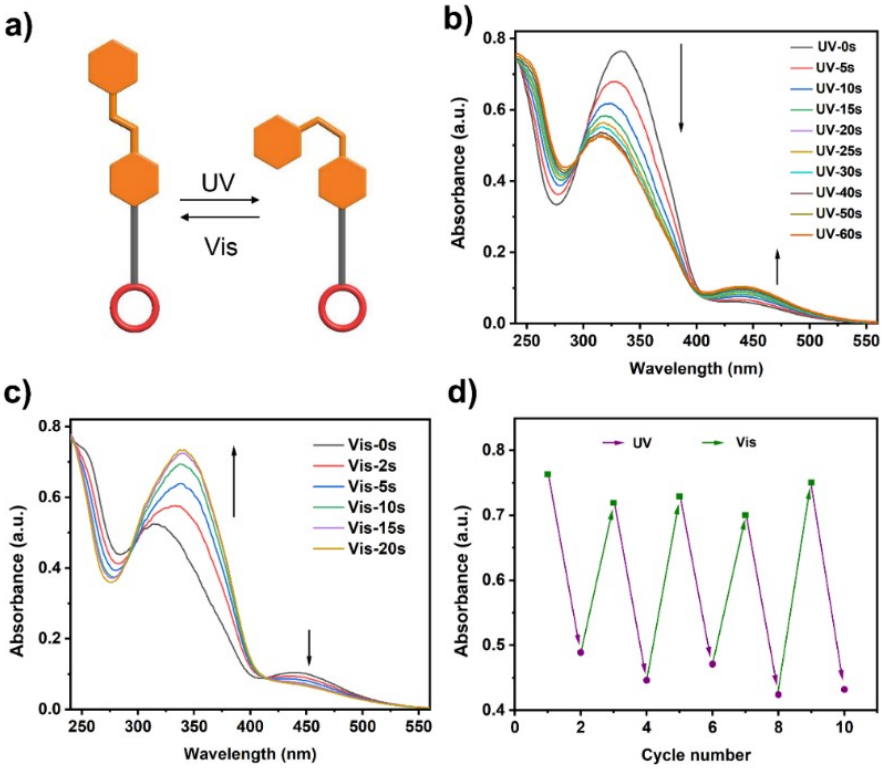


Figure S1. a) Schematic representation of *t*Azo-LA-TACN photoisomerization. b) UV-vis spectra of *t*Azo-LA-TACN photoisomerization after UV irradiation and c), d) the reversible switching of *t*Azo-LA-TACN upon alternative irradiation of UV and visible light. All the experiments were performed in the presence of *t*Azo-LA-TACN at 0.1 mM in DMSO/HEPES (20 mM HEPES, pH=8.0) (1/99, vol/vol) by irradiation of UV (365 nm, 5.0 mW·cm^{-2}, 60 s) and visible light (530 nm, 5.0 mW·cm^{-2}, 20 s).

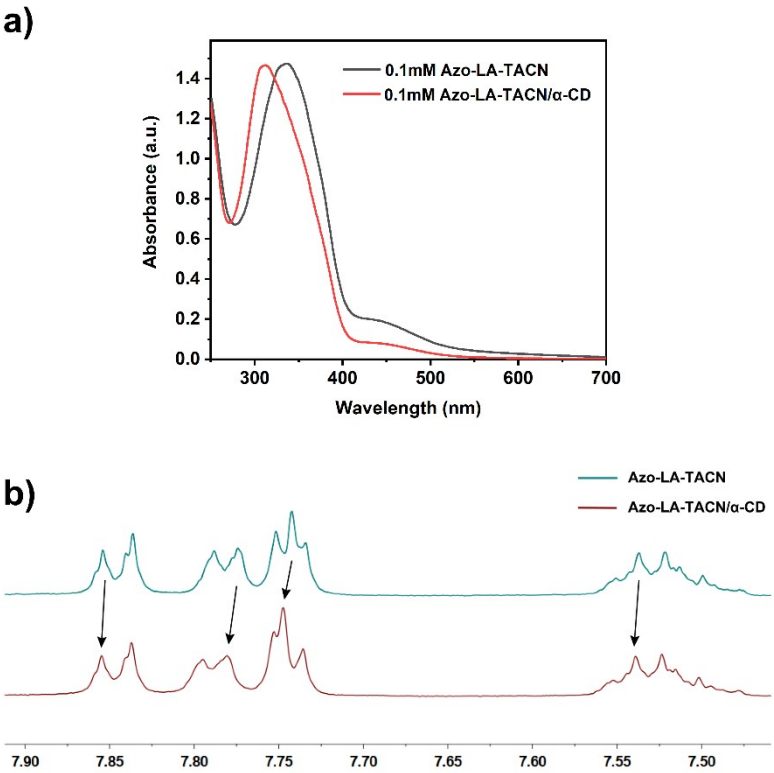


Figure S2. a) UV-vis spectra of *t*Azo-LA-TACN (0.1 mM) before (black) and after (red) addition of α/CD (0.1 mM) in DMSO/ HEPES (20 mM HEPES, pH=8.0). b) ^{1}H NMR spectra (500 MHz) of *t*Azo-LA-TACN (2.0 mM) before (blue) and after (red) addition of α/CD (2.0 mM) in DMSO-d_6/D_2O (2/1, vol/vol).

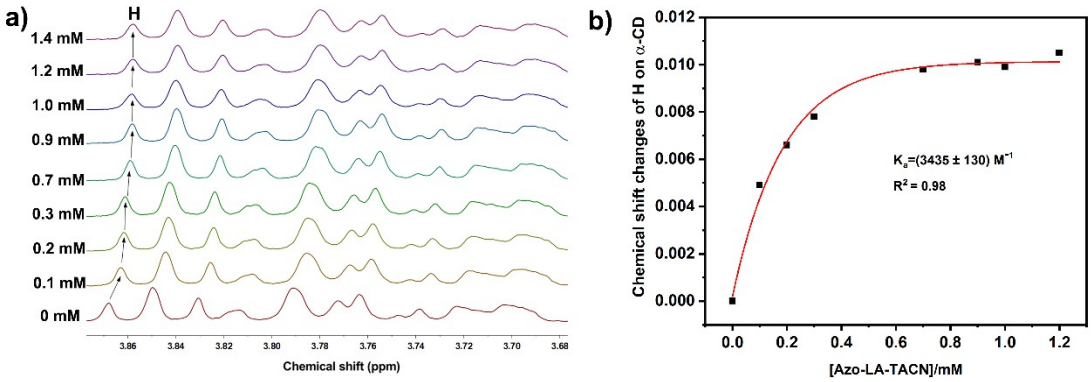


Figure S3. a) ^{1}H NMR spectra (D_2O/DMSO-d_6, vol/vol, 9:1, 500 MHz) of host α-CD recorded at a concentration of 1.00 mM in the presence of increasing concentrations of *t*Azo-LA-TACN (from 0 eq to 1.4 eq): 0 mM, 0.1 mM; 0.2 mM; 0.3 mM; 0.7 mM; 0.9 mM; 1.0 mM; 1.2 mM; 1.4 mM. b) Changes in the chemical shift corresponding to H on host α-CD as a function of added *t*Azo-LA-TACN. The red solid line was obtained from nonlinear least-squares fitting using eq. S8

The concentration of α-CD was kept at 1.0 mM. Upon addition of *t*Azo-LA-TACN at 0 mM, 0.1 mM; 0.2 mM; 0.3 mM; 0.7 mM; 0.9 mM; 1.0 mM; 1.2 mM; 1.4 mM in DMSO-d_6/D_2O (1/9, vol/vol), the chemical shift of *t*Azo-LA-TACN decreased. With a 1:1 stoichiometry,[2] ^{1}H NMR titration was used to determine the binding constant.[3] The binding constant K_a can be expressed by using concentration of a host ([H]), a guest ([G]) and a 1:1 complex ([HG]) as follows:

$$K_a = \frac{[HG]}{[H]\,[G]} \quad \text{(eq. S3)}$$

In the 1:1 system, the physical property (Y) that is related to the absolute concentration of each species can be represented as:

$$Y = Y_H[H] + Y_G[G] + Y_{HG}[HG] \quad \text{(eq. S4)}$$

where Y_H, Y_G and Y_{HG} are mole-dependent physical properties of pure H, pure G and pure HG, respectively. When pure G does not contribute to the physical property, one can simplify the equation as follows:

$$Y = Y_H[H] + Y_{HG}[HG] \quad \text{(eq. S5)}$$

The change of the physical property (ΔY) can then be derived as:

$$\Delta Y = (Y_{HG} - Y_H)[HG] = Y_{\Delta HG}[HG] \quad \text{(eq.S6)}$$

In this equation, the concentration of HG during a titration experiment is expressed with the total concentration of host ($[H]_0$) and the total concentration of guest ($[G]_0$) as follows:

$$[HG] = \frac{1}{2}(G0 + H0 + \frac{1}{K_a}) - \sqrt{(G0 + H0 + \frac{1}{K_a})2 + 4[H0][G0]}$$

(eq. S7)

In the NMR, quantity of chemical shift change (δ) becomes the corresponding property. Thus, the ^{1}H NMR titration data were fitted to an equation:

$$\Delta\delta = \delta_{\Delta HG}(\frac{[HG]}{H0}) = (\frac{\delta_{\Delta HG}}{H0})\{\frac{1}{2}(G0 + H0 + \frac{1}{K_a}) - \sqrt{(G0 + H0 + \frac{1}{K_a})2 + 4[H0}$$

}

(eq. S8)

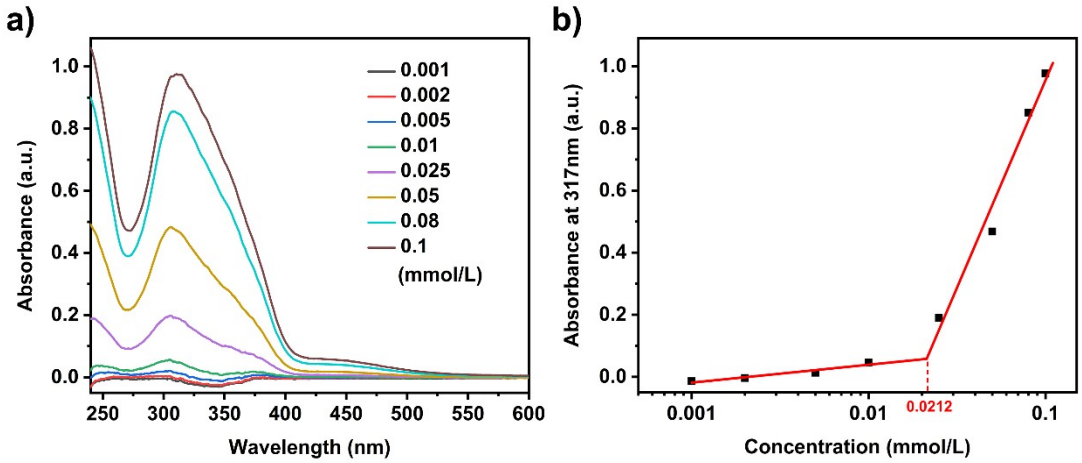


Figure S4. a) UV-vis spectra of *t*Azo-LA-TACN/α-CD (10^{-4} M) at different concentrations (from 0.001 mM to 0.1 mM) in DMSO/ HEPES (20 mM HEPES, pH=8.0). b) The critical assembling concentration (CAC) of *t*Azo-LA-TACN/α-CD by using UV-vis spectra data at 317 nm from the UV-vis spectra of a).

The critical aggregation concentration (CAC) was used to monitor the aggregation behavior of *t*Azo-LA-TACN/α-CD. Thus, we could obtain the CAC by measuring the UV-Vis absorption spectra of *t*Azo-LA-TACN/α-CD with increasing concentrations (from 0.001 mM to 0.1 mM), and the isolated absorption peaks at 317 nm were used for the measurement.

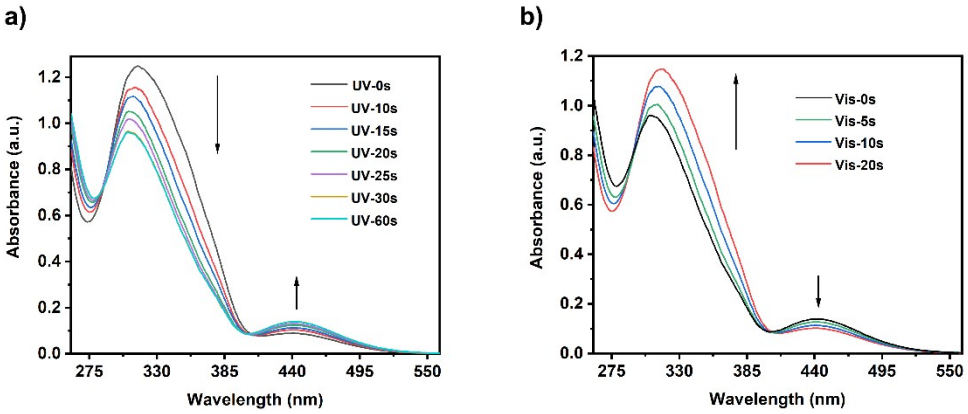


Figure S5. a) UV-vis spectra of *t*Azo-LA-TACN/α-CD photoisomerization after UV irradiation and b) the reversible switching of *t*Azo-LA-TACN/α-CD upon visible light. All the experiments were performed in the presence of *t*Azo-LA-TACN/α-CD at 0.1 mM in DMSO/ HEPES (20 mM HEPES, pH=8.0) by irradiation of UV (365 nm, 5.0 $mW \cdot cm^{-2}$, 60 s) and visible light (530 nm, 5.0 $mW \cdot cm^{-2}$, 30 s).

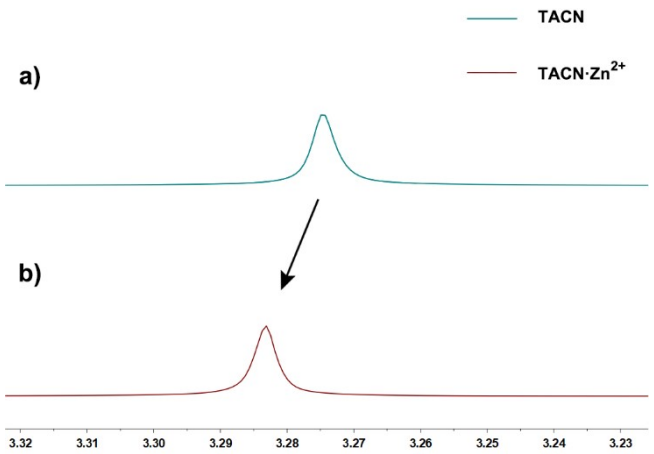


Figure S6. ^{1}H NMR spectra of TACN (5.0 mM) unit a) before and b) after addition of Zn^{2+} (5.0 mM) in D_2O.

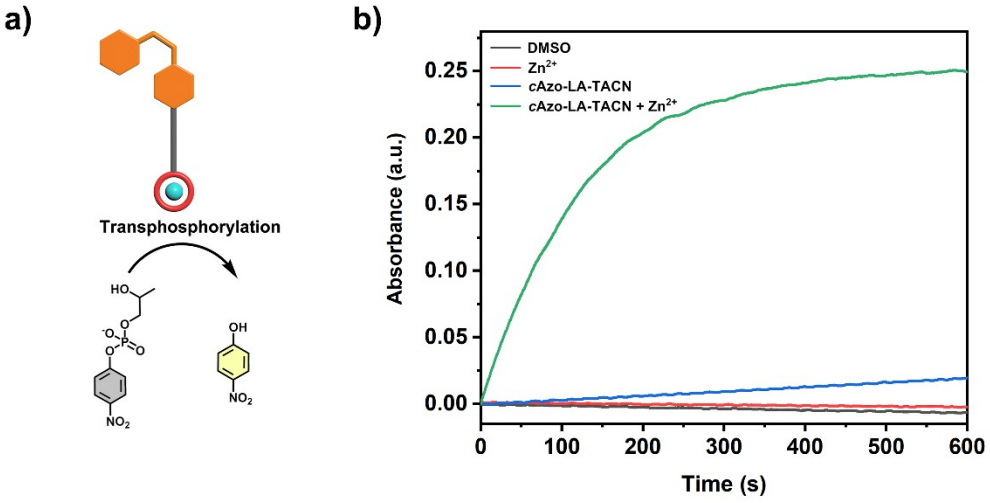


Figure S7. (a) Schematic representation of *c*Azo-LA-TACN·Zn^{2+} in the absence of lipid vesicles. b) Transphosphorylation of HPNP (0.1 mM) after addition of DMSO (black), Zn^{2+} (0.05 mM, red), *c*Azo-LA-TACN (0.05 mM, blue), *c*Azo-LA-TACN (0.05 mM) and Zn^{2+} (0.05 mM, green) (20 mM HEPES, pH=8.0).

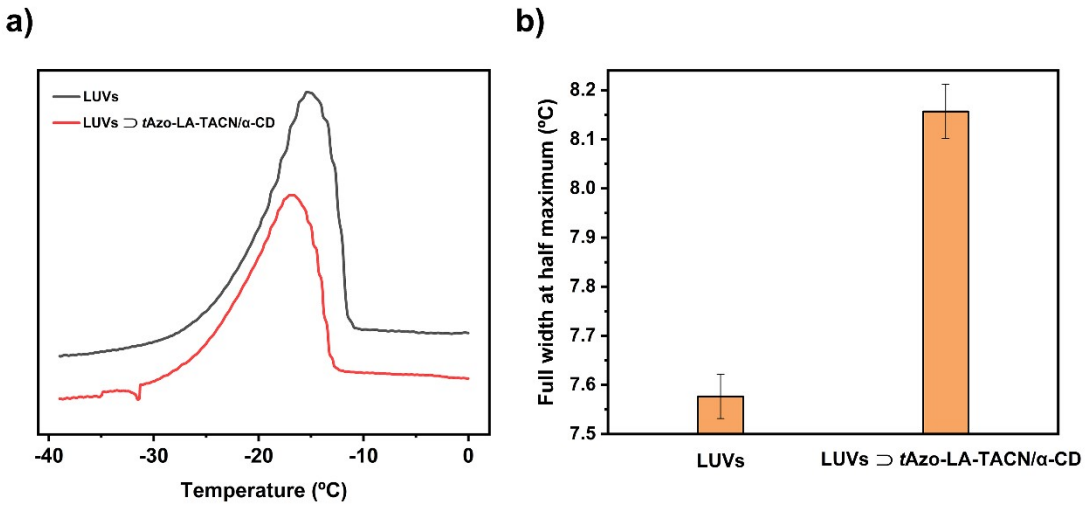


Figure S8. a) DSC analysis of LUVs (1.2 mM) and *t*Azo-LA-TACN/α-CD (7.5 μM *t*Azo-LA-TACN and 0.5 mM α-CD)-containing LUVs (1.2 mM) (20 mM HEPES, pH=8.0) and b) the corresponding full-width at half-maximum of the lipid phase transition.

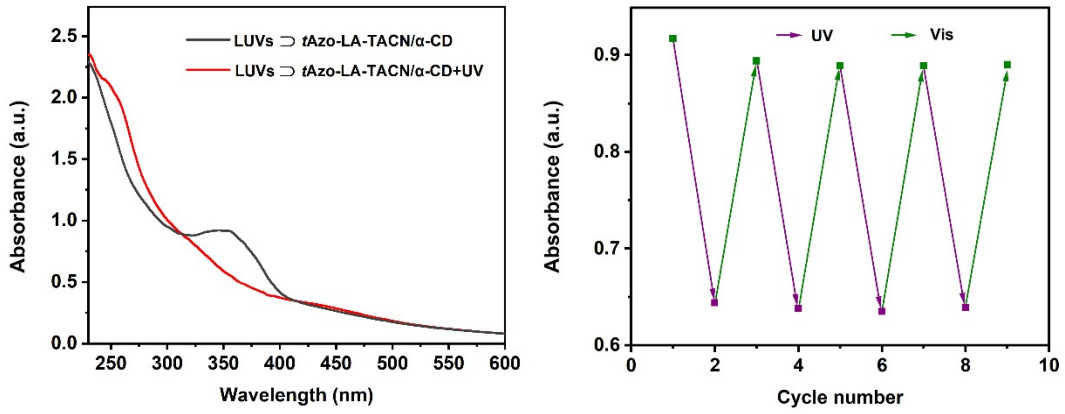


Figure S9. a) UV-vis spectra of *t*Azo-LA-TACN/α-CD (50 μM) photoisomerization in

the lipid membrane after UV irradiation for 60 s and b) the corresponding reversible switching upon the alternative irradiation of UV and visible light. All the experiments were performed in buffer solution (20 mM HEPES, pH =8.0) by irradiation of UV (365 nm, 5.0 mW·cm^{-2}, 60 s) and visible light (530 nm, 5.0 mW·cm^{-2}, 30 s).

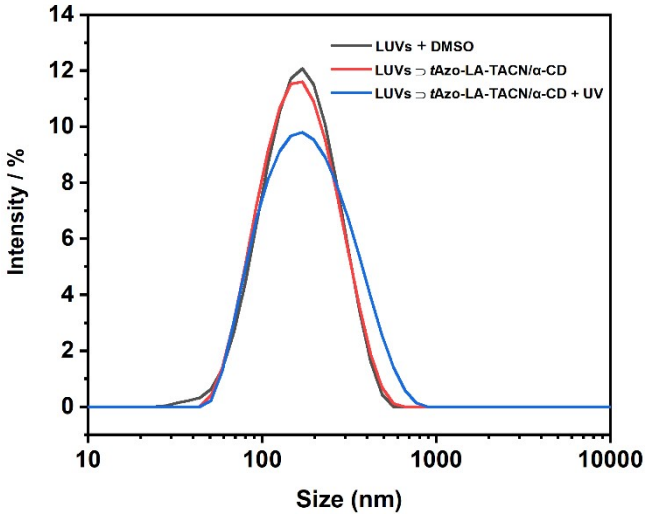


Figure S10. DLS analysis of LUVs (1.2 mM) after addition of DMSO (black), *t*Azo-LA-TACN/α-CD (7.5 μM *t*Azo-LA-TACN and 0.5 mM α-CD) (red), *t*Azo-LA-TACN/α-CD (7.5 μM *t*Azo-LA-TACN and 0.5 mM α-CD) and subsequent UV irradiation for 120 s (blue) (20 mM HEPES, pH=8.0).

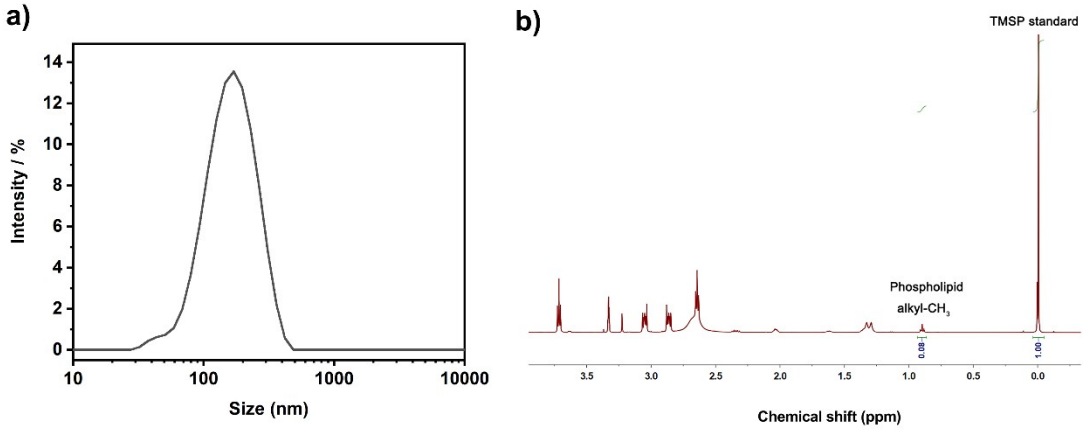


Figure S11. a) DLS measurement of DOPC/DOPE LUVs (1.2 mM) loaded with 1.75

mM HPNP, 0.25 mM Zn^{2+} (20 mM HEPES, pH=8.0). b) Partial ^1H NMR (500 MHz, 9:2:3 $CD_3OD/CDCl_3/H_2O$) spectra of LUVs for phospholipid concentration determination. TMSP = 3-(Trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt.

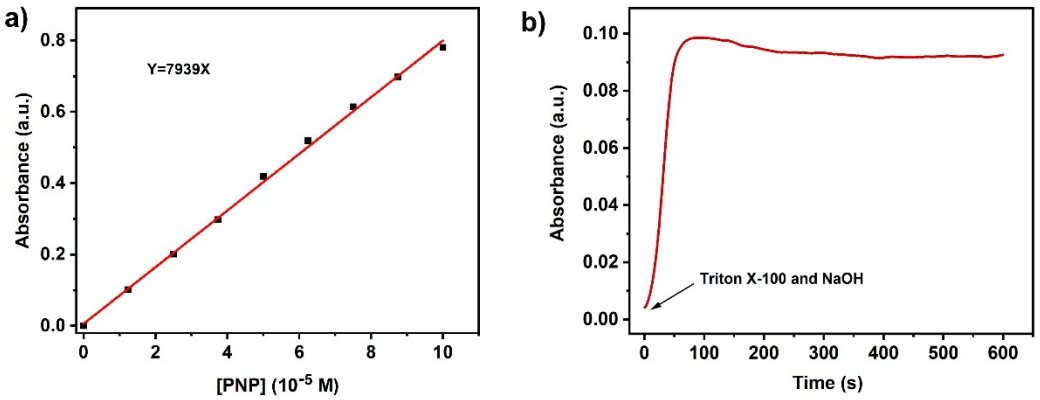


Figure S12. a) The absorbance-concentration standard curve of *p*-nitrophenol (PNP, the hydrolysate production of HPNP) and b) the lysis measurement of DOPC/DOPE LUVs (1.2 mM, 20 mM HEPES, pH=8.0) using 20% Triton X-100 and 1 M NaOH.

The concentration of HPNP inside the vesicles was characterized by adding 20% Triton X-100 (5 µl) to burst the DOPC/DOPE LUVs (300 µl, 1.2 mM) and 1 M NaOH (5 µl) to measure the complete reaction. As shown in Figure S12b, the overall bulk absorbance (A) of PNP after adding Triton X-100 and NaOH: A = 0.093. The overall bulk concentration of PNP (C_{PNP}) was calculated using the its UV-vis absorbance (A = 0.093, Figure S12b) and the standard curve (Y=7939X, Figure S12a):

$$C_{PNP} = \frac{A}{7939}$$

The overall bulk concentration of HPNP (C_{HPNP}):

$$C_{HPNP} = C_{PNP}$$

The internal average volume (V_{ves}) of a single vesicle (considered as a perfect water-filled sphere) was calculated using the average diameter (d = 200 nm) and the membrane thickness[4] (δ = 4 nm):

$$V_{ves} = \frac{4}{3}\pi\left(\frac{d}{2} - \delta\right)^3$$

The amount of the lipid (N_L) using the corresponding elution volume (V_1 = 18 ml), the

concentration of the lipid (C_L = 1.2 mM) and the Avogadro constant (N_A = 6.02 X 10^{23}):

$$N_L = V_1 C_L N_A$$

The amount of the vesicles (*Nves*) using the average area of per lipid[4] (S = 0.6 nm^2) and N_L:

$$Nves = \frac{N_L S}{2\ X\ 4\pi\left(\frac{d}{2}\right)^2}$$

The total internal volume (V_{tot}) using *Nves* and V_{ves}:

$$V_{tot} = Nves V_{ves}$$

The radio (r) of the total volume the total internal volume and the total internal volume using V_1 and V_{tot}:

$$r = \frac{V_1}{V_{tot}}$$

The internal concentration (C) of HPNP inside the vesicles:

$$C = rC_{HPNP}$$

where, A = 0.093, d = 200 nm, δ = 4 nm, V_1 = 18 ml, C_L = 1.2 mM, N_A = 6.02 X 10^{23}, S = 0.6 nm^2, and substituted in the above equations gives the concentration of HPNP inside the vesicles: C = 1.75 mM.

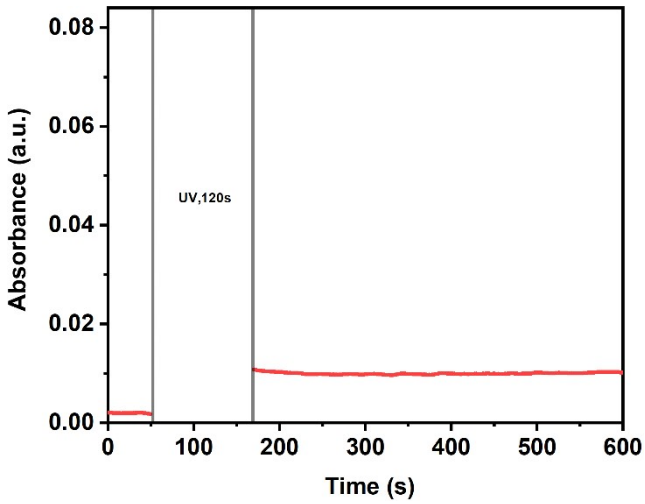


Figure S13. Time-dependent UV-vis spectra at 405 nm of LUVs (1.2 mM) (1.75 mM HPNP, 0.25 mM Zn^{2+}, 20 mM HEPES, pH=8.0) in the absence of *t*Azo-LA-TACN/α-CD (7.5 μM *t*Azo-LA-TACN and 0.5 mM α-CD) with UV irradiation for 120 s.

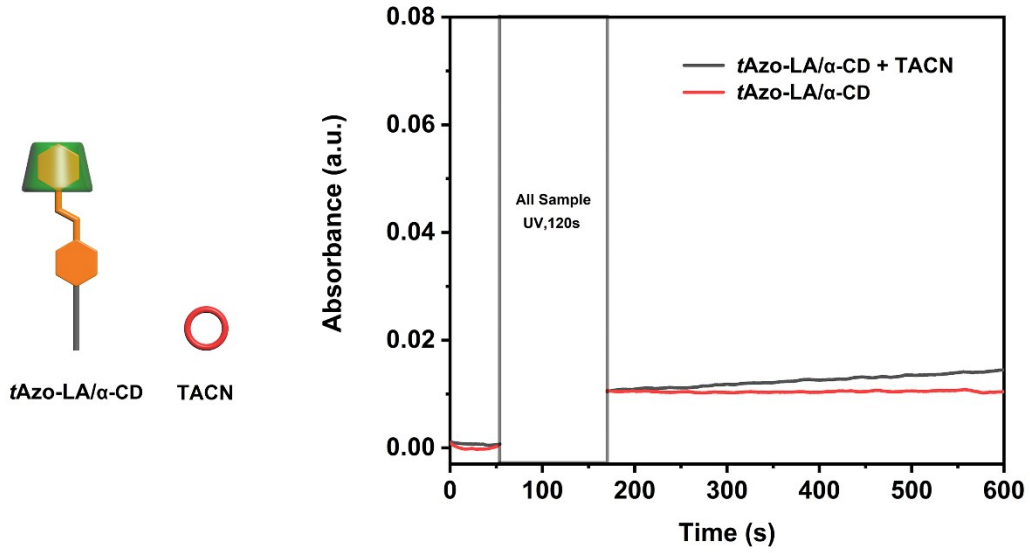


Figure S14. Time-dependent UV-vis spectra at 405 nm of LUVs (1.2 mM) (1.75 mM HPNP, 0.25 mM Zn^{2+}, 20 mM HEPES, pH=8.0) in the presence of *t*Azo-LA/α-CD (7.5 μM *t*Azo-LA and 0.5 mM α-CD, red) and TACN (7.5 μM, black) along with UV irradiation for 120 s.

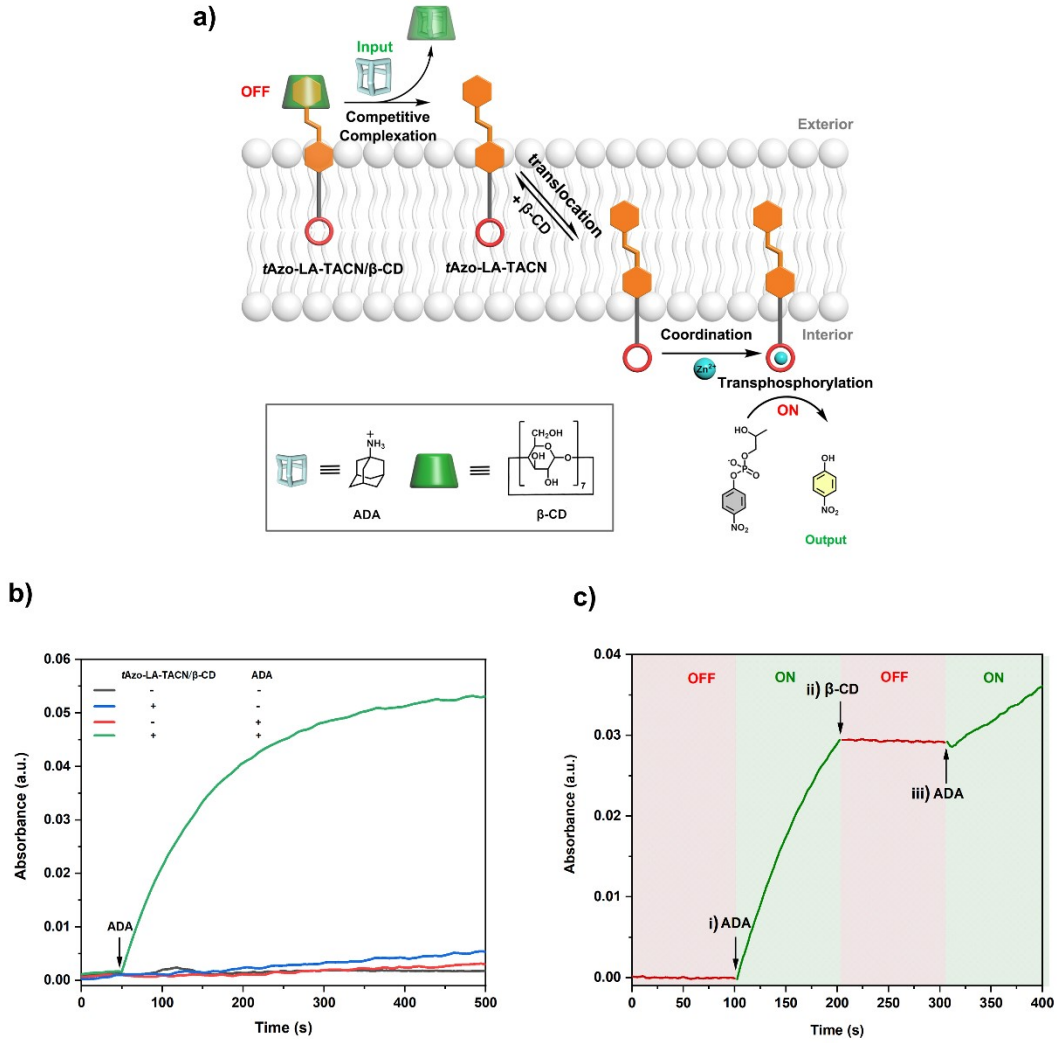


Figure S15 a) Schematic representation artificial signal transduction regulated by competitive supramolecular host-guest complexation. b) Time-dependent UV-vis spectra at 405 nm of LUVs (1.75 mM HPNP, 0.25 mM Zn^{2+}, 20 mM HEPES, pH=8.0) in the presence (+) or absence (-) of *t*Azo-LA-TACN/β-CD (7.5 μM *t*Azo-LA-TACN and 0.5 mM β-CD) and ADA (0.5 mM). c) Time-dependent UV-vis spectra at 405 nm of LUVs (1.75 mM HPNP, 0.25 mM Zn^{2+}, 20 mM HEPES, pH=8.0) in the presence of *t*Azo-LA-TACN/β-CD (7.5 μM *t*Azo-LA-TACN and 0.5 mM β-CD) after addition of i) 0.5 mM ADA ('ON' state), ii) 1.0 mM β-CD ('OFF' state), iii) 1.0 mM ADA ('ON' state)

8 ^{1}H NMR and ^{13}C NMR and ESI-MS Data.

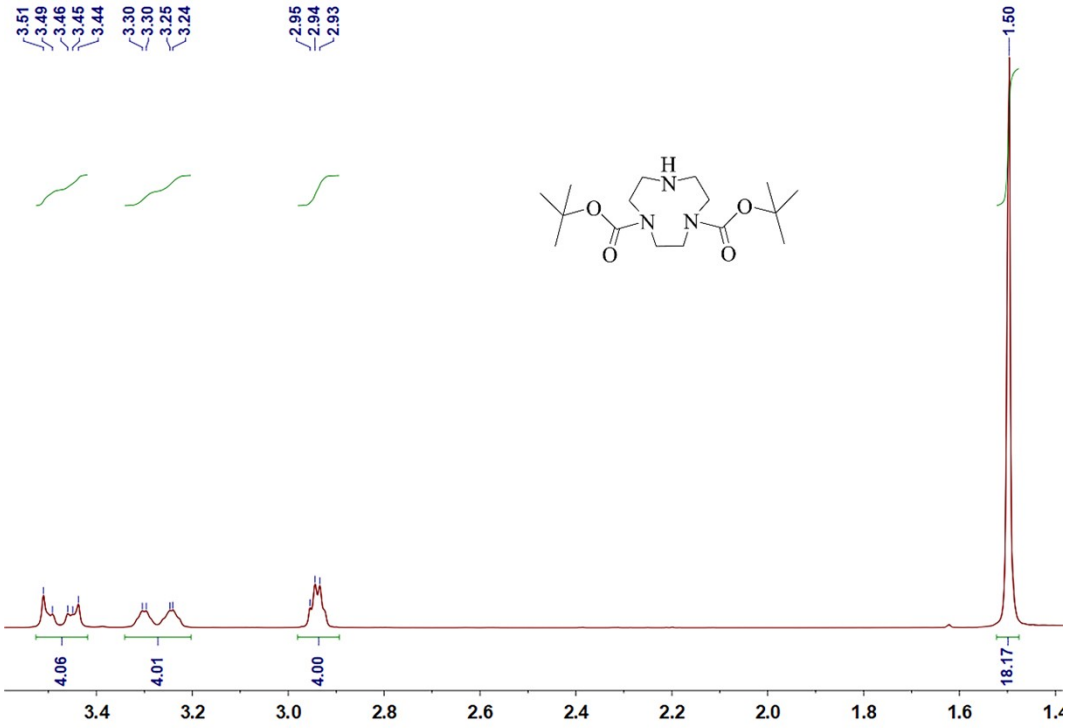


Figure S16. ^{1}H NMR spectrum of **TACN-2Boc** in $CDCl_3$

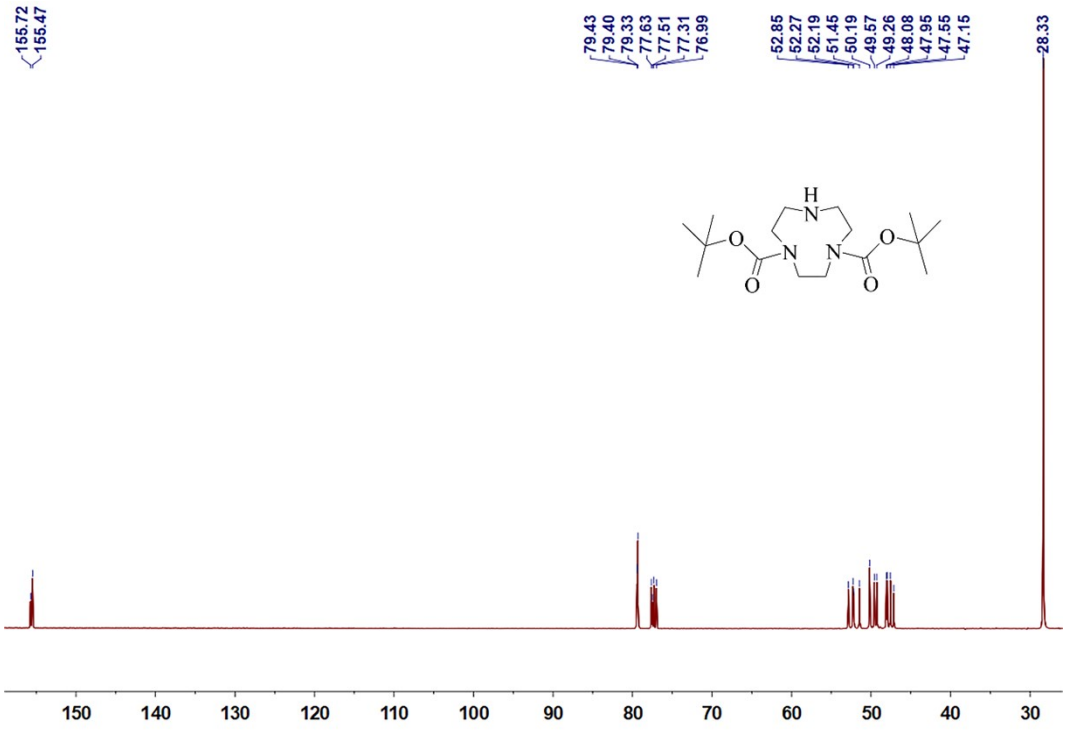


Figure S17. ^{13}C NMR spectrum of **TACN-2Boc** in $CDCl_3$

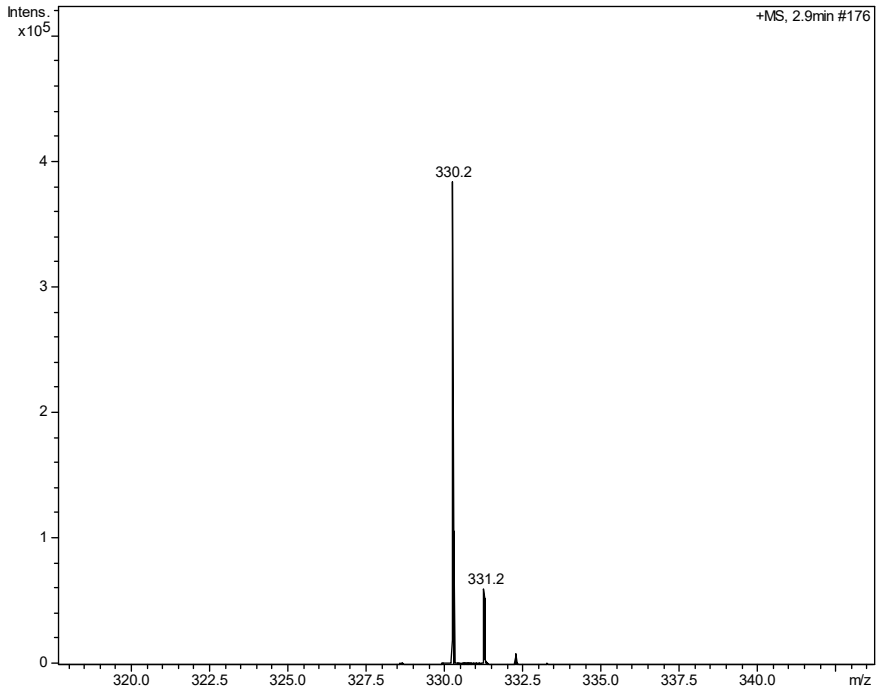


Figure S18. HR-MS (ESI) spectrum of **TACN-2Boc** in MeCN

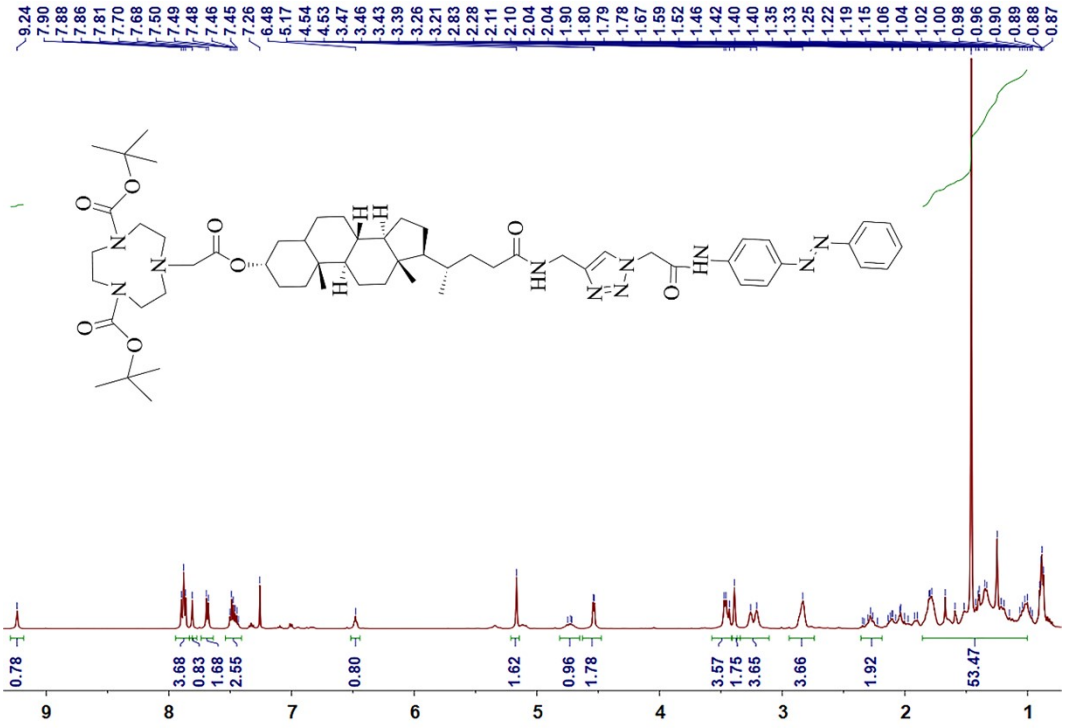


Figure S19. ^{1}H NMR spectrum of ***t*Azo-LA-TACN-2Boc** in $CDCl_3$

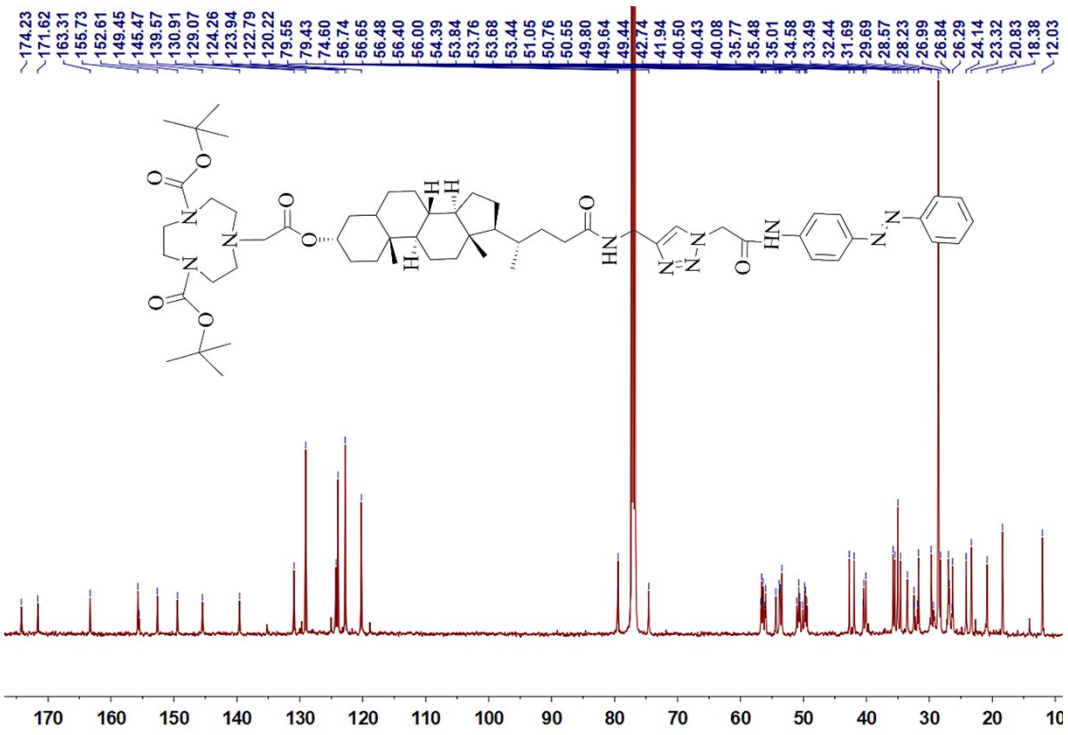


Figure S20. ^{13}C NMR spectrum of ***t*Azo-LA-TACN-2Boc** in $CDCl_3$

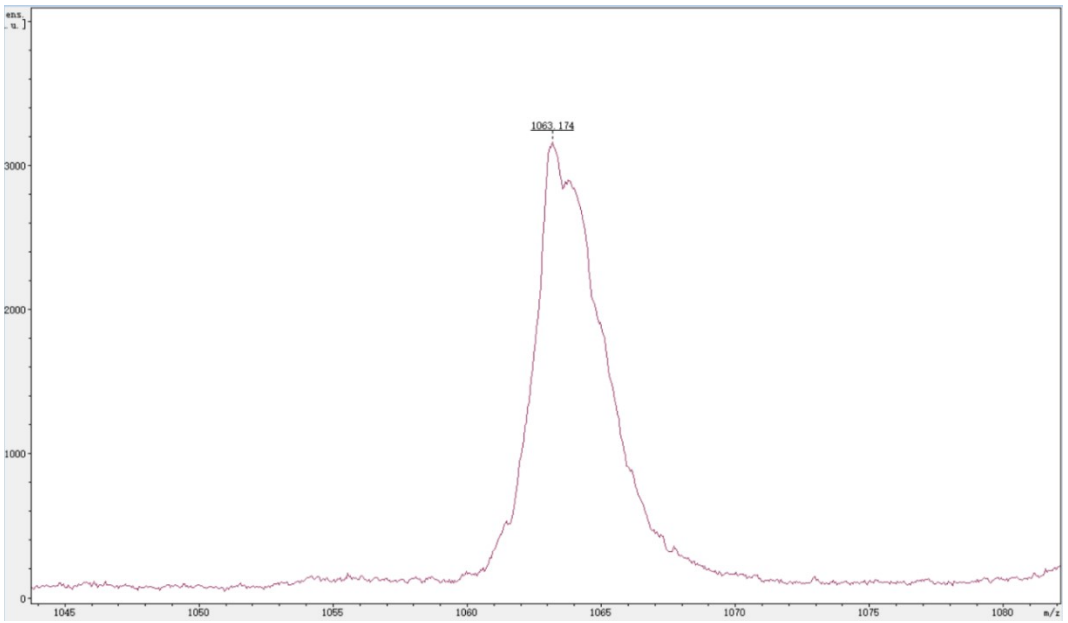


Figure S21. MALDI-TOF spectrum of ***t*Azo-LA-TACN-2Boc** in DCM.

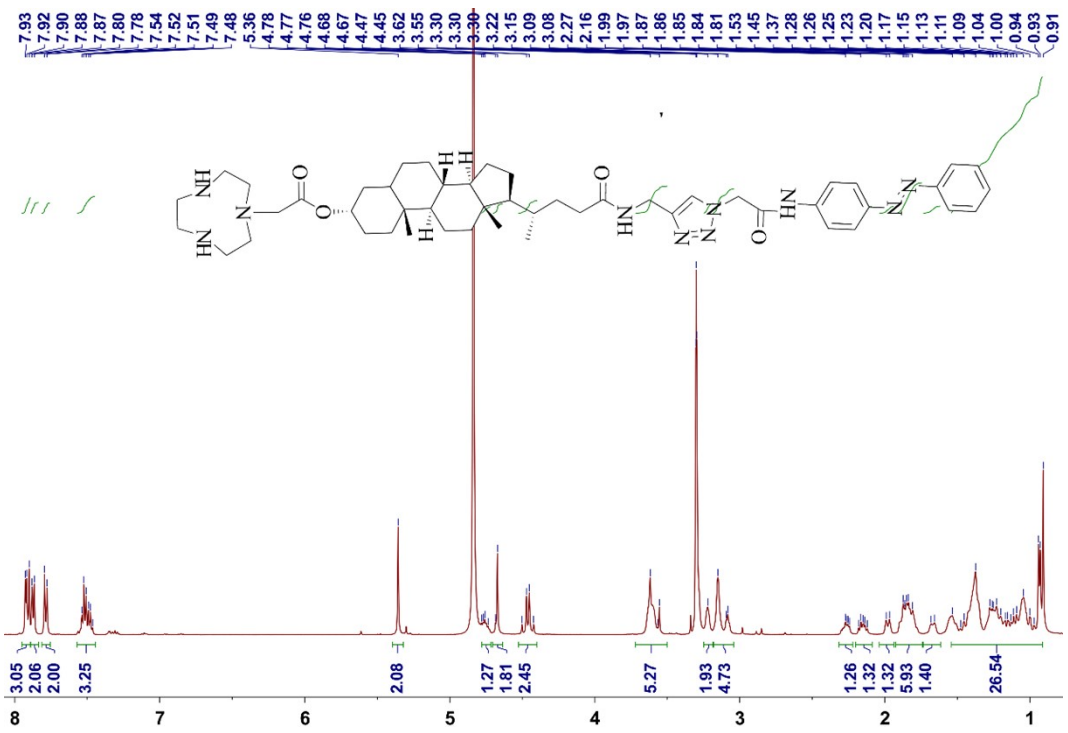


Figure S22. ^{1}H NMR spectrum of ***t*Azo-LA-TACN** in MeOD

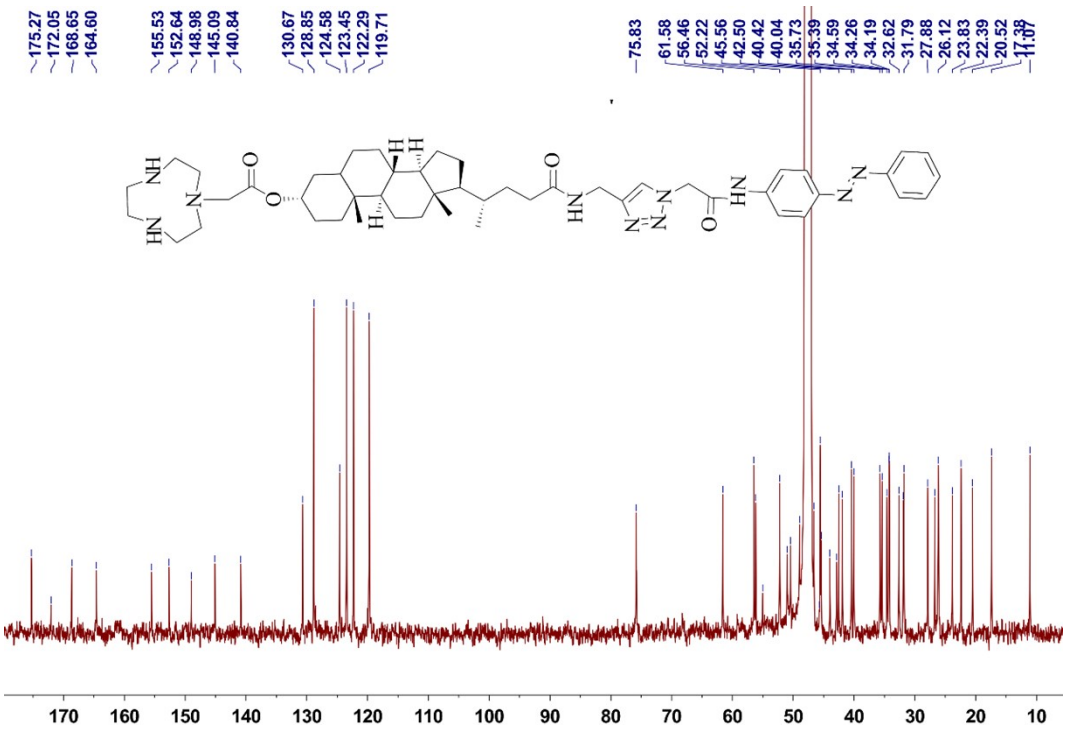


Figure S23. ^{13}C NMR spectrum of ***t*Azo-LA-TACN** in MeOD

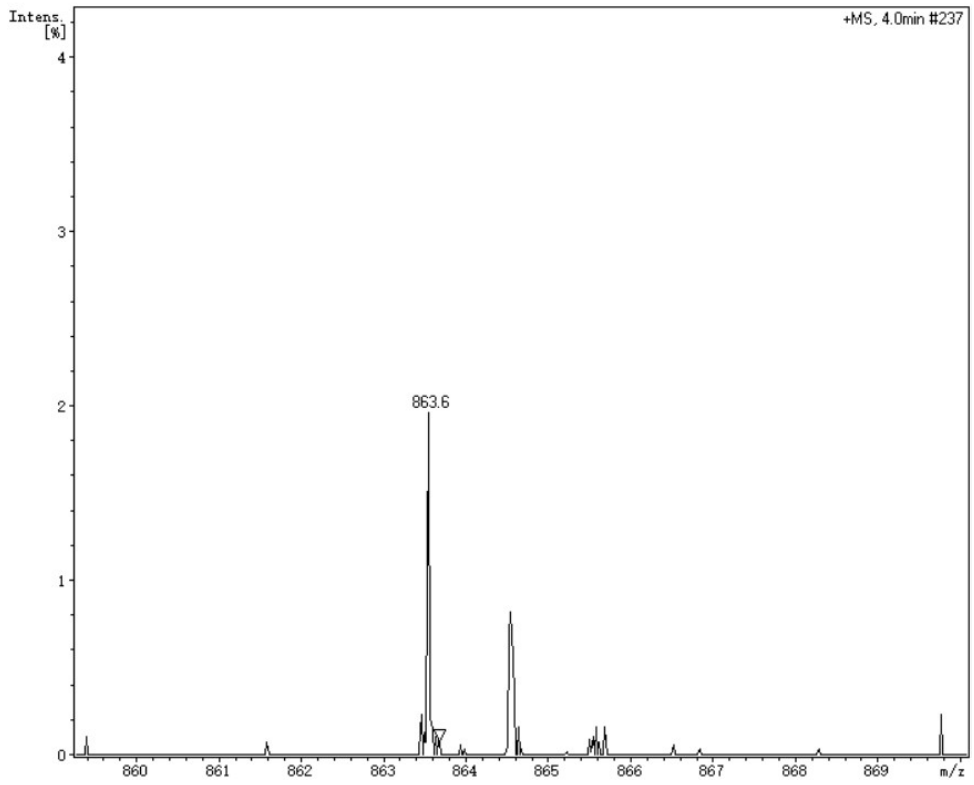


Figure S24. HR-MS (ESI) spectrum of ***t*Azo-LA-TACN** in MeCN

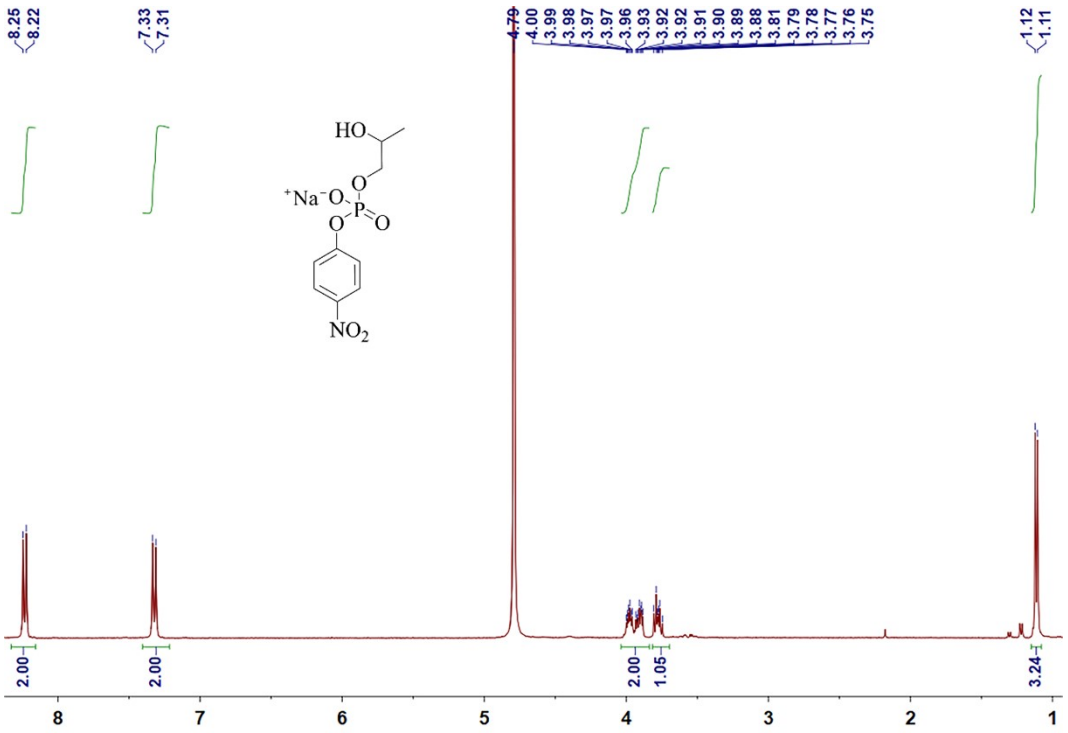


Figure S25. ^{1}H NMR spectrum of **HPNP** in D_2O

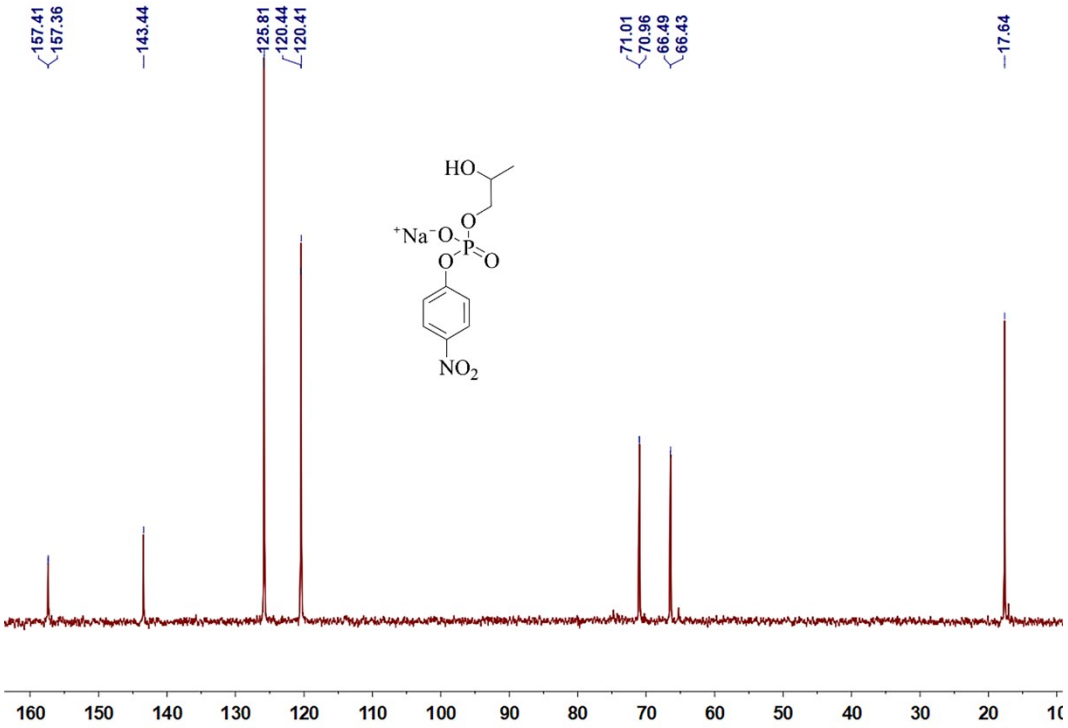


Figure S26. ^{13}C NMR spectrum of **HPNP** in D_2O

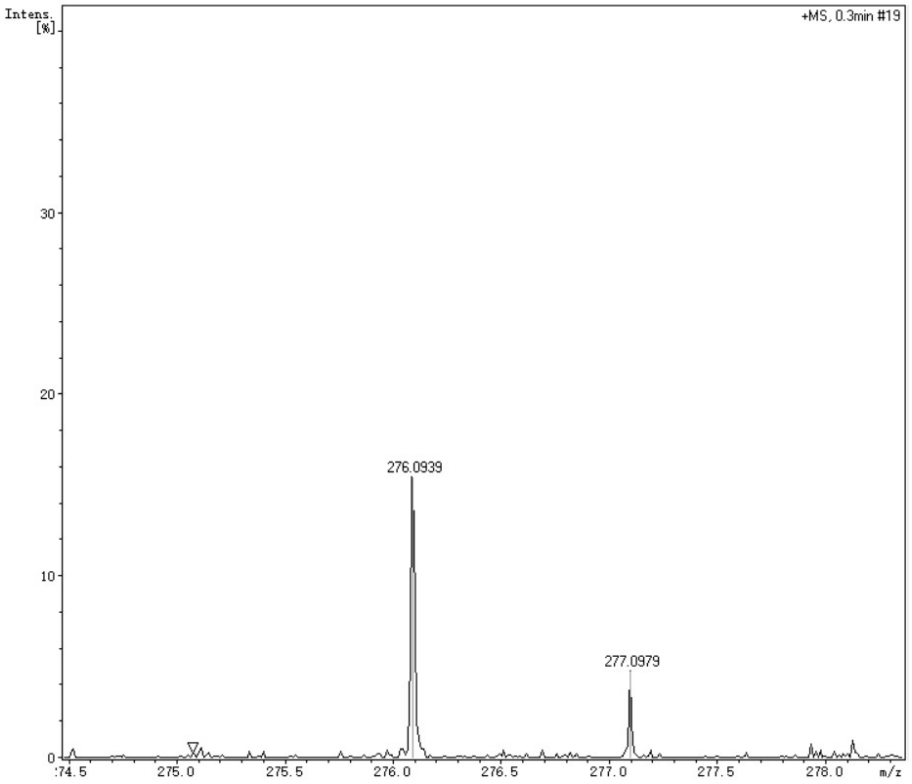


Figure S27. HR-MS (ESI) spectrum of **HPNP** in H_2O

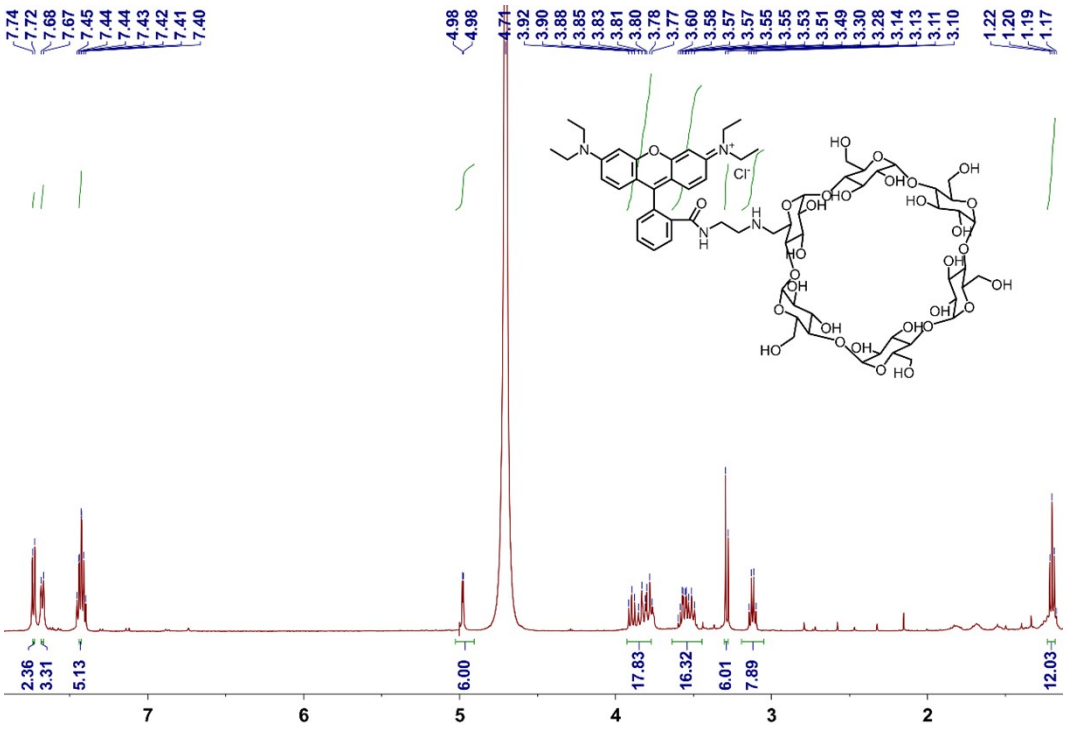


Figure S28. ^{1}H NMR spectrum of **α-CD-RhB** in D_2O

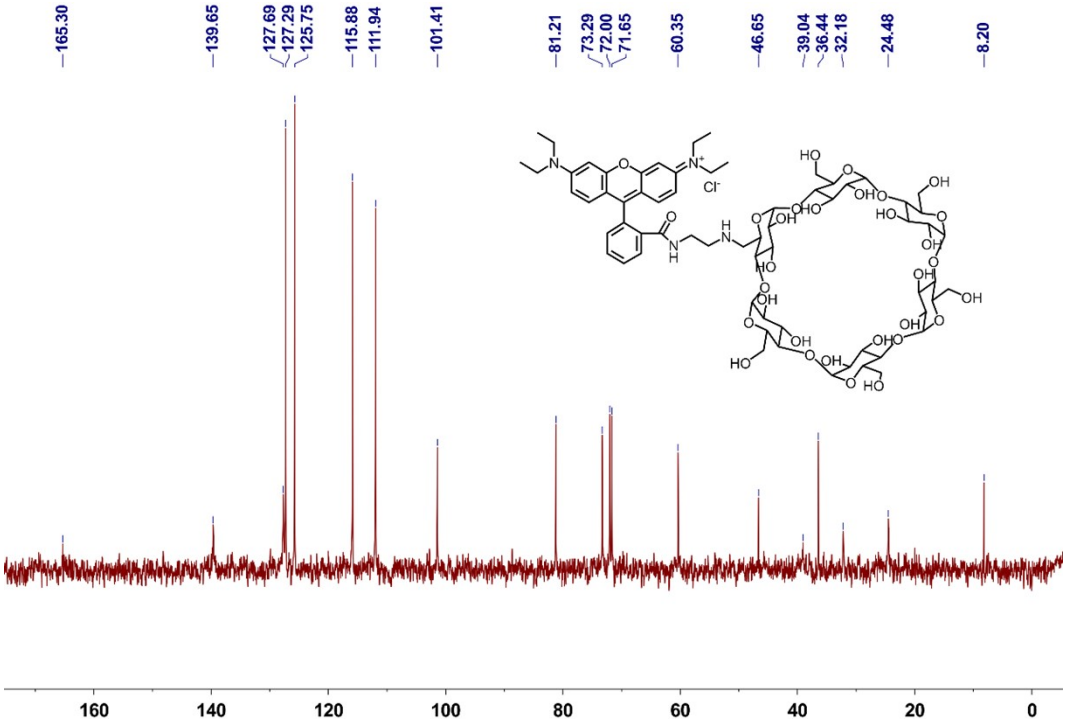


Figure S29. ^{13}C NMR spectrum of **α-CD-RhB** in D_2O

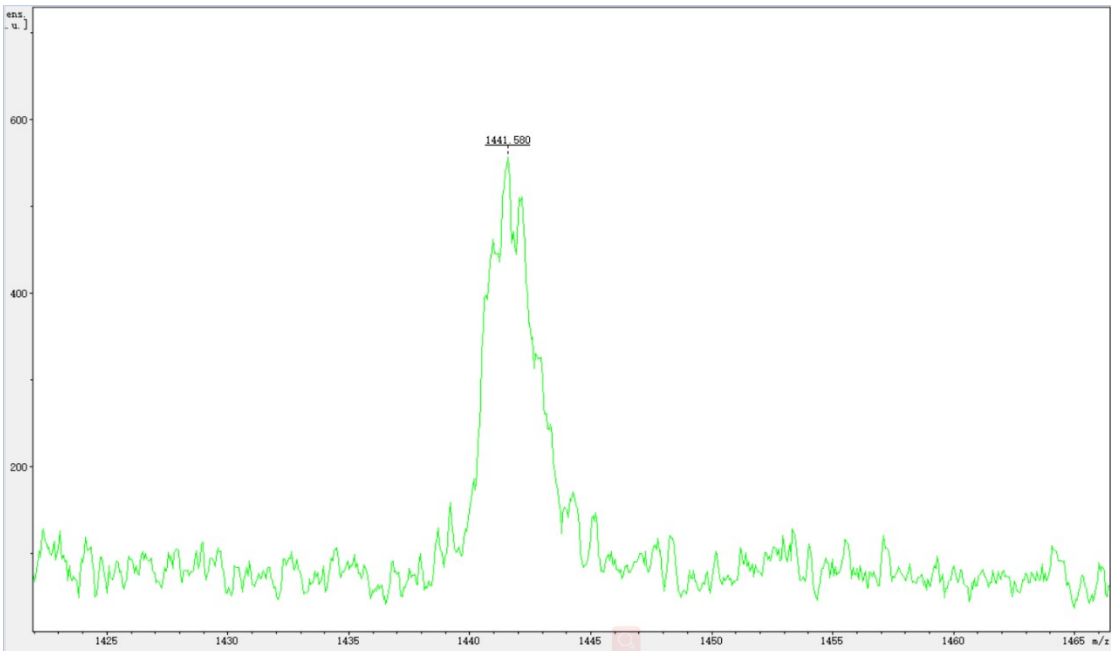


Figure S30. MALDI-TOF spectrum of **α-CD-RhB** in H_2O

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