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# Supporting Information

# Supramolecularly Regulated Artificial Transmembrane Signal Transduction for 'ON/OFF'-Switchable Enzyme Catalysis

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## Contents

## 1. General information

All the reagents and solvents were procured from Energy Chemical and Aladdin. 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. $^{1}$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). Dynamic light scattering (DLS) experiments were performed on DLS Zetasizer Nano Series from Malvern Company. IR spectra were reported on Bruker FTIR VERTEX 80V. UV-Visible spectra were obtained on UV-Vis Absorption Spectrometry UV-2450 from Shimadzu Company.

## 2. Fluorescence experiments

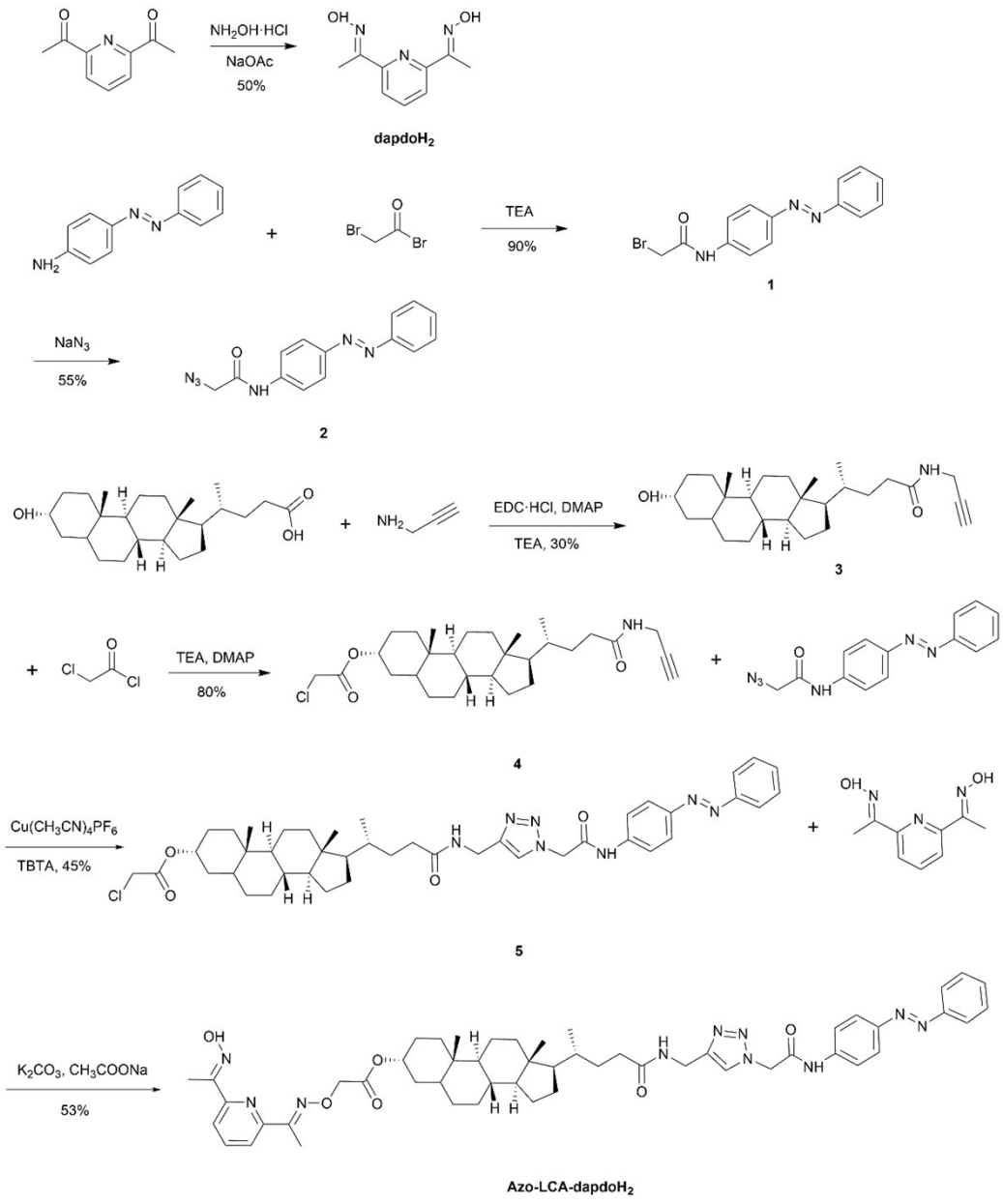
Fluorescence kinetics experiments were recorded according to the following parameters: excitation wavelength = 415 nm, emission wavelength = 510 nm, record for 8000s. Fluorescence intensity after addition of Triton X-100 and 1 M NaOH at t = 8000s was set to 1.0. Fluorescence spectrum experiments were recorded using the following parameters: emission wavelength = 510 nm, excitation range 390-450 nm. The emission and excitation slits were set at 5 nm for all LUVs experiments.

## 3. Preparation procedure of lipid vesicles containing Azo-LCA-dapdo$H_2$/β-CD

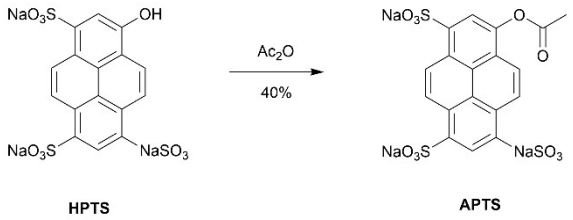
To a 10 mL glass sample bottle containing DOPC/DOPE lipids (1,2-Dioleoyl-sn-glycero-3-phosphocholine/1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine, in a 3:2 molar ratio) in dry chloroform, a chloroform solution of Azo-LCA-dapdo$H_2$ and a water of β-CD was added leading to 0.5 mol% loading (final concentrations of 1 mM lipids, and 5.0 μM Azo-LCA-dapdo$H_2$/β-CD). The solvent was removed completely under high vacuum for 2 h to obtain a thin lipid film. To the bottle was added a solution of

100 mM HEPES buffer at PH=7.0 containing 0.25 mM APTS and $ZnCl_2$ to hydrate the lipid. The mixture solution was sonicated for 1 min. The suspension was subjected to 5 times freeze-thaw cycles using liquid nitrogen and a thermostatic water bath at 35°C followed by extrusion for 19 times with 200 nm polycarbonate membrane, and were purified by Sephadex G-50 using 100 mM HEPES buffer at PH=7.0 to remove the APTS and $ZnCl_2$ outside the vesicles.

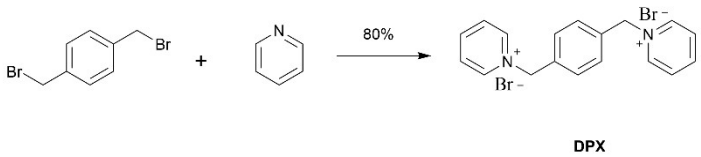
## 4. Synthetic procedures




**Scheme S1. Synthetic routes of Azo-LCA-dapdo$H_2$**




**Scheme S2. Synthetic routes of 8-acetoxypyrene-1,3,6-trisulfonatetrisodium salt (APTS)**




**Scheme S3. Synthetic routes of *p*-xylene-bis-pyridinium bromide (DPX)**


**Scheme S4. Synthetic routes of [$ZnCl_2$dapdo$H_2$]**

Synthesis of compound **1**

4-aminoazobenzene (440 mg, 2.26 mmol) was dissolved to a solution of dry TEA (0.38 mL) and dry THF (50 mL). At 0 ºC, bromoacetyl bromide (0.48 mL, 5.56 mmol) in dry THF (10 mL) was added dropwise via a dropping funnel. The solvents were removed in vacuo after the reaction mixture was stirred under $N_2$ at rt for 24 h. The obtained crude was extracted with DCM (100 mL) and water (50 mL). The organic phase was collected and dried over $Na_2SO_4$. The solvents were evaporated in vacuo. The resulting crude was further purified using column chromatography to obtain a yellowish red solid **1** (647 mg, 90%). $^1$H NMR (500 MHz, $CDCl_3$) δ = 8.31 (s, 1H), 7.95 (d, *J* = 8.8 Hz, 2H), 7.91 (d, *J* = 7.5 Hz, 2H), 7.72 (d, *J* = 8.7 Hz, 2H), 7.52 (t, *J* = 7.4 Hz, 2H), 7.49 –

7.42 (m, 1H), 4.06 (s, 2H). $^{13}$C NMR (125 MHz, $CDCl_3$) δ = 163.42, 152.64, 149.61, 139.31, 130.97, 129.11, 128.93, 124.02, 122.83, 122.13, 120.23, 120.02, 119.75, 29.44. HRMS (ESI): *m/z* calcd for $C_{14}H_{12}BrN_3OH^+$: 318.0242, Found: 318.0142.

Synthesis of compound **2**

To a solution of compound **1** (333.5 mg, 1.05 mmol) in DMF (10 mL), sodium azide (205 mg, 3.15 mmol) was added. The reaction mixture was allowed to stirred at 65 ºC for 24 h. The crude product was obtained via removing the solvent, which was further purified by extraction with DCM (80 mL) and water (20 mL). The final compound was obtained using column chromatography as a yellow solid **2** (162 mg, 55%). $^1$H NMR (400 MHz, $CDCl_3$) δ = 8.21 (s, 1H), 7.95 (d, *J* = 8.8 Hz, 2H), 7.93 – 7.88 (m, 2H), 7.73 (d, *J* = 8.8 Hz, 2H), 7.52 (dd, *J* = 9.7, 4.7 Hz, 2H), 7.49 – 7.43 (m, 1H), 4.20 (s, 2H). $^{13}$C NMR (125 MHz, $CDCl_3$) δ = 164.72, 152.64, 149.50, 139.22, 130.95, 129.11, 128.95, 124.04, 122.82, 122.18, 120.21, 119.97, 53.00. HRMS (ESI): *m/z* calcd for $C_{14}H_{12}N_6OH^+$: 281.1151, Found: 281.1215.

Synthesis of compound **3**

2-Propynylamine (440 mg, 7.94 mmol) was added to the mixture of lithocholic acid (2.00 g, 5.30 mmol), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC·HCl, 1.52 g, 7.96 mmol), dry triethylamine (TEA, 3.6 mL, 26.6 mmol), 4-dimethylaminopyridine (4 mg, 0.02 mmol) and dry DCM (300 mL). The resulting solution was washed with 1 M HCl (2 × 40 mL) and extracted with water (2 × 50 mL) after stirred at rt for 24 h. The organic phase was dried with $Na_2SO_4$ and the solvents were evaporated in vacuo to give crude. The white solid product **3** was obtained using column chromatography (615 mg, 30%). $^1$H NMR (500 MHz, $CDCl_3$) δ = 5.58 (s, 1H), 4.05 (dd, *J* = 5.2, 2.6 Hz, 2H), 3.62 (s, 1H), 2.25 (ddd, *J* = 16.6, 7.9, 3.8 Hz, 2H), 2.12 – 2.04 (m, 1H), 2.01 – 0.85 (m, 33H), 0.64 (s, 3H). $^{13}$C NMR (125 MHz, $CDCl_3$) δ = 173.17, 79.71, 71.87, 71.54, 56.50, 55.98, 42.75, 42.10, 40.44, 40.19, 36.46, 35.85, 35.45, 35.35, 34.58, 33.30, 31.57, 30.55, 29.18, 28.25, 27.19, 26.42, 24.21, 23.38, 20.83, 18.39, 12.06. HRMS (ESI): *m/z* calcd for $C_{27}H_{43}NO_2H^+$: 414.3372, Found:

414.3458.

Synthesis of compound **4**

The mixture of compound **3** (330 mg, 0.484 mmol), 4-dimethylaminopyridine (2.0 mg, 0.02 mmol) and dry TEA (70 mg, 0.0580 mmol) was dissolved in dry DCM (60 mL). At 0°C, chloroacetylchloride (58 mg, 0.58 mmol) in dry DCM (10 mL) was added into the mixture dropwise via a dropping funnel. The reaction was recovered to rt and stirred for 24 h. The mixture was extracted with water (50 mL) and dried over $Na_2SO_4$ followed removal of the organic solvents. The crude was purified using column chromatography to obtain a colorless oil liquid **4** (195 mg, 80%). $^1$H NMR (500 MHz, $CDCl_3$) δ = 5.78 (s, 1H), 4.89 – 4.72 (m, 1H), 4.03 (dt, *J* = 4.8, 3.4 Hz, 2H), 4.01 (s, 2H), 2.32 – 2.15 (m, 2H), 2.13 – 1.99 (m, 1H), 1.98 – 0.81 (m, 32H), 0.62 (s, 3H). $^{13}$C NMR (125 MHz, $CDCl_3$) δ = 173.27, 166.83, 79.83, 76.69, 71.36, 56.45, 56.05, 42.73, 41.88, 41.24, 40.42, 40.11, 35.76, 35.46, 34.92, 34.55, 33.26, 32.02, 31.58, 29.11, 28.22, 26.97, 26.45, 26.28, 24.17, 23.28, 20.84, 18.39, 12.05. HRMS (ESI): *m/z* calcd for $C_{29}H_{44}NO_3H^+$: 490.3088, Found: 490.3079

Synthesis of compound **5**

A mixture of compound **2** (50 mg, 0.180 mmol), compound **4** (88 mg, 0.180 mmol), TBTA (16 mg, 0.03 mmol) and $Cu(CH_3CN)_4PF_6$ (11 mg, 0.03 mmol) were added to dry DCM (30 mL). The reaction mixture was stirred under $N_2$ at rt for 24 h, and then washed with basic EDTA solution (0.1 M, 2 × 10 mL), water (2 × 20 mL), dried over $Na_2SO_4$ and filtered. The resultant filtrates were evaporated in vacuo. The residue was further purified using column chromatography to give a yellow solid **5** (62 mg, 45%). $^1$H NMR (500 MHz, $CDCl_3$) δ = 8.69 (s, 1H), 7.93 (dd, *J* = 12.0, 8.1 Hz, 4H), 7.82 (s, 1H), 7.70 (d, *J* = 8.7 Hz, 2H), 7.51 (dt, *J* = 21.8, 7.0 Hz, 3H), 6.27 (t, *J* = 5.8 Hz, 1H), 5.21 (s, 2H), 4.89 – 4.76 (m, 1H), 4.57 (dd, *J* = 14.8, 5.9 Hz, 2H), 4.05 (s, 2H), 2.35 – 2.23 (m, 2H), 2.17 – 2.10 (m, 1H), 1.85 – 1.30 (m, 25H), 0.95 – 0.90 (m, 6H), 0.63 (s, 3H). $^{13}$C NMR (125 MHz, $CDCl_3$) δ = 174.14, 166.84, 163.16, 152.60, 149.50, 145.58, 139.41, 130.97, 129.73, 129.10, 128.91, 124.29, 123.98, 122.81, 122.07, 120.37,

120.08, 56.42, 56.01, 53.53, 42.74, 41.88, 41.25, 40.41, 40.09, 35.76, 35.50, 35.03, 34.92, 34.56, 33.50, 32.03, 31.70, 29.70, 28.26, 26.96, 26.46, 26.28, 24.15, 23.27, 20.83, 18.37, 12.04. HRMS (ESI): *m/z* calcd for $C_{43}H_{56}ClN_7O_4$ $H^+$: 770.4161, Found: 770.4059.

Synthesis of **dapdoH$_2$**

To a solution of hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$, 257 mg, 3.68 mmol) and sodium acetate (36.72 mg, 0.459 mmol) in water (20 mL) was added 2,6-diacetylpyridine (600 mg, 3.68 mmol). The obtained reaction was refluxed for 1.5 h before stirred at rt for 24 h. The white precipitate was collected by filtration and washed with water. The white solid product **dapdoH$_2$** was obtained using column chromatography (326 mg, 50%). $^1$H NMR (500 MHz, DMSO-*d6*) δ = 11.52 (s, 2H), 7.84 – 7.74 (m, 3H), 2.26 (s, 6H). $^{13}$C NMR (125 MHz, DMSO-*d6*) δ = 154.76, 153.91, 137.22, 119.59, 10.59. HRMS (ESI): *m/z* calcd for $C_9H_{11}N_3O_2H^+$: 194.0930, Found: 194.0835.

Synthesis of **Azo-LCA-dapdoH$_2$**

At 0 ºC, **dapdoH$_2$** (13 mg, 0.050 mmol) and potassium carbonate (20 mg, 0.150 mmol) were added to DMF (6 mL). The reaction mixture was warmed to rt and stirred for 1 h, before a solution of compound **5** (40 mg, 0.050 mmol) in DMF (6 mL) was added dropwise. The final mixture was stirred under $N_2$ at rt for 24 h. After removal of the solvents, the residues was added to DCM (50 mL), washed with water (2 × 10 mL), dried over $Na_2SO_4$ and filtered. The resultant filtrates were evaporated in vacuo. The residue was purified using column chromatography to gain a yellow solid **Azo-LCA-dapdoH$_2$** (24 mg, 53%). $^1$H NMR (500 MHz, $CDCl_3$) δ = 9.76 (s, 1H), 7.93 – 7.80 (m, 5H), 7.79 (d, *J* = 7.8 Hz, 1H), 7.66 (d, *J* = 8.7 Hz, 2H), 7.56 (t, *J* = 7.8 Hz, 1H), 7.51 – 7.37 (m, 3H), 7.31 (dd, *J* = 5.7, 4.5 Hz, 1H), 5.47 (s, 1H), 5.16 (d, *J* = 16.5 Hz, 2H), 4.86 – 4.77 (m, 1H), 4.77 – 4.69 (m, 2H), 4.62 – 4.43 (m, 2H), 3.78 – 3.70 (m, 1H), 2.40 (d, *J* = 12.9 Hz, 3H), 2.37 (d, *J* = 8.4 Hz, 3H), 2.26 (ddd, *J* = 29.6, 14.9, 9.4 Hz, 2H), 2.10 – 2.03 (m, 1H), 1.50 – 0.84 (m, 31H), 0.60 – 0.44 (m, 3H). $^{13}$C NMR (125

MHz, $CDCl_3$) δ = 174.98, 169.93, 163.43, 157.63, 156.81, 153.51, 152.72, 152.58, 149.39, 145.27, 139.65, 136.29, 130.94, 129.08, 128.91, 128.70, 128.00, 124.27, 123.95, 123.85, 122.80, 120.21, 120.08, 119.90, 75.15, 71.28, 56.42, 55.47, 53.34, 42.54, 41.57, 40.37, 40.09, 35.53, 35.24, 34.92, 34.38, 32.89, 31.93, 31.58, 29.71, 28.18, 26.81, 26.32, 26.11, 24.07, 23.22, 20.70, 18.25, 11.95, 11.23, 10.46. HRMS (ESI): *m/z* calcd for $C_{52}H_{66}N_{10}O_6$ $H^+$: 927.5245, Found: 927.5196.

Synthesis of **APTS**

A mixture of 8-hydroxypyrene-1,3,6-trisulfonatetrisodium salt (HPTS, 0.65 g, 1.25mmol), anhydrous sodium acetate (12.5 mg, 0.15 mmol) and acetic anhydride (10 mL) was refluxed for 2 days. A solution of THF including 10% (v/v) of acetic acid (10 mL) was added to the resulting reaction mixture at rt. The precipitate was filtrated and washed with cold acetone (3 × 10 mL) and diethylether (2 × 10 mL) to obtain pale brown solid product (**APTS**, 0.428 g, 40%). $^1$H NMR (500 MHz, $D_2O$) δ = 9.27 (d, *J* = 9.8 Hz, 1H), 9.10 (dd, *J* = 26.8, 9.8 Hz, 2H), 8.99 (d, *J* = 9.6 Hz, 1H), 8.34 (s, 1H), 8.12 (d, *J* = 9.6 Hz, 1H), 2.56 (s, 3H). $^{13}$C NMR (125 MHz, $D_2O$) δ = 173.15, 143.35, 137.86, 135.83, 129.22, 128.55, 127.00, 126.12, 124.98, 124.58, 124.30, 124.17, 123.47, 122.75, 119.92, 20.54.

Synthesis of **DPX**

A solution of pyridine (1.5 mL, 18.6mmol) and 1,4-bis(bromomethyl)benzene (8.4 mmol, 2.21g) in dry acetonitrile (15 mL) was heated at 65 ºC overnight. The solid was collected by filtration after the mixture was cooled to rt. The obtained solid was washed with diethyl ether (2 × 30 mL) to gain *p*-xylene-bis-pyridinium bromide (**DPX**, 2.8g, 80%).$^1$H NMR (500 MHz, $D_2O$) δ = 9.09 (d, *J* = 5.8 Hz, 4H), 8.68 (t, *J* = 7.8 Hz, 2H), 8.21 (t, *J* = 7.1 Hz, 4H), 7.73 (s, 4H), 6.02 (s, 4H). $^{13}$C NMR (125 MHz, $D_2O$) δ = 146.39, 144.60, 134.49, 130.42, 128.75, 64.00. HRMS (ESI): *m/z* calcd for $C_{18}H_{18}N_2^{2+}$: 131.0730, Found: 131.0709.

## 5. Addition data

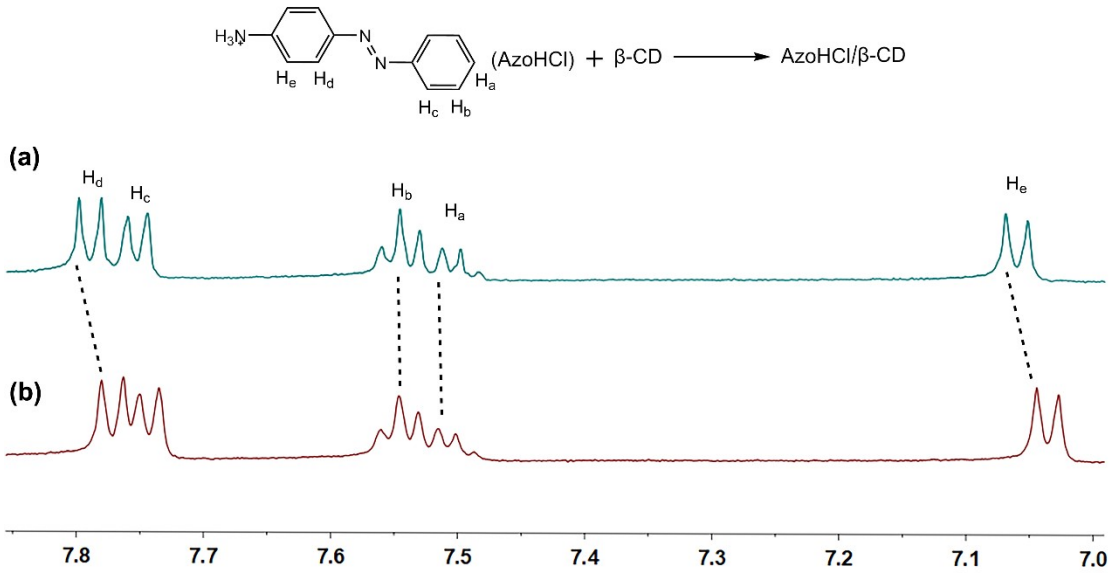



**Figure S1**. $^{1}$H NMR spectra of (a) 1.0 mM AzoHCl and (b) 1.0 mM AzoHCl/β-CD in $D_2O$ and DMSO-*d*6 (9/1, vol/vol).

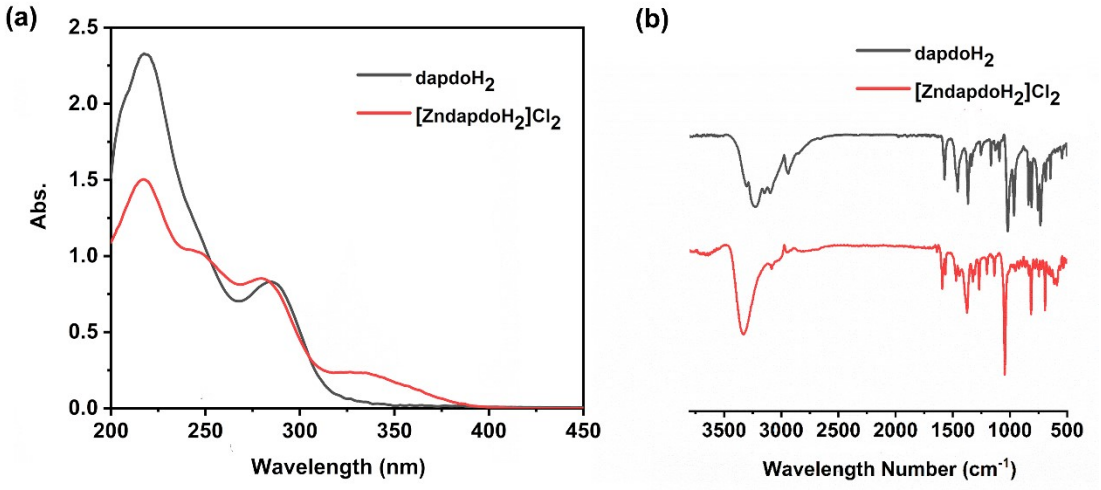



**Figure S2**. (a) UV-absorption and (b) IR spectra of dapdo$H_2$ and [Zndapdo$H_2$]$Cl_2$.

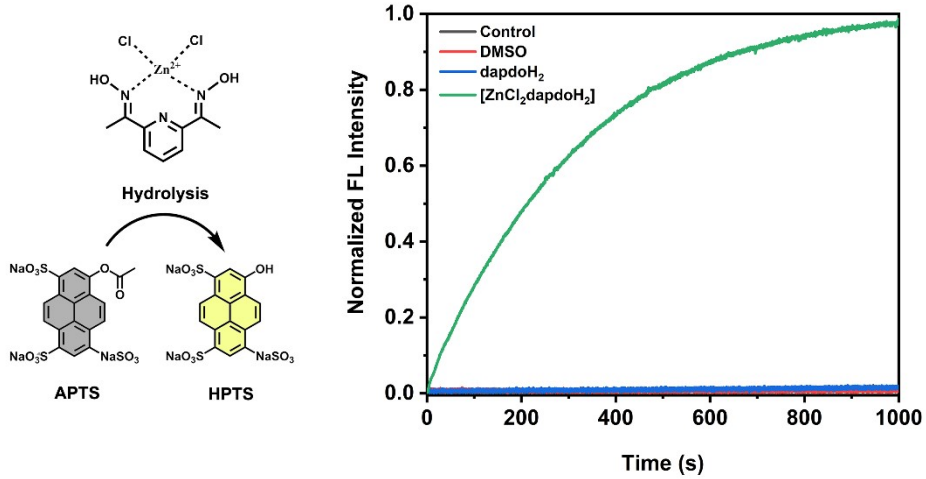



**Figure S3**. Catalytic efficacy of [ZndapdoH$_2$]Cl$_2$ in the absence of lipid vesicles. Time-dependent fluorescence spectra in buffer solution containing 0.5 μM APTS (100 mM HEPES, PH =7.0). Hydrolysis of APTS after addition of DMSO, 50 μM dapdoH$_2$, 50 μM [ZndapdoH$_2$]Cl$_2$ ($\lambda_{ex}$= 415 nm, $\lambda_{em}$=510 nm).

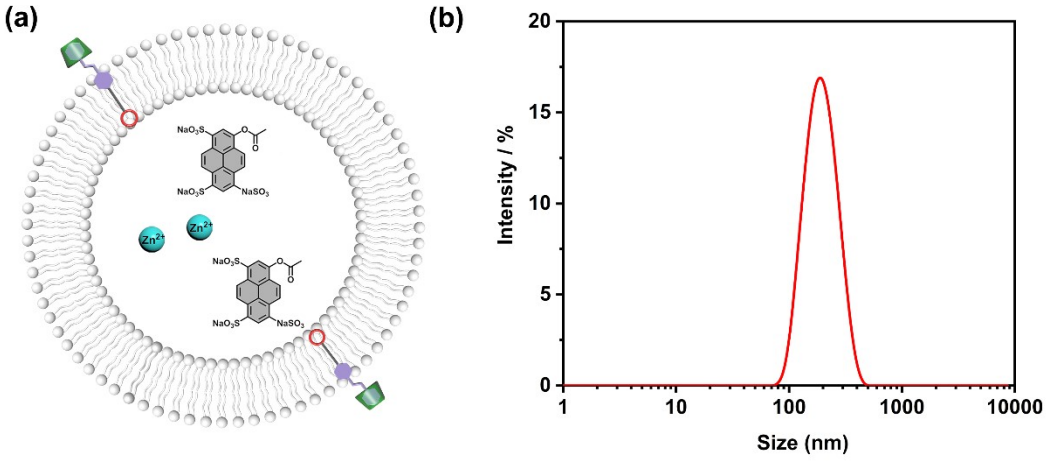



**Figure S4**. (a) Schematic model and (b) Dynamic Light Scattering (DLS) analysis of DOPC/DOPE LUVs loaded with 0.25 mM APTS, 0.25 mM ZnCl$_2$ and 5.0 μM Azo-LCA-dapdoH$_2$/β-CD.

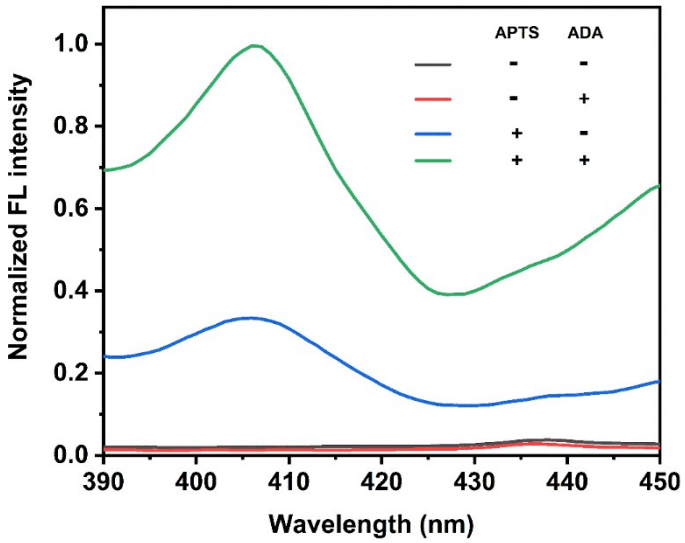



**Figure S5**. Fluorescence spectra of DOPC/DOPE LUVs (0.25 mM $ZnCl_2$, 100 mM HEPES, PH=7.0) containing 5.0 μM Azo-LCA-dapdo$H_2$/β-CD in the presence (+) and absence (-) of APTS. LUVs containing 0.25 mM APTS before (blue line) and after (8000s, green line) addition of 1.0 mM ADA. LUVs lacking 0.25 mM APTS before (black line) and after (8000s, red line) addition of 1.0 mM ADA ($\lambda_{ex}$= 415 nm, $\lambda_{em}$=510 nm).

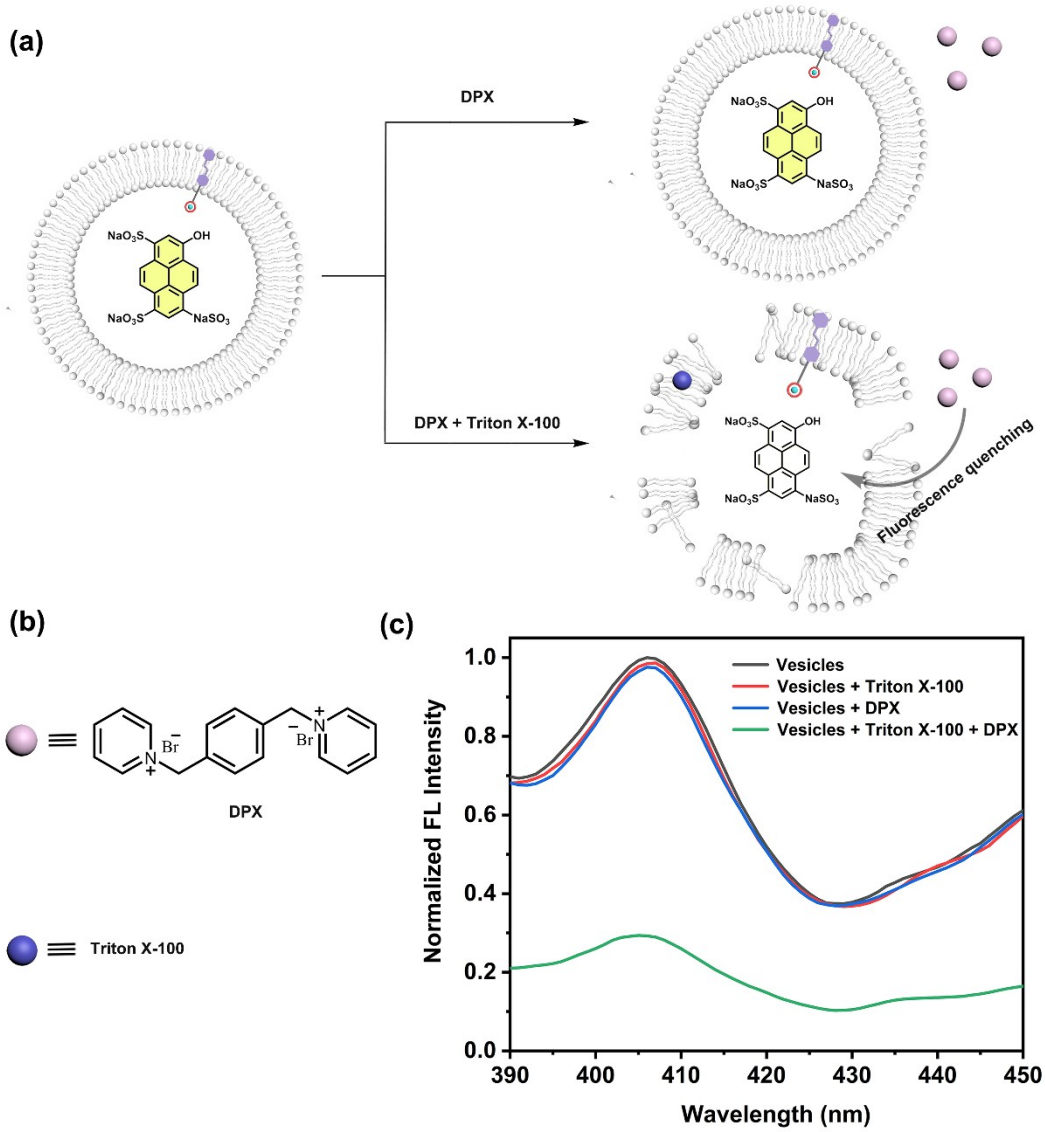



**Figure S6**. (a) Schematic model showing DPX quenching assay for vesicle integrity study. (b) Chemical structure of the quencher DPX. (c) Fluorescence spectra of DOPC/DOPE LUVs in the 'ON' state for 8000s followed by addition of 1.0 mM Triton X-100, 2.5 mM DPX, 1.0 mM Triton X-100 and 2.5 mM DPX (0.25 mM APTS, 0.25 mM $ZnCl_2$, 100 mM HEPES, PH =7.0, $\lambda_{ex}$= 415 nm, $\lambda_{em}$=510 nm).

## 6. $^{1}H$ and $^{13}C$ and ESI-MS analysis

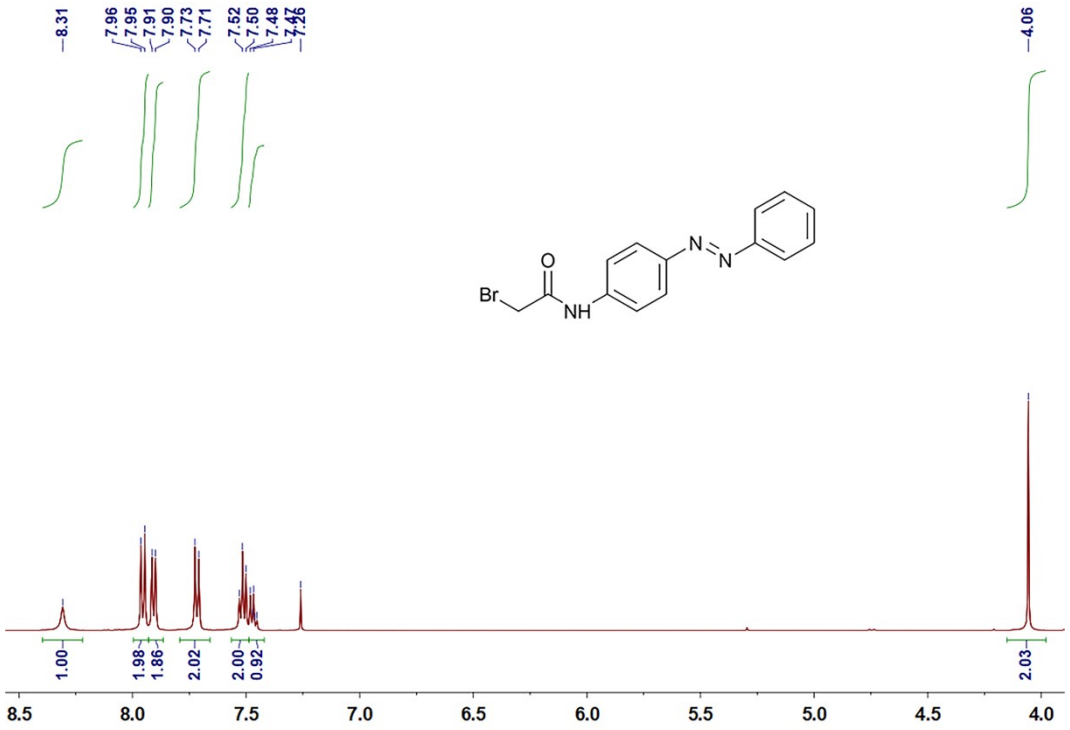



**Figure S7**. $^{1}H$ NMR spectrum of compound **1** in $CDCl_3$

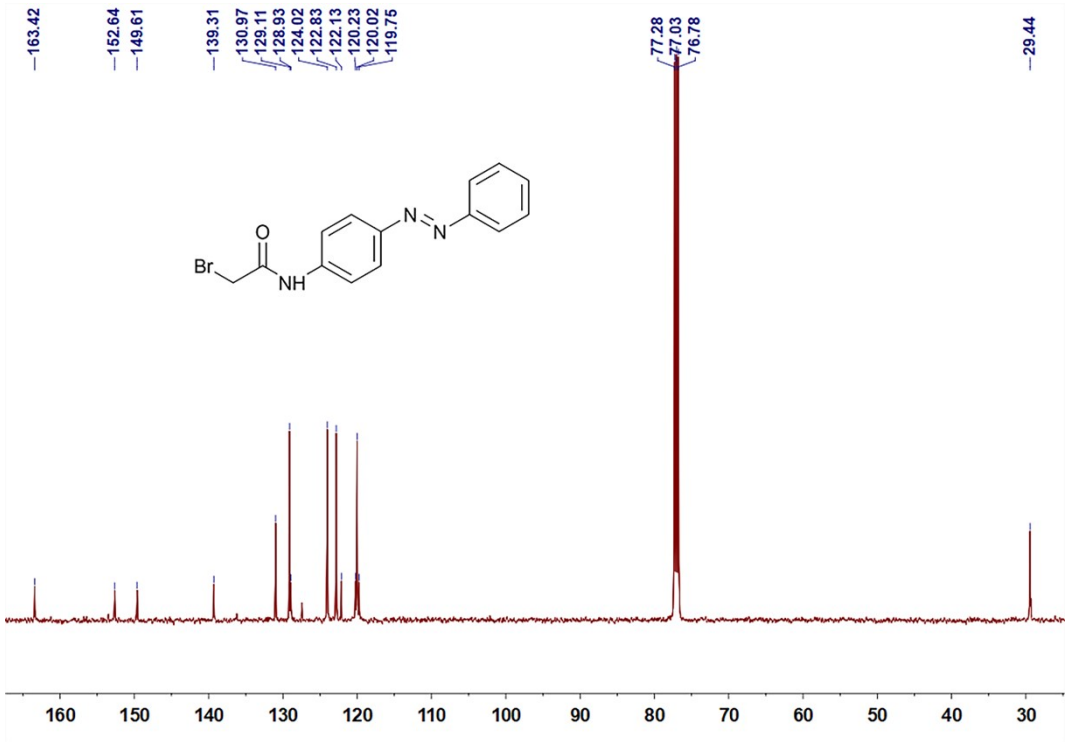



**Figure S8**. $^{13}C$ NMR spectrum of compound **1** in $CDCl_3$

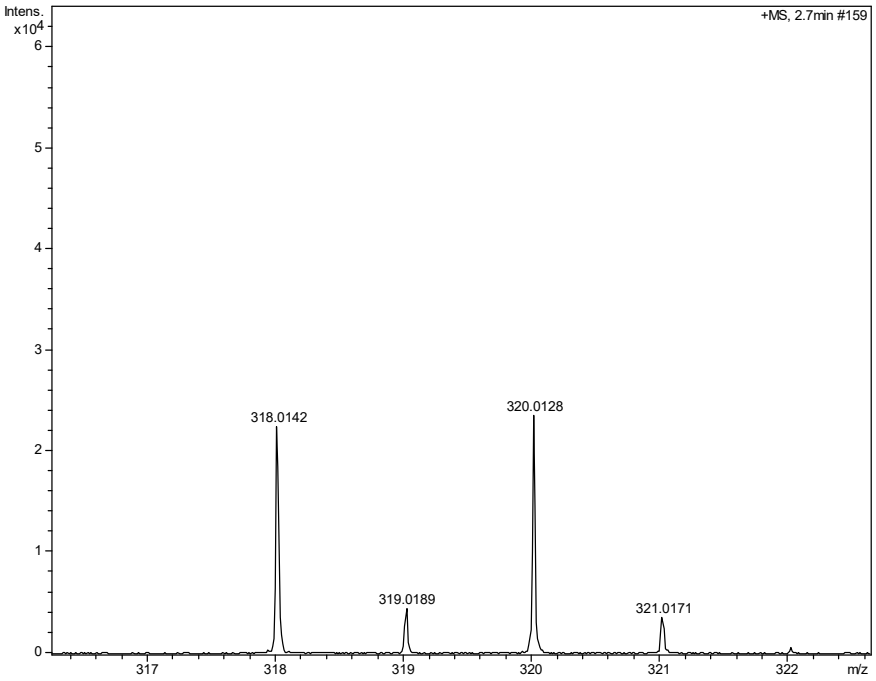



**Figure S9**. HR-MS (ESI) spectrum of compound **1** in MeCN

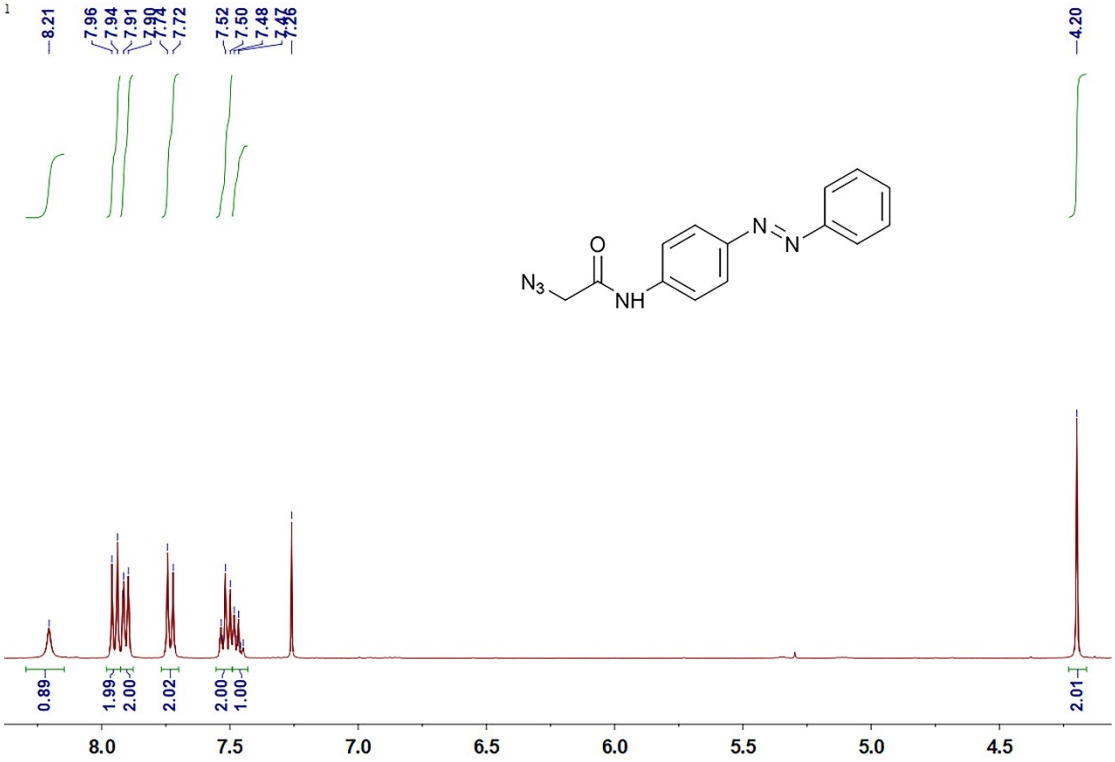



**Figure S10**. $^{1}H$ NMR spectrum of compound **2** in $CDCl_3$

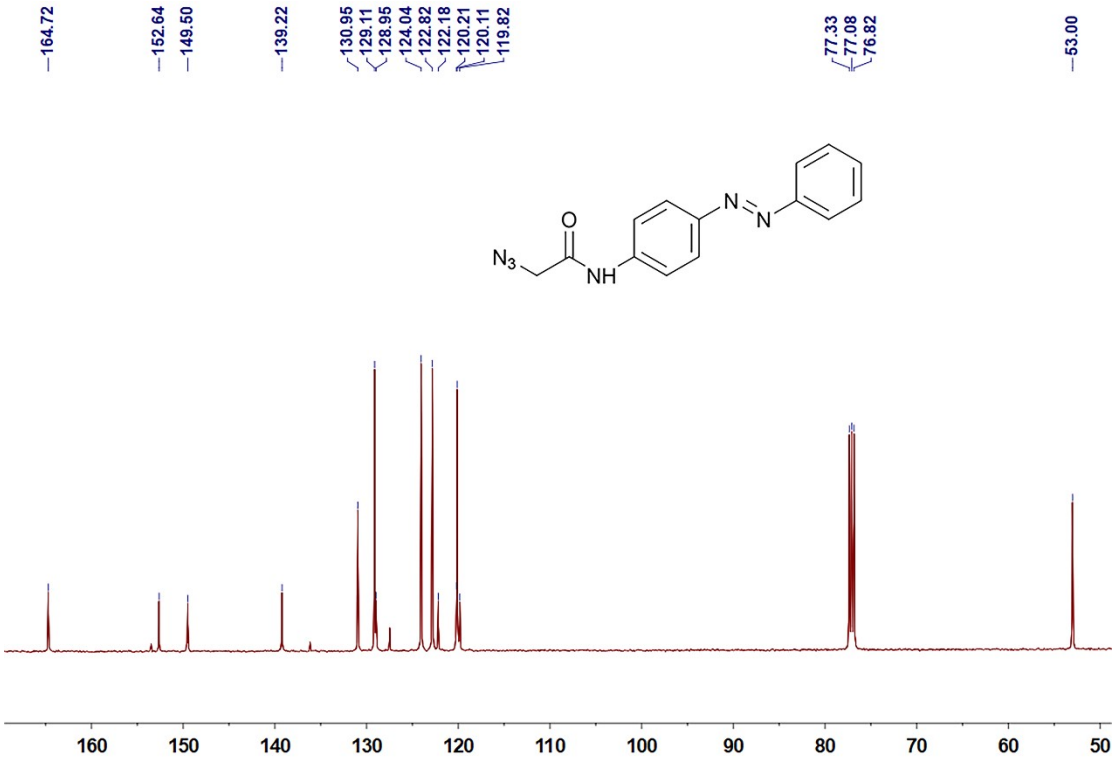



**Figure S11**. $^{13}C$ NMR spectrum of compound **2** in $CDCl_3$

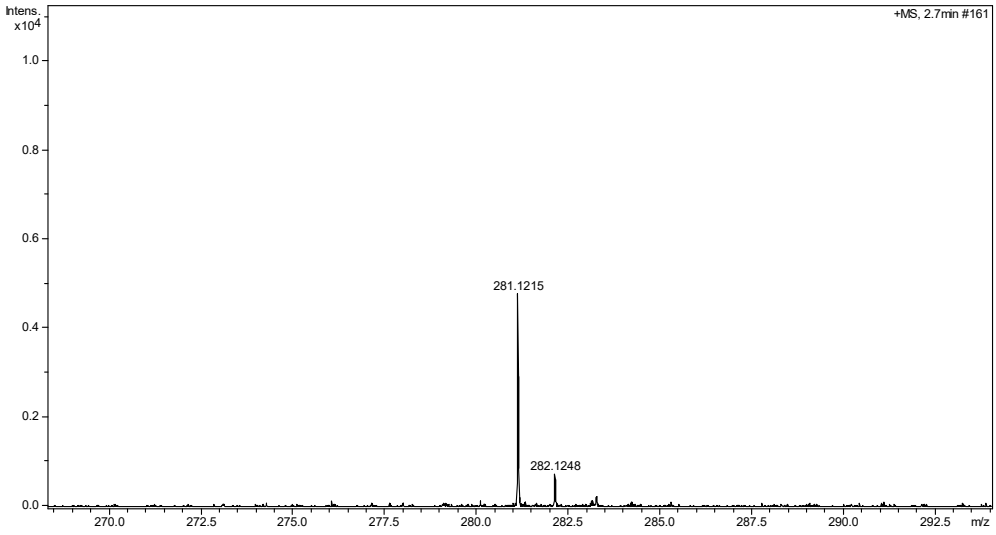



**Figure S12**. HR-MS (ESI) spectrum of compound **2** in MeCN

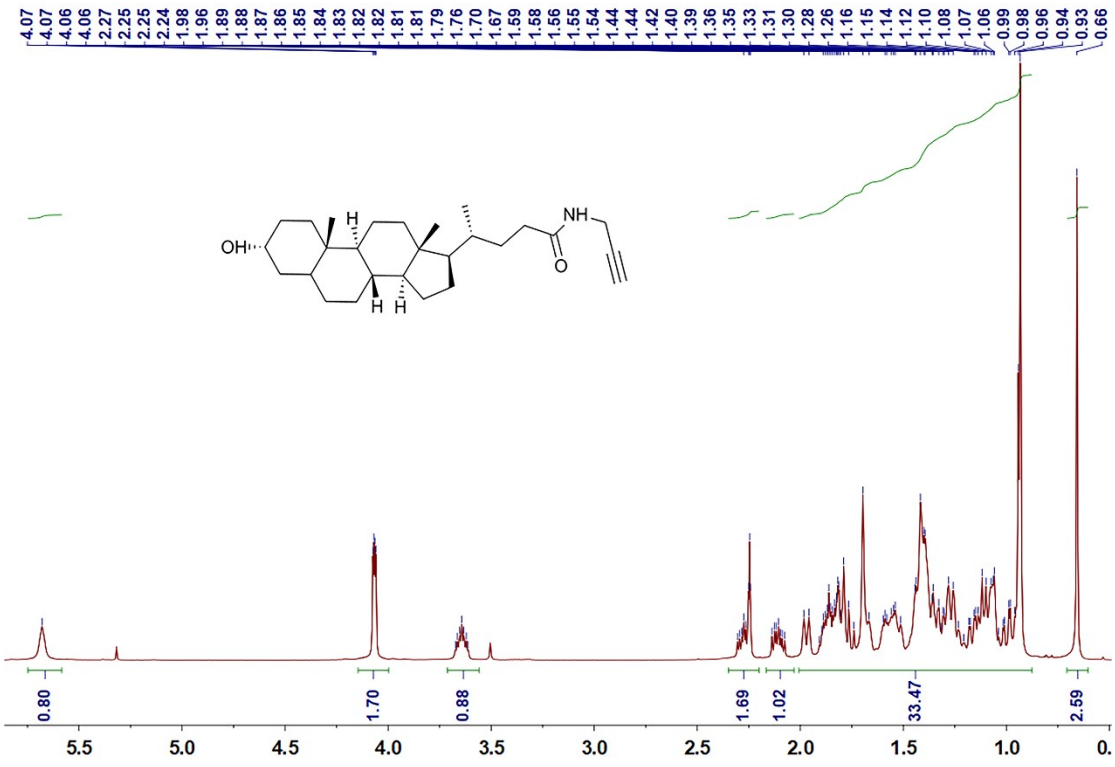



**Figure S13**. $^{1}H$ NMR spectrum of compound **3** in $CDCl_3$

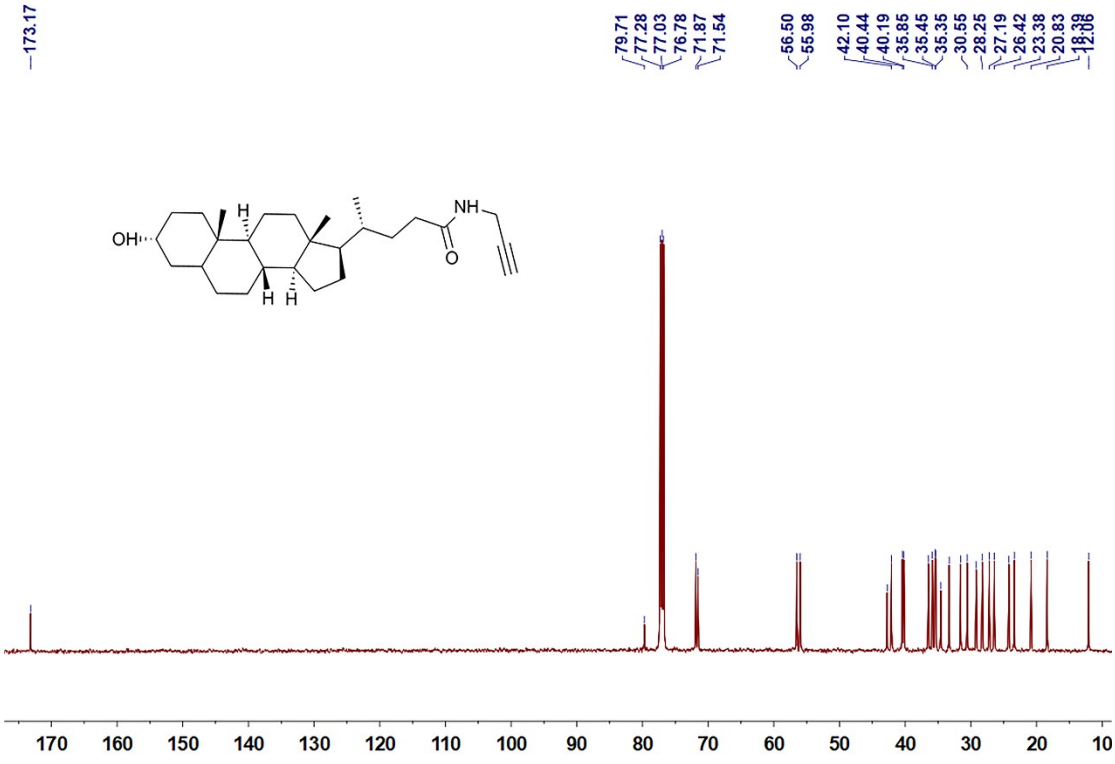



**Figure S14**. $^{13}C$ NMR spectrum of compound **3** in $CDCl_3$

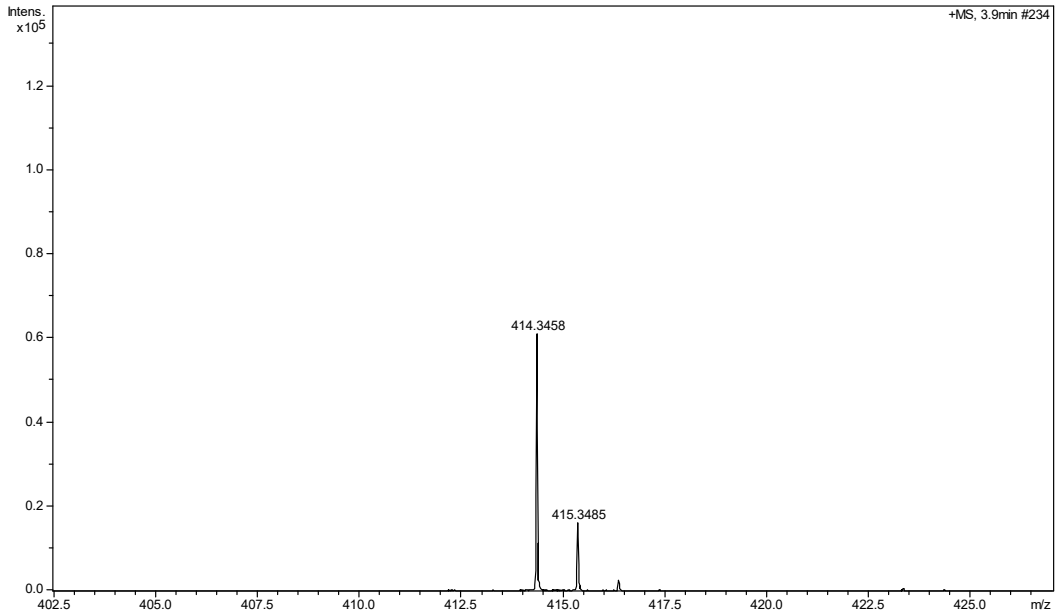



**Figure S15**. HR-MS (ESI) spectrum of compound **3** in MeCN

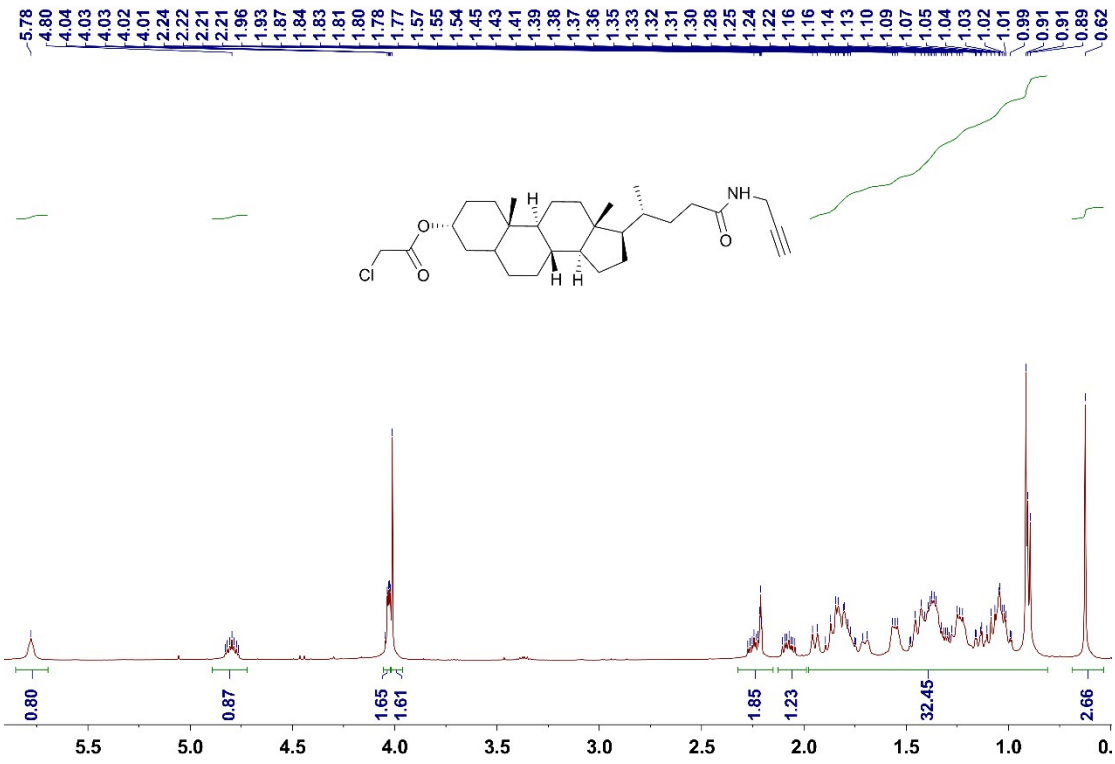



**Figure S16**. $^{1}$H NMR spectrum of compound **4** in $CDCl_3$

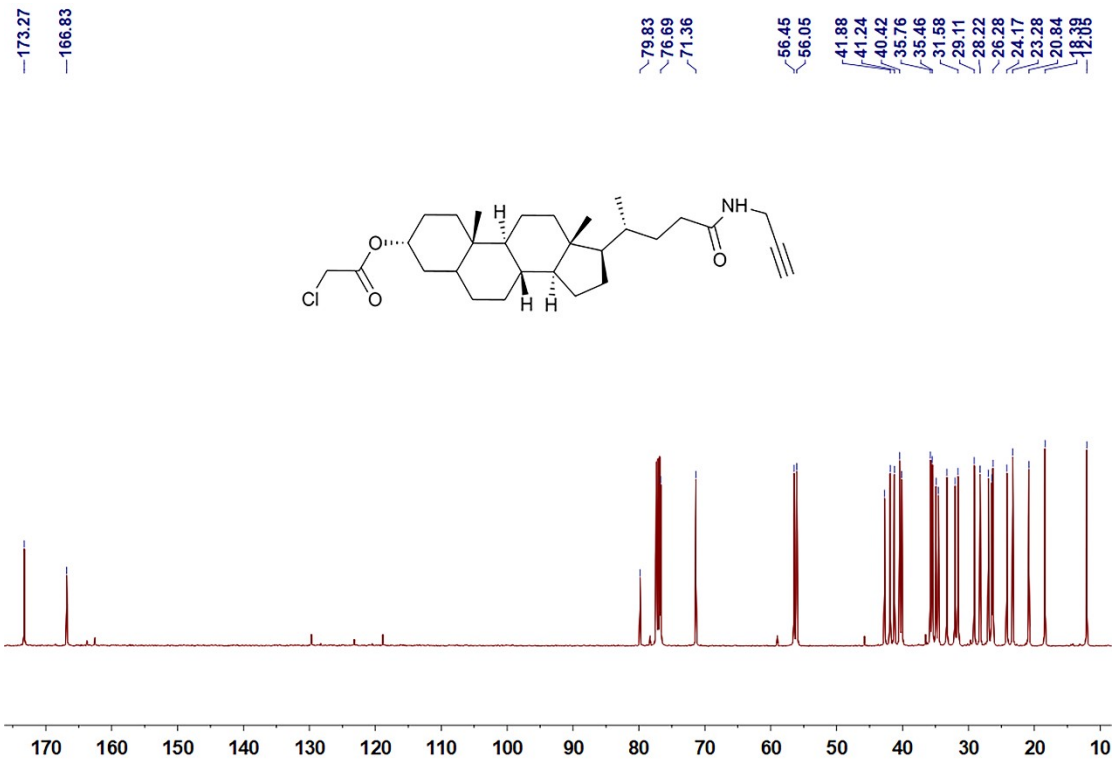



**Figure S17**. $^{13}C$ NMR spectrum of compound **4** in $CDCl_3$

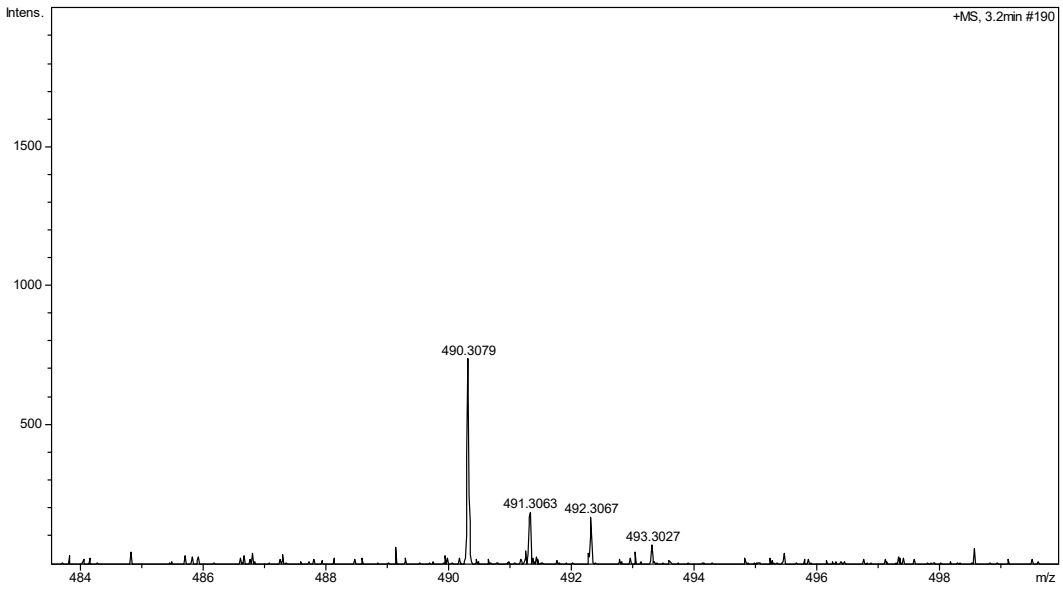



**Figure S18**. HR-MS (ESI) spectrum of compound **4** in MeCN

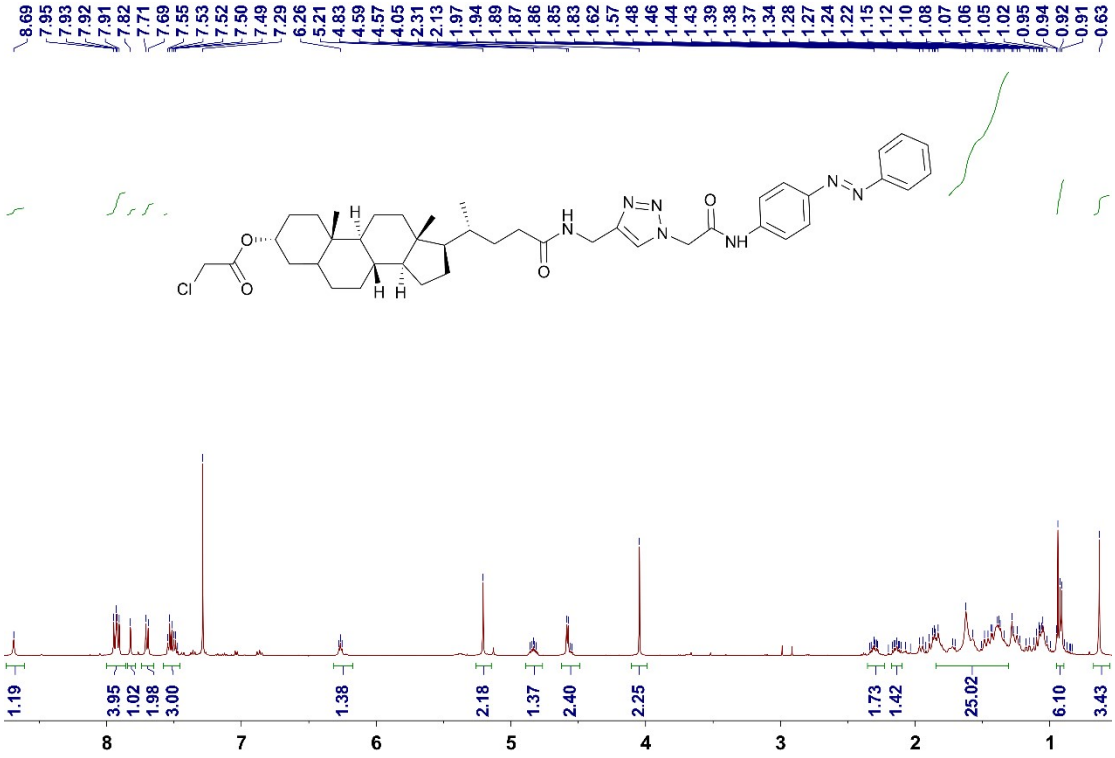


**Figure S19**. $^{1}H$ NMR spectrum of compound **5** in $CDCl_3$

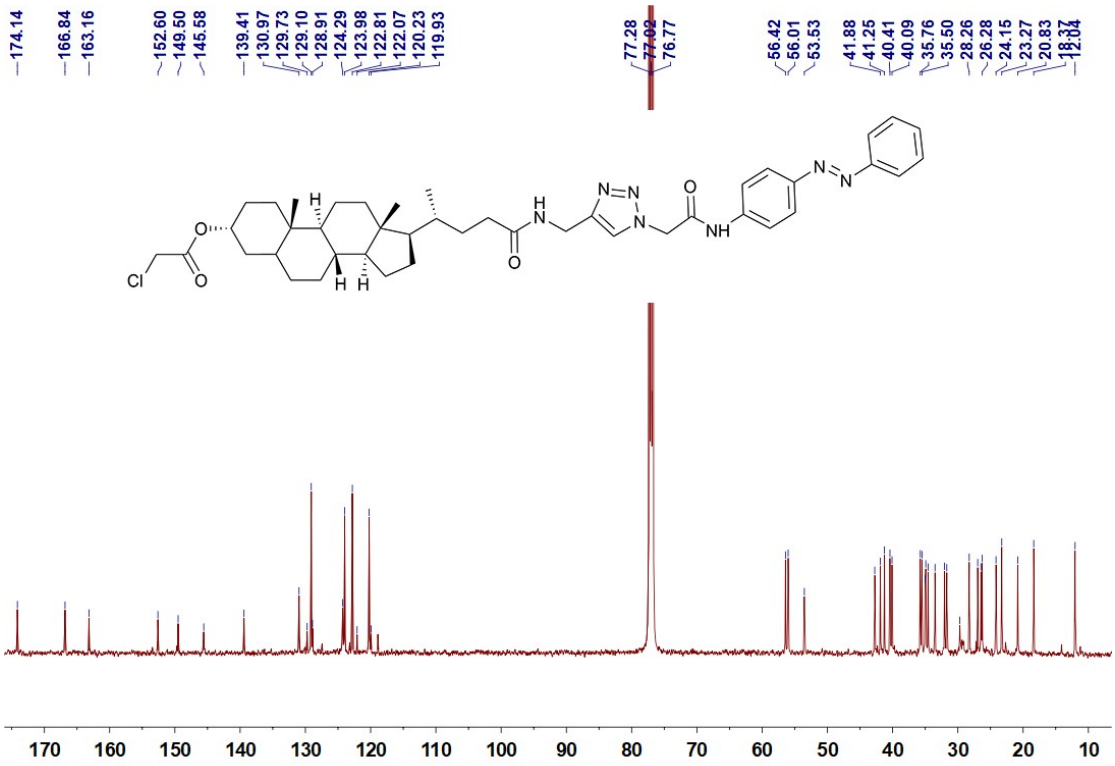


**Figure S20**. $^{13}C$ NMR spectrum of compound **5** in $CDCl_3$

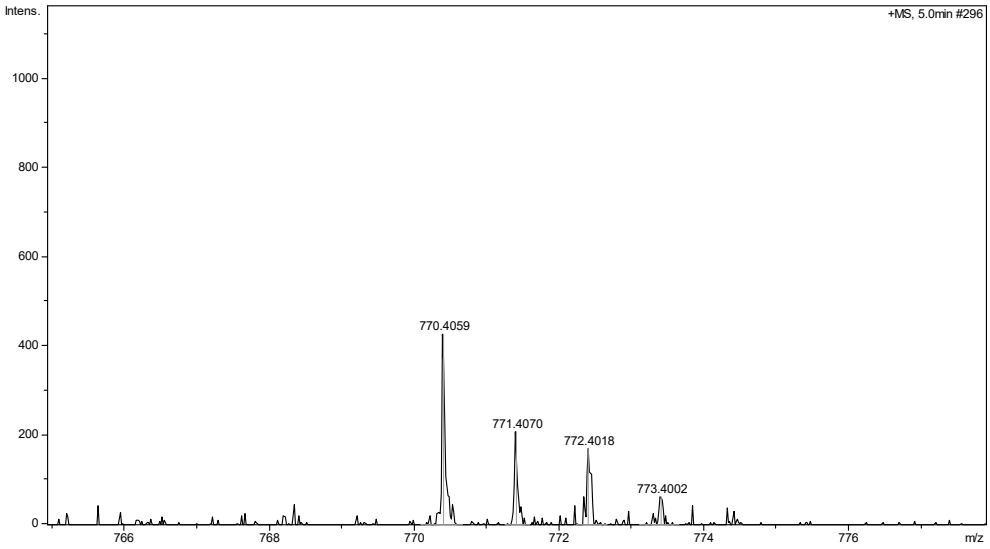



**Figure S21**. HR-MS (ESI) spectrum of compound **5** in MeCN

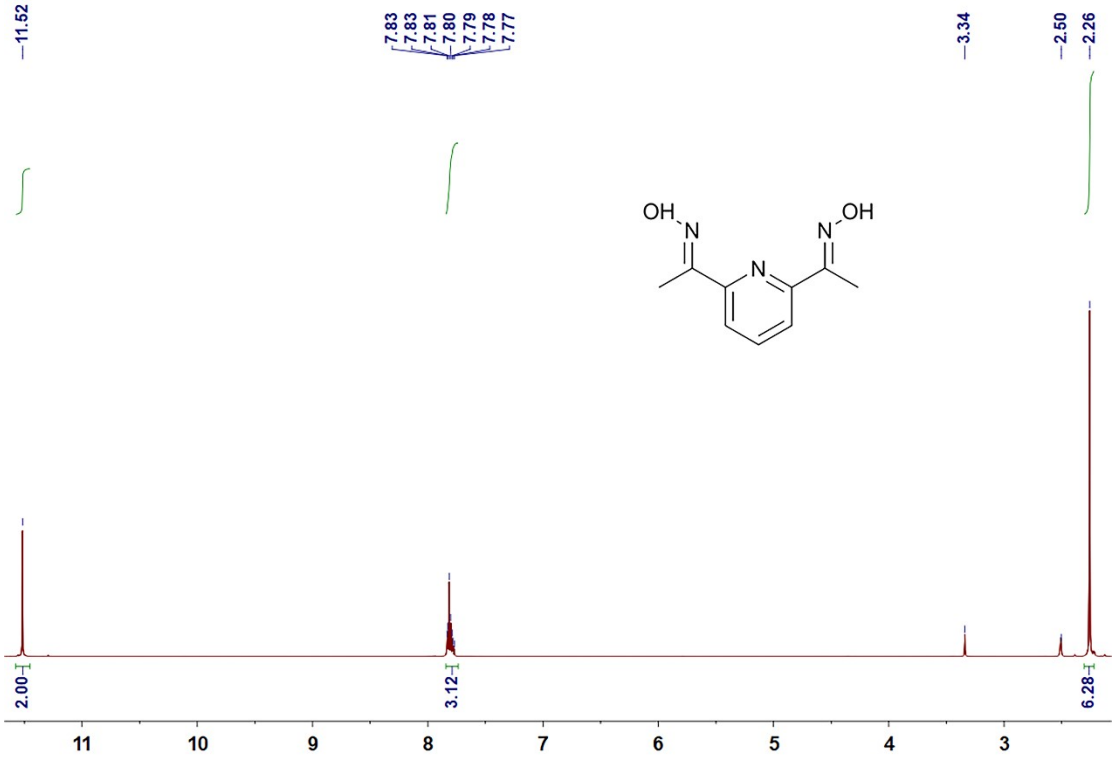



**Figure S22**. $^{1}$H NMR spectrum of **dapdoH$_2$** in DMSO-*d*6

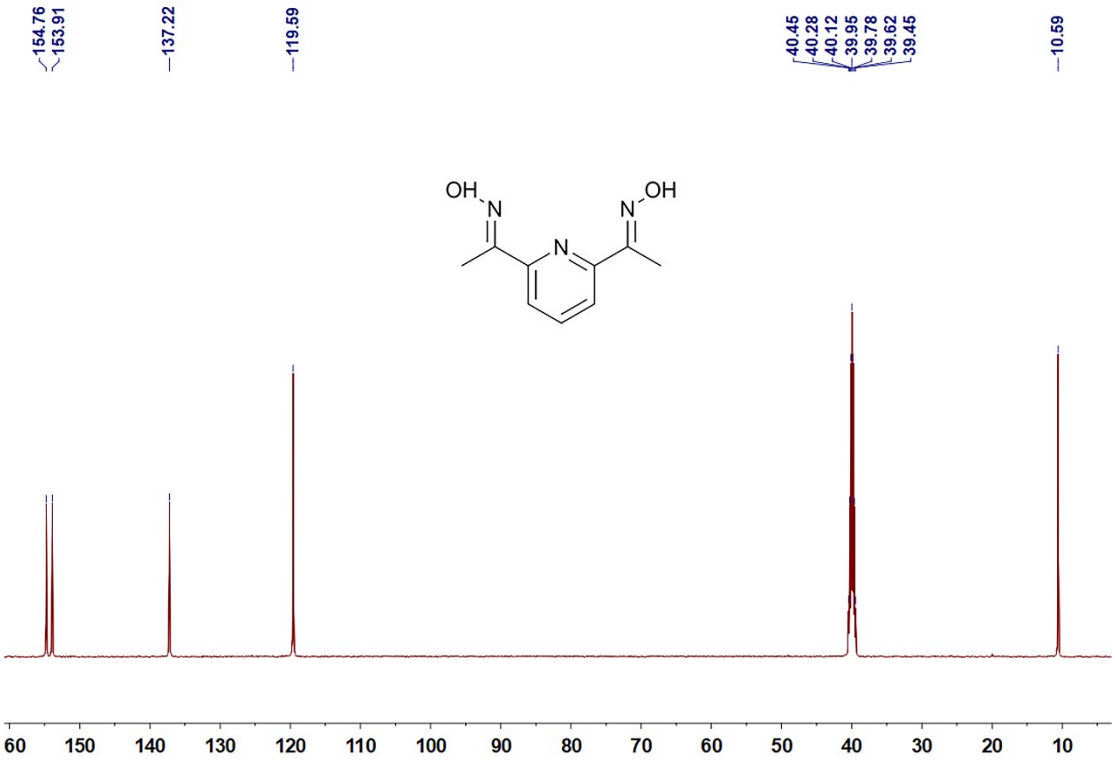



**Figure S23**. $^{13}$C NMR spectrum of **dapdoH$_2$** in DMSO-*d*6

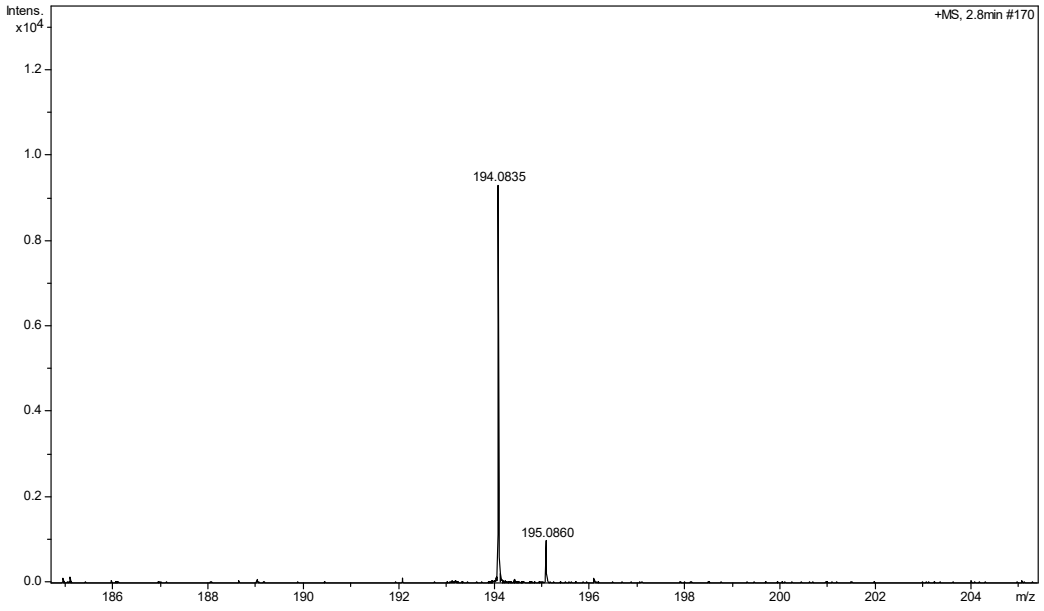



**Figure S24**. HR-MS (ESI) spectrum of **dapdoH$_2$** in MeCN

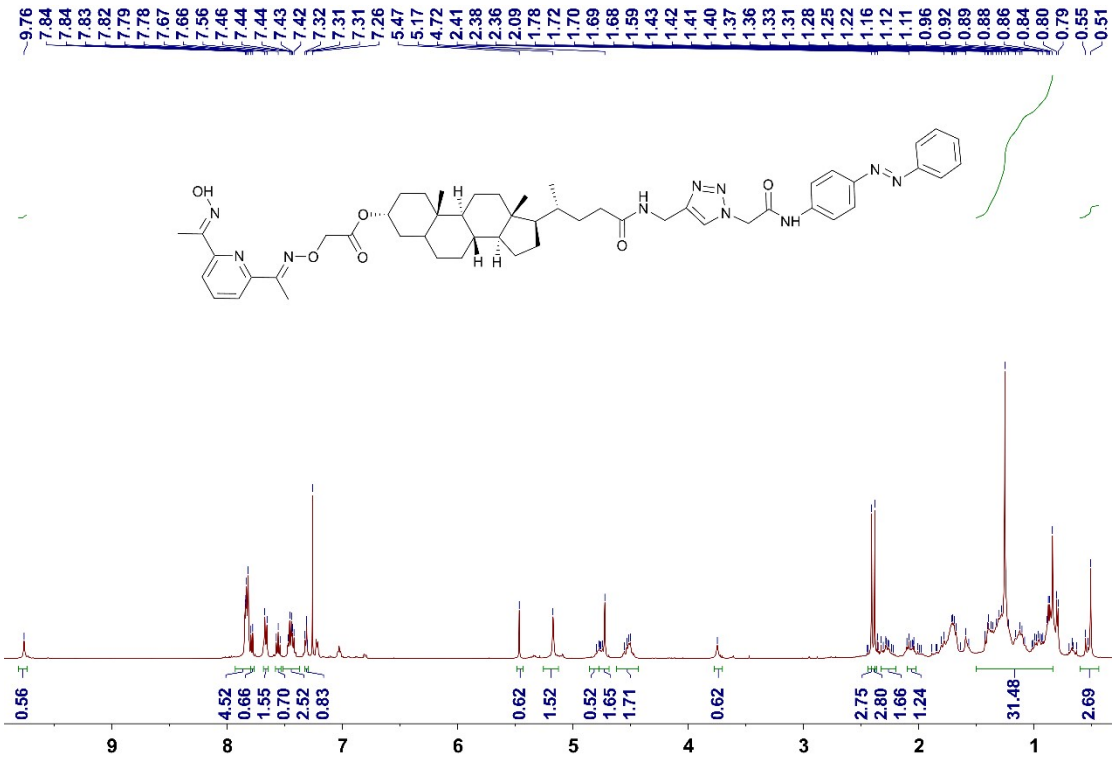


**Figure S25**. $^{1}$H NMR spectrum of **Azo-LCA-dapdoH$_2$** in $CDCl_3$

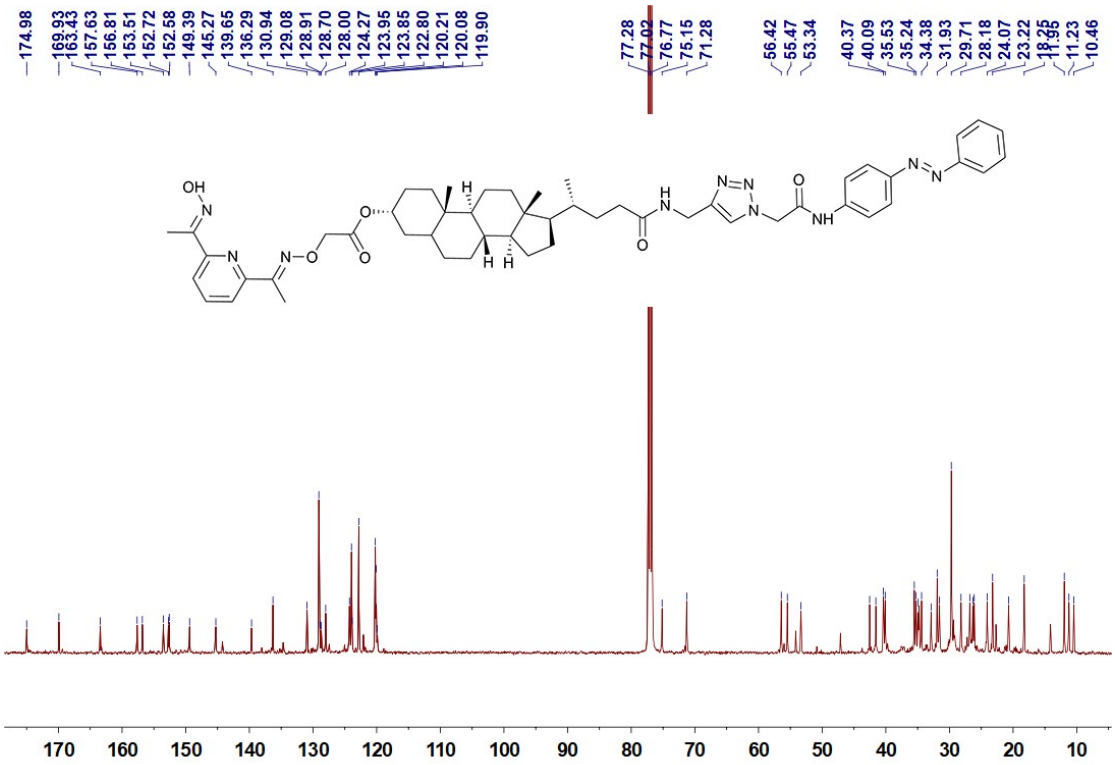


**Figure S26**. $^{13}$C NMR spectrum of **Azo-LCA-dapdoH$_2$** in $CDCl_3$

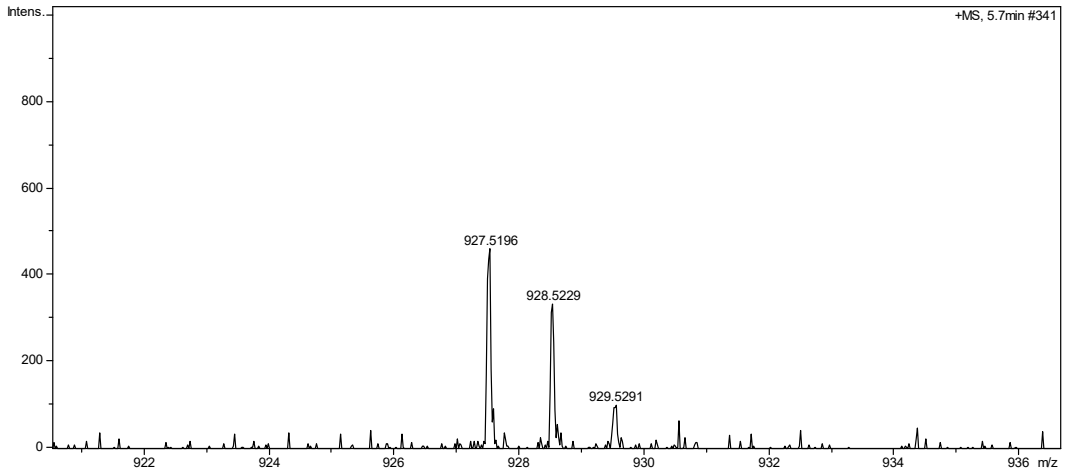



**Figure S27**. HR-MS (ESI) spectrum of compound **Azo-LCA-dapdoH$_2$** in MeCN

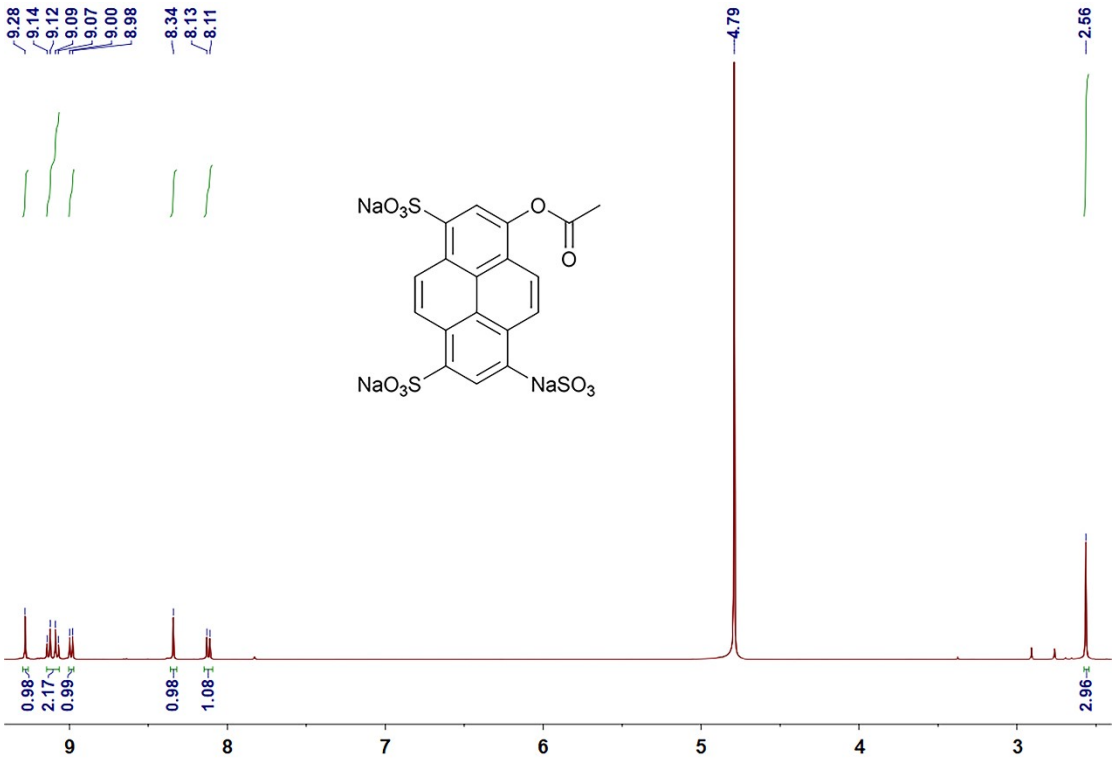



**Figure S28**. $^1$H NMR spectrum of **APTS** in $D_2O$

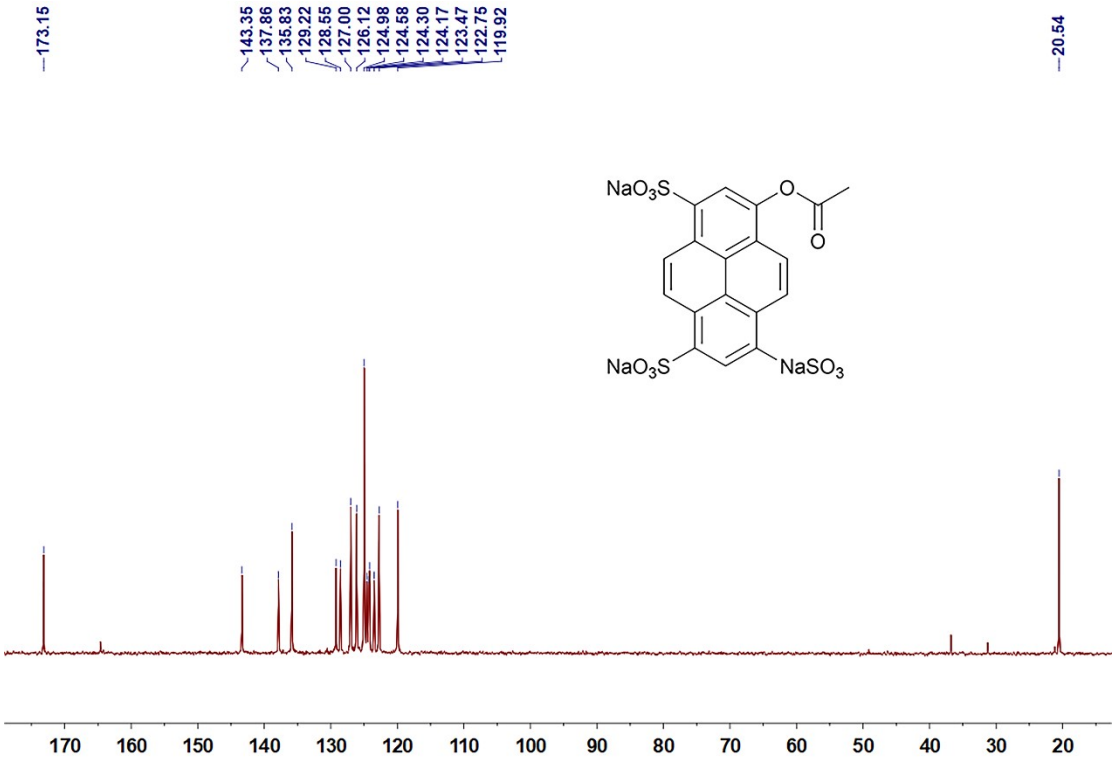



**Figure S29**. $^{13}C$ NMR spectrum of **APTS** in $D_2O$

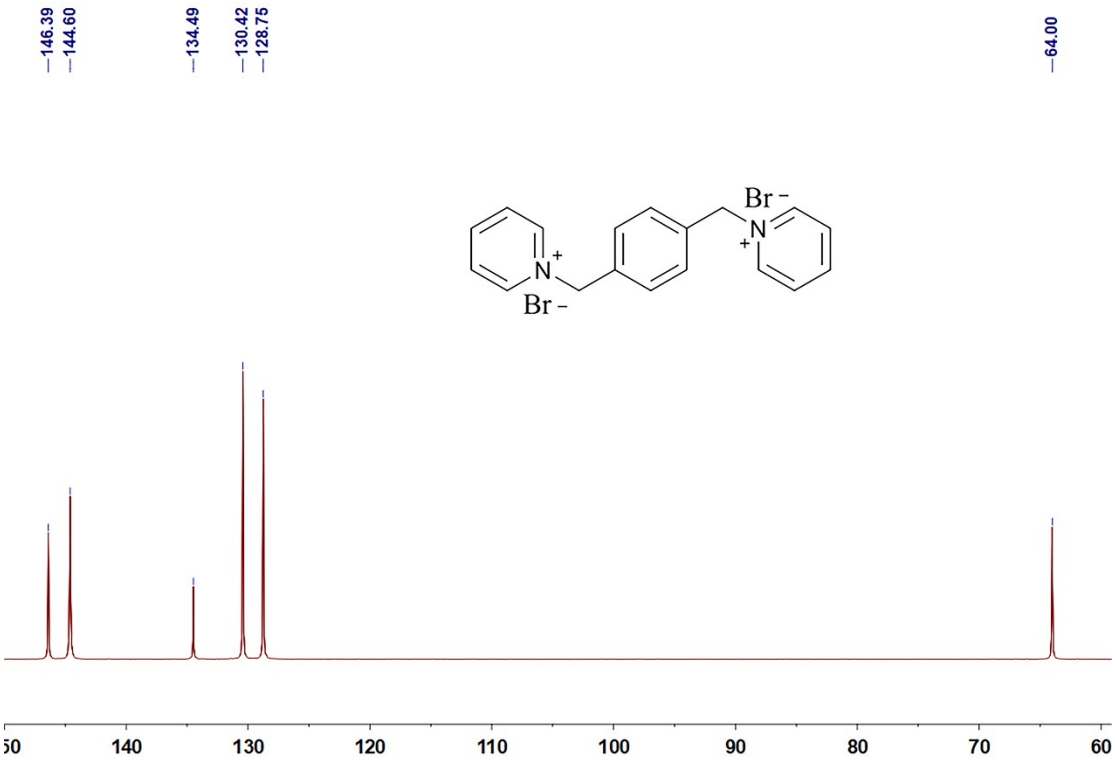



**Figure S30**. $^{1}H$ NMR spectrum of **DPX** in $D_2O$

**Figure S31**. $^{13}C$ NMR spectrum of **DPX** in $D_2O$

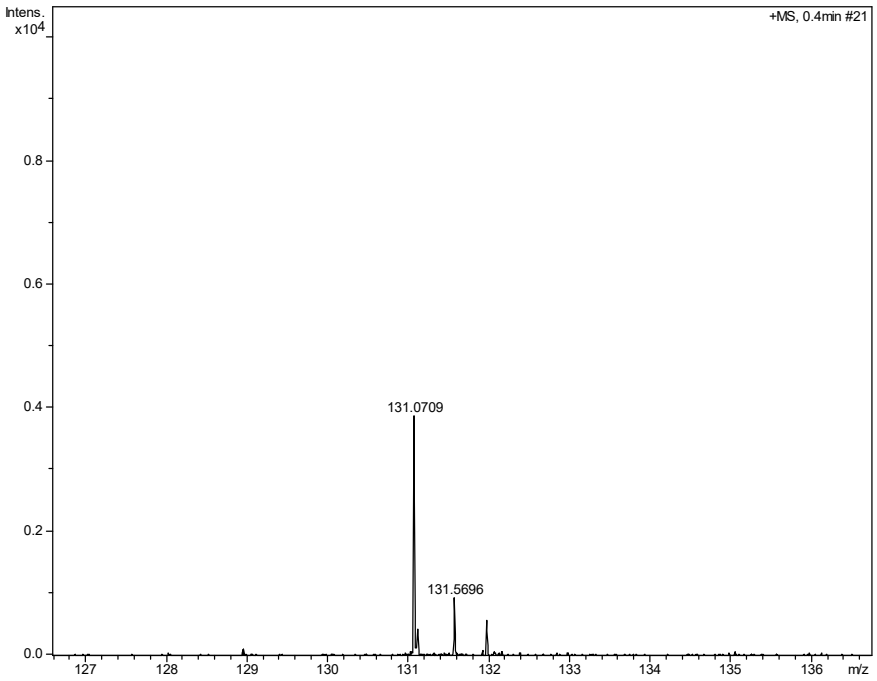



**Figure S32**. HR-MS (ESI) spectrum of **DPX** in MeCN