Supporting Information

Novel Biotin-linked Amphiphilic Calix[4]arene-based Supramolecular Micelles as Doxorubicin Carriers for Boosted Anticancer Activity

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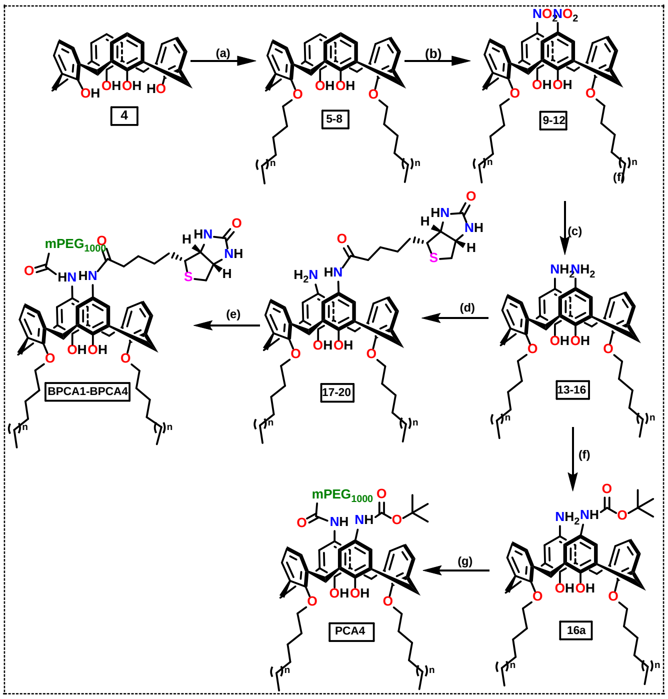


Scheme s1 Synthesis route of amphiphilic calix[4]arene derivatives. Reagents and conditions: (a) 1-iodoalkane, anhydrous K_2CO_3, acetonitrile, reflux; (b) 65% HNO_3/HOAc, DCM, 0 °C, N_2; (c) NH_2-NH_2.H_2O, Pd/C, EtOH, reflux; (d) biotin-N-succinimide ester, DMAP, DMF, rt; (e) $mPEG_{1000}$-NHS, DMAP, DMF, rt; (f) $(Boc)_2O$, NEt_3, MeOH/THF, 0-4 °C; (g) $mPEG_{1000}$-NHS, NEt_3, DMF/THF, rt.

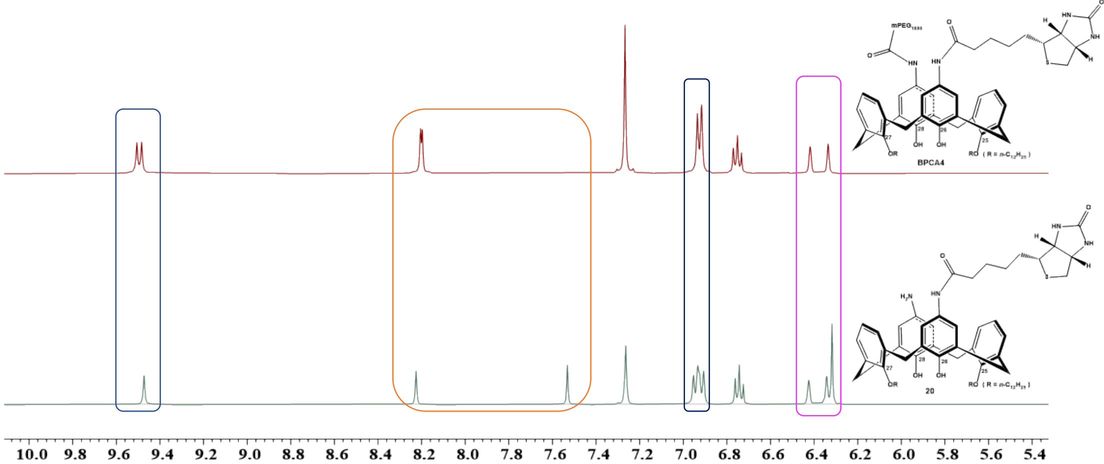


Figure S1 ^{1}H NMR comparison diagram of compound **BPCA4** and **20**

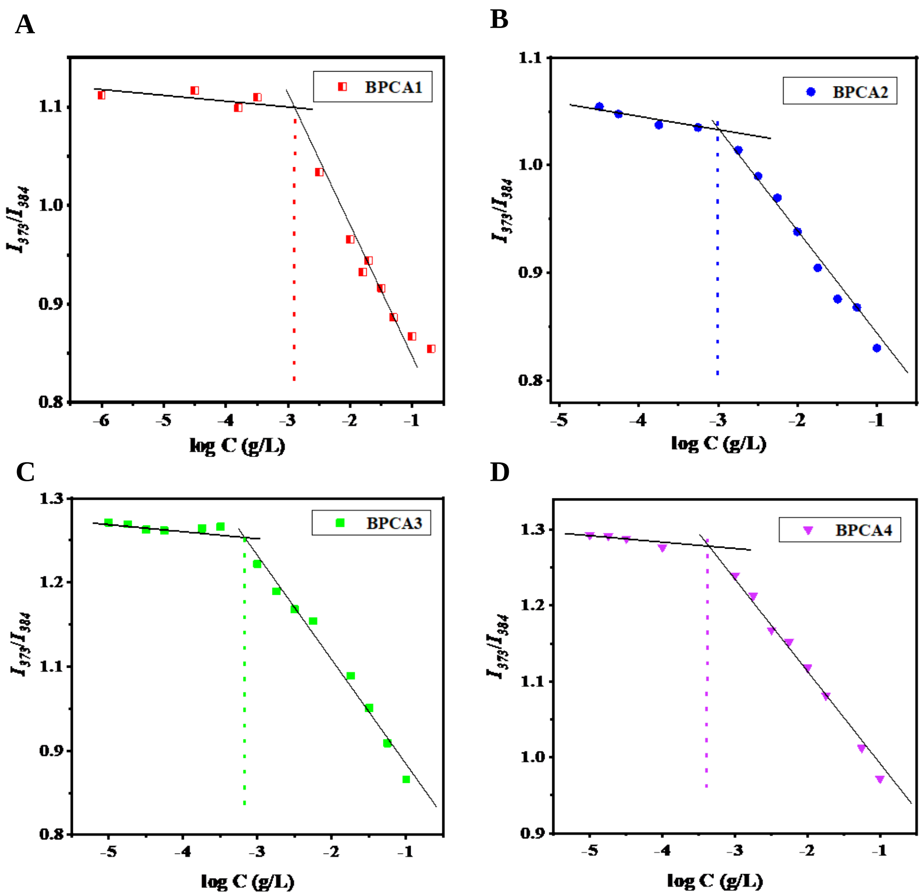


Figure S2 Plots of intensity ratio (I_{373}/I_{384}) from fluorescence emission spectra of pyrene versus log C of compounds **BPCA1-BPCA4**

Table S1 Particle Size and Zeta Potential of BPCA4 and BPCA4-DOX (n = 3).

Micelles	Mean Diameter(nm)	PDI	Zeta (mV)
BPCA4	78.5 ±2.0	0.220±0.121	2.91±0.03
BPCA4-DOX	112.2±0.2	0.266±0.020	5.27±0.02

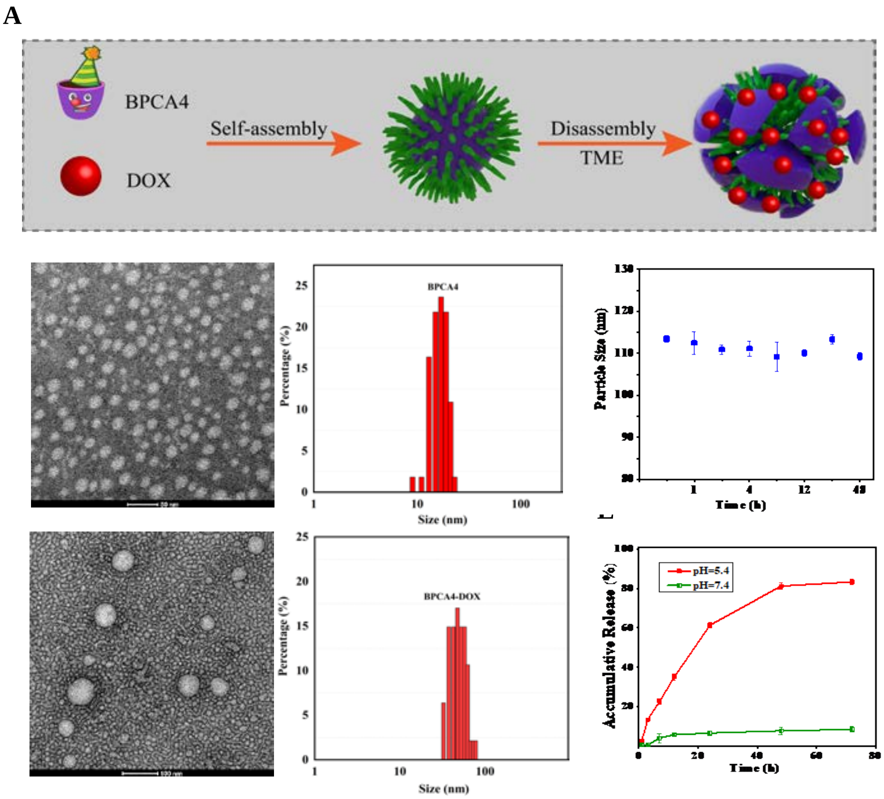


Fig. S3 Characterizations of **BPCA4** and **BPCA4-DOX micelles: A.** Schematic illustration of synthetic **BPCA4-DOX** formed by assembly**; B.** TEM image of **BPCA4** and **BPCA4-DOX**micelles; **C. Size distribution of BPCA4** and **BPCA4-DOX** micelles in TEM image**;** D. Stability of **BPCA4-DOX** micelles in PBS (0.15M, pH 7.4) at 4 °C; **E.** Accumulated DOX-release profiles of **BPCA4-DOX** micelles at different solution pHs.

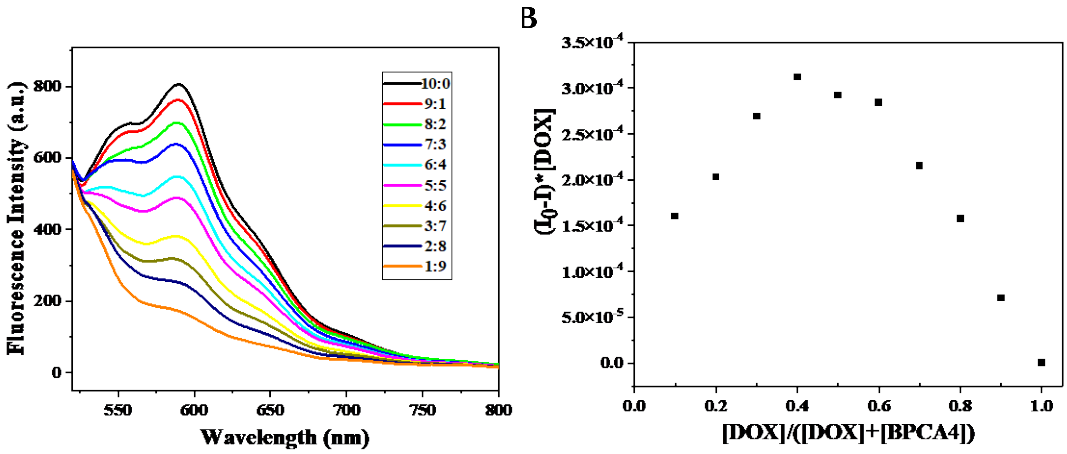


Figure S4 (A) The fluorescence emission intensity curve of DOX in DMF/PBS with the changes of **DOX/BPCA4** molar ratio. The total concentration of DOX and **BPCA4** is 1.84 μM. (B) Job's plot based on fluorescent data for the fluorescence emission intensity of DOX + BPCA4.

Table S2 IC_{50} values of BPCA4-DOX and PCA4-DOX against selected cell lines

Tumor types	Cells	In vitro cytotoxic activity (IC_{50} in μM)[a]		
		BPCA4-	**PCA4-DOX**	DOX·HCl [b]
Human cervical cancer	HeLa	2.45 ± 0.18	6.06 ± 1.08	0.85 ± 0.03
Human breast cancer	MCF-7	1.83 ± 0.15	12.03 ± 1.05	0.50 ± 0.03
Human breast cancer	MDA-MB-231	1.65 ± 0.18	7.45 ± 0.64	0.71 ± 0.04
Mouse breast cancer	4T1	2.18 ± 0.29	27.40 ± 0.8	0.64 ± 0.04

[a] Data represents the mean ± standard deviation of triplicate

[b] Doxorubicin hydrochloride as the positive control

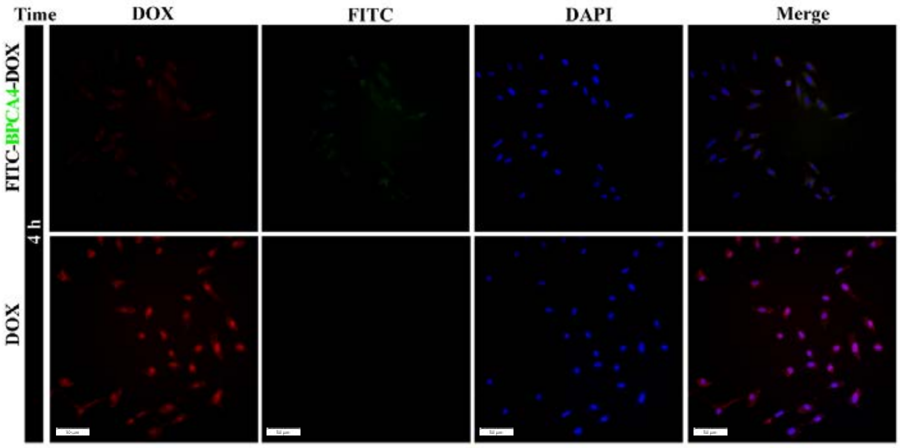


Figure.S5 Inverted fluorescence microscope images of MCF-10A cells after treatment 4h with **FITC-BPCA4-DOX**; red (DOX), green (FITC-labeled micelles), blue (DAPI stained nucleus), Merge (colocalized DOX, FITC and DAPI).

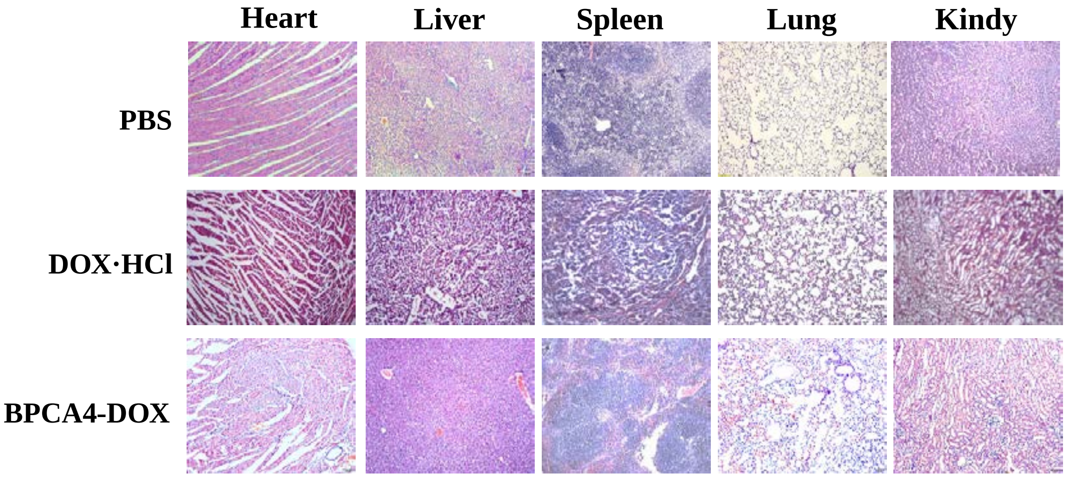


Figure S6. Histopathologic analysis of heart, liver, spleen, lung, and kidney organs after H&E staining, which were harvested from mice treated with PBS, free DOX·HCl and **BPCA4-DOX**).

Experimental section

General Information

Materials and methods

All reagents and solvents were purchased as analytical grade and used without further purification. The reaction was monitored by thin-layer chromatography (TLC) and visualized under UV light (254 nm). Silica gel (300-400 mesh) for column chromatography was purchased from Shanghai Titan Scientific Co., Ltd. ^{1}H NMR and ^{13}C NMR spectra were recorded in Chloroform-d on Bruker AV-400 NMR spectrometers using TMS as internal standard. The chemical shifts were reported as δ values in parts per million (ppm), and coupling constants (J) were given in hertz (Hz). The peak pattern abbreviations are as follows: s, singlet; brs, broad singlet; d, doublet; q, quadruplet; dd, doublet of doublet; t, triplet; m, multiplet. HRMS data were obtained using a (UHR-T0F) maXis 4G instrument. Melting points were determined with capillaries with a YRT-3 microscope apparatus and were uncorrected. Distilled water was used in the experiments. Experimental cells were obtained from the Cell Bank, Chinese Academy of Sciences. Inverted fluorescence microscope (U-REL-T,TH4-200,TX2-ILL100) Olympus, Japan. All the reagents and kits used in the cell experiments are commercially available.

General procedure for the preparation of compounds

Compound **3** (Biotin N-succinimide ester) was synthesized by the reaction of biotin **1** with N-hydroxy succinimide **2** according to the literature procedures[37]. calix[4]arene **4** was prepared by the reverse Friedel-Crafts reaction of *p-tert*-butyl calix[4] arene as previously reported[22].

General procedure for the preparation of compounds 5-8

To a stirred suspension of calix[4]arene **4** (2.06 g, 5.0 mmol), potassium carbonate (3.26 g, 15.5 mmol) in CH_3CN (40 mL) was slowly added 1-iodohexane (1.66 mL, 11 mmol) or 1-iodooctane (1.99 mL, 11 mmol) or 1-bromodecane (2.34 mL, 11 mmol) or 1-iododecane (2.71 mL, 11 mmol) and heated at reflux for 48 h. The solution was diluted with an equal volume of water and extracted 3 times with CH_2Cl_2. The combined organics were rinsed with brine and dried with anhydrous sodium sulfate and filtered. The solvent was removed under reduced pressure, followed by anhydrous ethanol was added. The resulting precipitated solid was collected by filtration to afford white product **5-8**.

25,27-bis-hexyloxy-calix[4]arene-26, 28-diol (5). White solid, yield: 69.2% (1.99 g); m.p.: 149.8 – 150.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (s, 2H), 7.07 (d, *J* = 7.6 Hz, 4H), 6.92 (d, *J* = 7.8 Hz, 4H), 6.74 (t, *J* = 7.6 Hz, 2H), 6.66 (t, *J* = 7.6 Hz, 2H), 4.33 (d, *J* = 13.0 Hz, 4H), 4.01 (t, *J* = 7.0Hz, 4H), 3.39 (d, *J* = 12.8 Hz, 4H), 2.12 – 2.04 (m, 4H), 1.75 – 1.68 (m, 4H), 1.51 – 1.38 (m, 8H), 0.97 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.47, 152.08, 133.58, 128.98, 128.50, 128.30, 125.36, 119.03, 76.96, 31.87, 31.52, 30.11, 25.80, 22.78, 14.29; HR-MS(ESI) calculated for $C_{40}H_{48}O_4$ ([M +Na]$^+$) : 615.3450, found: 615.3452.

25,27-bis-octyloxy-calix[4]arene-26, 28-diol (6). White solid, yield: 77.4% (2.38 g); m.p.: 111.6 – 112.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 2H), 7.05 (d, *J* = 7.6 Hz, 4H), 6.91 (d, *J* = 7.6 Hz, 4H), 6.73 (t, *J* = 7.6 Hz, 2H), 6.64 (t, *J* = 7.6 Hz, 2H), 4.31 (d, *J* = 12.8 Hz, 4H), 3.99 (t, *J* = 6.6 Hz, 4H), 3.37 (d, *J* = 12.8 Hz, 4H), 2.10 – 2.03 (m, 4H), 1.73 – 1.64 (m, 4H), 1.48 – 1.42 (m, 4H), 1.38 (m, , 4H), 1.34 – 1.30 (m, 8H), 0.90 (t, *J* = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.50, 152.13, 133.62, 129.01, 128.52, 128.33, 125.38, 119.06, 76.96, 32.12, 31.57, 30.17, 29.67, 29.51, 26.15, 22.85, 14.28; HR-MS(ESI) calculated for $C_{44}H_{56}O_4$ ([M +Na]$^+$): 671.4076, found: 671.4060.

25,27-bis-decyloxy-calix[4]arene-26, 28-diol (7). White solid, yield: 80.2% (2.74 g); m.p.: 139.5 – 141.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 2H), 7.05 (d, *J* = 7.6 Hz, 4H), 6.91 (d, *J* = 7.6 Hz, 4H), 6.73 (t, *J* = 7.6 Hz, 2H), 6.64 (t, *J* = 7.6 Hz, 2H), 4.31 (d, *J* = 12.8 Hz, 4H), 3.99 (t, *J* = 6.6 Hz, 4H), 3.37 (d, *J* = 12.8 Hz, 4H), 2.10 – 2.02 (m, 4H), 1.73 – 1.65 (m, 4H), 1.49 – 1.42 (m, 4H), 1.36 (d, *J* = 7.0 Hz, 4H), 1.33 – 1.25 (m, 16H), 0.88 (t, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.45, 152.06, 133.59, 128.97, 128.48, 128.27, 125.35, 119.01, 76.91, 32.07, 31.53, 30.14, 29.85, 29.82, 29.67, 29.52, 26.11, 22.82, 14.26; HR-MS(ESI) calculated for $C_{48}H_{64}O_4$ ([M +Na]$^+$) :727.4702, found: 727.4687.

25,27-bis-dodecyloxy-calix[4]arene-26,28-diol (8). White solid, yield: 89.9% (3.36 g); m.p.: 116.6 – 117.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (s, 2H), 7.05 (d, *J* = 7.6 Hz, 4H), 6.91 (d, *J* = 8.0 Hz, 4H), 6.73 (t, *J* = 7.6 Hz, 2H), 6.64 (t, *J* = 7.6 Hz, 2H), 4.31 (d, *J* = 13.2 Hz, 4H), 3.99 (t, *J* = 7.0 Hz, 4H), 3.37 (d, *J* = 13.2 Hz, 4H), 2.10 – 2.03 (m, 4H), 1.73 – 1.65 (m, 4H), 1.49 – 1.42 (m, 4H), 1.35 – 1.26 (m, 28H), 0.88 (t, *J* = 6.8 Hz , 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.50, 152.13, 133.63, 129.01, 128.52, 128.32, 125.38, 119.06, 76.95, 32.10, 31.58, 30.18, 29.94, 29.90, 29.86, 29.85, 29.71, 29.55, 26.15, 22.86, 14.29; HR-MS(ESI) calculated for $C_{52}H_{72}O_4$ ([M +Na]$^+$):783.5328, found: 783.5301.

General procedure for the preparation of compounds 9-12

Glacial acetic acid (3.5 mL) was added to the solution of compound **5** (1.24 g, 2.01 mmol) in 40 mL CH_2Cl_2 at 0 ^{o}C under a nitrogen atmosphere and stirred for 20 min. 65% nitric acid (0.72 mL) was then added slowly and the suspension was stirred for an additional 20 min. The cooling bath was removed and reaction mixture was stirred at room temperature overnight. The solution was diluted with water and extracted 3 times with CH_2Cl_2. The combined organics were rinsed with brine and dried with anhydrous sodium sulfate and and evaporated to dryness. The crude product purified by flash chromatography on silica gel (petroleum ether / ethyl acetate = 10/1) to give product **9**.

5,11-bis-nitro-25,27-bis-hexyloxy-calix[4]arene-26, 28-diol (9). White solid; yield: 41.3% (590 mg); m.p.: 225.3 – 226.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.39 (s, 2H), 8.05 (s, 4H), 6.99 (d, *J* = 7.6 Hz, 4H), 6.85 (t, *J* = 7.6 Hz, 2H), 4.29 (d, *J* = 13.2 Hz, 4H), 4.04 (t, *J* = 6.6Hz, 4H), 3.51 (d, *J* = 13.2 Hz, 4H), 2.11 – 2.04 (m, 4H), 1.74 – 1.67 (m, 4H), 1.49 – 1.40 (m, 8H), 0.96 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.78, 151.84, 139.82, 131.95, 129.72, 128.43, 125.98, 124.66, 77.40, 31.76, 31.35, 30.06, 25.73, 22.75, 14.23; HR-MS(ESI) calculated for $C_{40}H_{46}N_2O_8$ ([M +Na]$^+$): 705.3152, found: 705.3137.

5,11-bis-nitro-25,27-bis-octyloxy-calix[4]arene-26, 28-diol (10). Prepared from compound **6** (1.04 g, 1.64 mmol) and 65% nitric acid (0.59 mL) according to general procedure. White solid; yield: 40.7 % (491 mg); m.p.: 184.7 – 185.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.39 (s, 2H), 8.03 (s, 4H), 6.98 (d, *J* = 7.6 Hz, 4H), 6.84 (t, *J* = 7.6 Hz, 2H), 4.28 (d, *J* = 13.2 Hz, 4H), 4.03 (t, *J* = 6.6 Hz, 4H), 3.50 (d, *J* = 13.2 Hz, 4H), 2.10 – 2.03 (m, 4H), 1.73 – 1.65 (m, 4H), 1.50 – 1.43 (m, 4H), 1.40 – 1.35 (m, 4H), 1.33 – 1.29 (m, 8H), 0.88 (t, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.77, 151.84, 139.83, 131.95, 129.72, 128.43, 125.98, 124.65, 77.34, 32.01, 31.36, 30.08, 29.52, 29.42, 26.03, 22.77, 14.21; HR-MS(ESI) calculated for $C_{44}H_{54}N_2O_8$ ([M +Na]$^+$): 761.3778, found: 761.3749.

5,11-bis-nitro-25,27-bis-decyloxy-calix[4]arene-26, 28-diol (11). Prepared from compound **7** (1.18 g, 1.67 mmol) and 65% nitric acid (0.60 mL) according to general procedure. White solid; yield: 39.2 % (520 mg); m.p.: 164.7 – 165.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.40 (s, 2H), 8.03 (s, 4H), 6.98

(d, *J* = 7.8 Hz, 4H), 6.84 (t, *J* = 7.8 Hz, 2H), 4.28 (d, *J* = 13.2 Hz, 4H), 4.03 (t, *J* = 7.0 Hz, 4H), 3.50 (d, *J* = 13.6 Hz, 4H), 2.10 – 2.03(m, 4H), 1.73 – 1.65 (m, 4H), 1.50 – 1.43 (m, 4H), 1.39 – 1.34 (m, 4H) , 1.31 – 1.25 (m, 16H), 0.86 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.77, 151.84, 139.83, 131.95, 129.72, 128.42, 125.98, 124.65, 77.32, 32.03, 31.36, 30.09, 29.77, 29.76, 29.56, 29.47, 26.03, 22.78, 14.22; HR-MS(ESI) calculated for $C_{48}H_{62}$ N_2O_4 ([M +Na]$^+$) :817.4403, found: 817.4384.

5,11-bis-nitro-25,27-bis-dodecyloxy-calix[4]arene-26,28-diol (12). Prepared from compound **8** (1.63 g, 2.14 mmol) and 65% nitric acid (0.77 mL) according to general procedure. White solid; yield: 30.0 % (550 mg); m.p.: 135.8 – 136.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.40 (s, 2H), 8.03 (s, 4H), 6.98 (d, *J* = 7.6 Hz, 4H), 6.84 (t, *J* = 7.6 Hz, 2H), 4.28 (d, *J* = 13.2 Hz, 4H), 4.03 (t, *J* = 6.6 Hz, 4H), 3.50 (d, *J* = 13.2 Hz, 4H), 2.10 – 2.03 (m, 4H), 1.73 – 1.66 (m, 4H), 1.50 – 1.43 (m, 4H), 1.39 – 1.36 (m, 4H), 1.33 – 1.25 (m, 24H), 0.87 (t, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.76, 151.84, 139.84, 131.95, 129.72, 128.43, 125.98, 124.65, 77.32, 32.03, 31.37, 30.09, 29.82, 29.79, 29.76, 29.56, 29.47, 26.03, 22.79, 14.22; HR-MS(ESI) calculated for $C_{52}H_{70}$ N_2O_4 ([M +Na]$^+$) : 873.5029, found: 873.5001.

General procedure for the preparation of compounds 13-16

To a solution of **9** (300 mg, 0.44 mmol) and 10% Pd/C (50 mg) in ethanol (20 mL) was added slowly 80% hydrazine hydrate (0.8 mL) and stirred at 50 °C for 8 h. The solution was filtered and the filter cake was washed with a small amount of ethanol, followed by concentration under reduced pressure to afford compound **13**.

5,11-bis-amino-25,27-bis-hexyloxy-calix[4]arene-26,28-diol (13). White solid; yield: 95.0 % (260 mg); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (s, 2H), 6.91 (d, *J* = 7.6 Hz, 4H), 6.74 (t, *J* = 7.6 Hz, 2H), 6.45 (s, 4H), 4.29 (d, *J* = 12.8 Hz, 4H), 3.95 (t, *J* = 7.0 Hz, 4H), 3.23 (d, *J* = 12.8 Hz, 4H), 3.09 (bs, 4H), 2.09 – 2.01 (m, 4H), 1.67 – 1.60 (m, 4H), 1.46 – 1.37 (m, 8H), 0.93 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.32, 146.25, 138.01, 133.78, 129.28, 128.86, 125.14, 116.02, 77.33，76.93，31.85, 31.56, 30.02, 25.72, 22.75, 14.26; HR-MS(ESI) calculated for $C_{40}H_{50}N_2O_4$ ([M +Na]$^+$) : 645.3668, found: 645.3667.

5,11-bis-amino-25,27-bis-octyloxy-calix[4]arene-26,28-diol (14). Prepared from compound **10** (420 mg, 0.66 mmol) and 10% Pd/C (70 mg) according to general procedure. White solid; yield: 95.9% (370 mg); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (s, 2H), 6.91 (d, *J* = 7.6 Hz, 4H), 6.74 (t, *J* = 7.6 Hz, 2H), 6.46 (s, 4H), 4.29 (d, *J* = 12.8 Hz, 4H), 3.95 (t, *J* = 6.8 Hz, 4H), 3.23 (d, *J* = 12.8 Hz, 4H), 3.06 (bs, 4H), 2.09 – 2.02 (m, 4H), 1.67 – 1.59 (m, 4H), 1.46 – 1.41 (m, 4H), 1.38 – 1.34 (m, 4H), 1.33 – 1.28 (m, 8H), 0.89 (t, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.32, 146.26, 137.95, 133.79, 129.29, 128.87, 125.15, 116.04, 77.34, 76.91, 32.06, 31.57, 30.06, 29.63, 29.45, 26.05, 22.80, 14.25; HR-MS(ESI) calculated for $C_{44}H_{58}N_2O_4$ ([M +Na]$^+$) : 701.4294, found: 701.4284.

5,11-bis-amino-25,27-bis-decyloxy-calix[4]arene-26,28-diol (15). Prepared from compound **11** (354 mg,0.45 mmol) and 10% Pd/C (60 mg) according to general procedure. White solid; yield: 98.7% (323 mg); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (s, 2H), 6.91 (d, *J* = 7.6 Hz, 4H), 6.73 (t, *J* = 7.6 Hz, 2H), 6.45 (s, 4H), 4.29 (d, *J* = 12.8 Hz, 4H), 3.95 (t, *J* = 6.8 Hz, 4H), 3.23 (d, *J* = 12.8 Hz, 4H), 3.04 (brs, 4H), 2.09 – 2.01 (m, 4H), 1.67 – 1.59 (m, 4H), 1.46 – 1.39 (m, 4H), 1.37 – 1.34 (m, 4H), 1.32 – 1.26 (m, 16H), 0.88 (t, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.30, 146.34, 137.74, 133.79, 129.29, 128.88, 125.17, 116.12, 77.34,76.91,32.07, 31.57, 30.07, 29.83, 29.80, 29.68, 29.51, 26.05, 22.83, 14.27; HR-MS(ESI) calculated for $C_{48}H_{66}N_2O_4$ ([M +Na]$^+$) : 757.4920, found: 757.4911.

5,11-bis-amino-25,27-bis-dodecyloxy-calix[4]arene-26,28-diol (16). Prepared from compound **12** (503 mg, 0.59 mmol) and 10% Pd/C (85 mg) according to general procedure. White solid; yield: 98.6% (461 mg); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (s, 2H), 6.91 (d, *J* = 7.8 Hz, 4H), 6.74 (t, *J* = 7.8 Hz, 2H), 6.45 (s, 4H), 4.28 (d, *J* = 12.8 Hz, 4H), 3.95 (t, *J* = 7.2 Hz, 4H), 3.23 (d, *J* = 13.0 Hz, 4H), 2.09 – 2.01 (m, 4H), 1.66 – 1.59 (m, 4H), 1.46 – 1.41 (m, 4H), 1.37 – 1.26 (m, 28H), 0.88 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.32, 146.20, 138.08, 133.81, 129.29, 128.86, 125.15, 115.99,77.33,76.90, 32.06, 31.58, 30.07, 29.88, 29.85, 29.82, 29.80, 29.68, 29.51, 26.05, 22.82, 14.26; HR-MS(ESI) calculated for $C_{52}H_{74}N_2O_4$ ($[M+Na]^+$) :813.5546, found: 813.5533.

General procedure for the preparation of compound 16a

To a stirred suspension of compound **16** (60 mg, 0.075 mmol) in methanol (4 mL) was dropped triethylamine (26 µL, 0.19 mmol) in ice bath for 10 min, followed by slow addition of di-*tert*-butyl bicarbonate (16.5 mg, 0.075 mmol) in 4 ml THF and reacted for 4 h. The solvent was removed under reduced pressure and the residue was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate = 4/1) to obtained compound **16a**.

5-Boc-amino-11-amino-25,27-bis-dodecyloxy-calix[4]arene-26,28-diol (16a). White solid; yield: 46.41% (31 mg); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 1H), 7.62 (s, 1H), 7.04 (s, 2H), 6.92 – 6.89 (m, 4H), 6.72 (t, *J* = 7.6 Hz, 2H), 6.48 (s, 2H), 6.24 (brs, 1H), 4.28 (dd, *J* = 12.8, 3.2 Hz, 4H), 3.96 (t, *J* = 6.8 Hz, 4H), 3.27 (dd, *J* = 31.6, 13.2 Hz, 4H), 2.08 – 2.01 (m, 4H), 1.68 – 1.60 (m, 4H), 1.50 (s, 9H), 1.46 – 1.41 (m, 4H), 1.38 – 1.33 (m, 4H), 1.30 – 1.27 (m, 24H), 0.88 (t, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.24, 152.23, 149.44, 146.56, 133.70, 133.42, 129.57, 129.17, 128.97, 128.93, 128.71, 125.21, 116.30, 32.04, 31.55, 31.51, 30.07, 29.86, 29.83, 29.80, 29.78, 29.65, 29.49, 28.52, 26.04, 22.79, 14.23, 14.22.

General procedure for the preparation of compounds 17-20

To a solution of **13** (100 mg, 0.16 mmol) in N,N-dimethylformamide (5 mL) was added slowly biotin N-succinimidyl ester (100 mg, 0. 29 mmol) with catalytic amount of 4-dimethylaminopyridine and stirred at room temperature. After the reaction was completed, the mixture was filtered and the filtrate was concentrated under reduced pressure to give crude product, which was purified by flash chromatography on silica gel (dichloromethane / methanol = 30/1) to yield product **17**.

5-biotinamino-11-amino-25,27-bis-hexyloxy-calix[4]arene-26,28-diol (17). White solid; yield: 55.7% (76 mg); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.54 (s, 1H), 8.28 (s, 1H), 7.58 (s, 1H), 7.31 (s, 2H), 7.00 – 6.96 (m, 4H), 6.79 (t, *J* = 7.6 Hz, 2H), 6.47 (s, 1H), 6.39 (s, 1H), 6.37 (s, 2H), 4.31 (dd, *J* = 8.8, 4.8 Hz, 4H) , 4.15 (dd, *J* = 16.8, 12.8 Hz, 4H), 3.92 (t, *J* = 6.4 Hz, 4H), 3.34 (d, *J* = 12.4 Hz, 2H), 3.22 (d, *J* = 12.6 Hz, 2H), 3.14 – 3.10 (m, 1H), 3.02 (q, *J* = 7.6 Hz, 1H), 2.83 (dd, *J* = 12.8, 4.8 Hz, 1H), 2.58 (d, *J* = 12.4 Hz, 1H), 2.23 (t, *J* = 7.4 Hz, 2H), 2.01 – 1.94 (m, 4H), 1.74 – 1.67 (m, 4H), 1.62 – 1.57 (m, 2H), 1.48 – 1.32 (m, 12H), 1.16 (t, *J* = 7.6 Hz, 2H), 0.94 (t, *J* = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.88, 163.26, 152.45, 149.03, 144.07, 141.15, 134.83, 134.16, 131.44, 129.32, 129.03, 128.71, 128.21, 125.55, 120.52, 115.01, 76.91, 61.57, 59.72, 55.95, 31.79, 31.25, 30.08, 28.80, 28.60, 25.71, 25.64, 22.72, 14.56; HR-MS(ESI) calculated for $C_{50}H_{64}N_4O_6S$ ($[M+Na]^+$) : 871.4444, found: 871.4436.

5-acylaminobiotin-11-amino-25,27-bis-octyloxy-calix[4]arene-26,28-diol (18). Pepared from compound **14** (109 mg, 0.16 mmol) and biotin N-succinimidyl ester (100 mg, 0. 29 mmol) according to general procedure. White solid; yield: 49.5% (72 mg); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.54 (s, 1H), 8.28 (s, 1H), 7.59 (s, 1H), 7.31 (s, 2H), 7.00 – 6.95 (m, 4H), 6.79 (t, *J* = 7.6Hz, 2H), 6.47 (s, 1H), 6.39 (s, 1H), 6.37 (s, 2H), 4.32 – 4.29 (m, 1H), 4.18 – 4.11 (m, 5H), 3.91 (t, *J* = 6.2 Hz, 4H), 3.22 (d, *J* = 12.6 Hz, 2H), 3.14 – 3.09 (m, 1H), 2.82 (dd, *J* = 12.4, 5.0 Hz, 1H), 2.58 (d, *J* = 12.4 Hz, 1H), 2.23 (t,

J = 7.4 Hz, 2H), 2.01 – 1.94 (m, 4H), 1.72 – 1.66 (m, 7.2 Hz, 4H), 1.62 –1.57 (m, 2H), 1.47 – 1.41 (m, 4H), 1.41 – 1.34 (m, 6H), 1.33 – 1.28 (m, 10H), 0.87 (t, J = 7.0 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.86, 163.25, 152.43, 149.02, 144.10, 141.07, 134.82, 134.16, 131.44, 129.32, 129.04, 128.68, 128.18, 125.82, 125.57, 120.47, 115.02, 76.86, 61.56, 59.71, 55.96, 31.98, 31.26, 30.13, 29.54, 29.39, 28.80, 28.59, 25.98, 25.71, 22.71, 14.51; HR-MS(ESI) calculated for $C_{54}H_{72}N_4O_6S$ ([M +Na]$^+$): 927.5070, found: 927.5052.

5-acylaminobiotin-11-amino-25,27-bis-decyloxy-calix[4]arene-26,28-diol (19). Prepared from compound **15** (118 mg, 0.16 mmol) and biotin N-succinimidyl ester (100 mg, 0. 29 mmol) according to general procedure. White solid; yield: 44.1% (68 mg); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.50 (s, 1H), 8.23 (s, 1H), 7.63 (s, 1H), 7.27 (s, 2H), 6.96 – 6.91 (m, 4H), 6.75 (t, J = 7.6 Hz, 2H), 6.43 (s, 1H), 6.39 (s, 2H), 6.35 (s, 1H), 4.29 – 4.24 (m, 1H), 4.1 – 4.10 (m, 5H), 3.87 (t, J = 6.0 Hz, 4H), 3.20 (d, J = 12.8 Hz, 2H), 3.11 – 3.05 (m, 1H), 2.78 (dd, J = 12.4, 5.0 Hz, 1H), 2.54 (d, J = 12.4 Hz, 1H), 2.19 (t, J = 7.2 Hz, 2H), 1.97 – 1.89 (m, 4H), 1.70 –1.61 (m, 4H), 1.59 –1.51 (m, 2H), 1.45 –1.36 (m, 4H), 1.35 – 1.23 (m, 14H), 1,23 –1.90 (m 10H) , 0.81 (t, J = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.85, 163.25, 152.41, 149.01, 144.82, 141.70,134.72, 134.17, 131.46, 129.32, 129.08, 128.73, 128.15, 125.58, 120.45, 115.70, 76.85, 61.56, 59.71, 55.96, 31.94, 31.22, 30.14, 29.75, 29.71, 29.59, 29.37, 28.81, 28.60, 25.98, 25.71, 22.68, 14.50; HR-MS(ESI) calculated for $C_{58}H_{80}N_4O_6S$ ([M +Na]$^+$): 983.5696, found: 983.5683.

5-acylaminobiotin-11-amino-25,27-bis-dodecyloxy-calix[4]arene-26,28-diol (20). Prepared from compound **16** (630 mg, 0.79 mmol) and biotin N-succinimidyl ester (420 mg, 1.23 mmol) according to general procedure. White solid; yield: 42.0% (340 mg); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.47 (s, 1H), 8.23 (s, 1H), 7.53 (s, 1H), 7.26 (s, 2H), 6.95 –6.91 (m, 4H), 6.74 (t, J = 7.6 Hz, 2H), 6.43 (s, 1H), 6.34 (s, 1H), 6.32 (s, 2H), 4.28 –4.25 (m, 1H), 4.14 – 4.06 (m, 5H), 3.86 (t, J = 6.0 Hz, 4H), 3.16 (d, J = 12.4 Hz, 2H), 3.07 (m, 1H), 2.78 (dd, J = 12.4, 5.0 Hz, 1H), 2.54 (d, J = 12.4 Hz, 1H), 2.18 (t, J = 7.2 Hz, 2H), 1.97 – 1.89 (m, 4H), 1.68 –1.61 (m, 4H), 1.58 – 1.51 (m, 2H), 1.45 –1.40 (m, 4H), 1.33 –1.24 (m, 12H), 1.22 –1.17 (m, 20H), 0.80 (t, J = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.33, 162.76, 151.90, 148.50, 143.59, 140.60, 134.32, 133.64, 130.97, 128.82, 128.53, 128.17, 127.65, 125.07, 119.89, 114.49, 76.32, 61.07, 59.22, 55.46, 31.43, 30.77, 29.66, 29.26, 29.22, 29.14, 28.86, 28.32, 28.10, 25.50, 25.22, 22.19, 13.98; HR-MS(ESI) calculated for $C_{62}H_{88}N_4O_6S$ ([M +Na]$^+$): 1039.6322, found: 1039.6300.

General procedure for the preparation of compounds BPCA1-BPCA4

Compound **17** (76 mg, 0.089 mmol) was dissolved in N,N-dimethylformamide (5 mL) with stirring. To this solution was added slowly N,N-dimethylformamide solution (2 mL) containing $mPEG_{1000}$-NHS (135 mg), and the solution was left to stir for 48 h at room temperature. The solvent was removed and the residue was purified by flash chromatography on silica gel (dichloromethane/methanol = 50/1) to afford a light-yellow viscous product **BPCA1** (42 mg, 50.71%).

According to general procedure, **BPCA2-BPCA4** was prepared from compound **18-20** (72 mg, 0.079 mmol/68 mg, 0.071 mmol/260 mg, 0.255 mmol) and $mPEG_{1000}$-NHS (120 mg/106 mg/400 mg) in the yields of 37.0%, 49.0% and 48.5%, respectively.

General procedure for the preparation of contrasted compound PCA4

To a stirred solution of compound **16a** (31 mg, 0.033 mmol) in N, N-dimethylformamide (2 mL) was dropped triethylamine (5 µL, 0.038 mmol), followed by slow addition of $mPEG_{1000}$-NHS (35 mg) in THF (4 ml) and reacted at room temperature for 4 h. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate = 15/8) to obtain compound **PCA4** in the yield of 39.9%.

Preparation and characterization of unloaded (blank) and DOX-loaded micelles

Preparation of DOX-loaded and unloaded (blank) micelles

20 mg of doxorubicin hydrochloride (DOX · HCl) was dissolved in 6 mL of ultrapure water. And then, 15 µL of triethylamine was added and stirred at room temperature for 6 h. The solution was centrifuged to discard the supernatant, wash the precipitate with ultrapure water, and centrifuge again until the pH is neutral. The supernatant was collected and freeze-dried to obtain *free doxorubicin*.

20 mg of **BPCA4** (or **PCA4)** and 2 mg of DOX were dissolved in 2 ml of DMSO by ultrasound method. Then, the solution was put into a dialysis bag (MWCO: 1000Da) using 500 mL of distilled water as the external dialysate and dialyzed for 24 h, followed by the collection of the dialysate, centrifugation, and cold freeze-drying to obtain drug-loaded **BPCA4-DOX and PCA4-DOX** micelles, which were stored in a refrigerator at 4 °C. The un-loaded micelles **BPCA1-BPCA4** were prepared with 20 mg of **BPCA1-BPCA4** and 2 mg of DOX in 2 ml of DMSO using similar dialysis techniques.

Determination of DOX Loading Capacity by the self-assembly ability

To prepare the samples, the DOX-loaded calixarene micelles were centrifuged (5000 r/min, 15 min), the supernatant was collected, and the supernatant was freeze-dried to obtain a lyophilized powder. The lyophilized powder was dissolved in methanol, and the absorbance at 490 nm was measured at 25 °C using a microplate reader. The content of DOX was calculated from a standard curve.

The encapsulation efficiency (DLE) and drug loading content (DLC) were calculated by the following equations:

$$\text{DLC (\%)} = \frac{Weight\ of\ encapsulated\ drug\ in\ micelles}{Total\ mass\ of\ micelles\ and\ encapsulated\ drugs} \times 100\%$$

$$\text{EE (\%)} = \frac{Weight\ of\ the\ drug\ contained\ in\ micelles}{Total\ mass\ of\ micelles\ and\ encapsulated\ drugs} \times 100\%$$

Hydrodynamic Diameter and ζ-Potential Measurements

The average particle size and ζ-potential of self-assembled micelles were determined using a nanoparticle-Zeta potentiometer (Nicomp 380/ZLS) with the following specifications: Sampling time: automatic; the number of measurements: 3 per sample; viscosity: 0.933 cP; refractive index: 1.333; scattering angle: 90°; λ = 633 nm; temperature: 25 °C. Instrument performance was checked with 90 nm monodisperse latex beads (Coulter) or DTS 50 standard solution (Malvern) for DLS before each series of experiments.

Transmission Electron Microscopy

TEM micrographs of unloaded (blank) and DOX-loaded calixarene micelles were taken using a transmission electron microscope (Tecnai Spirit G2 TWIN). The sample was prepared according to the following procedure: 0.1 mL of the micelles' suspension was placed on the front side of the copper mesh, allowed to stand for 15 min, and the excess suspension was blotted dry with filter paper. 0.1 ml of 4% uranyl acetate negative dye was dropped onto the front side of the copper mesh, stained for 3 min, and then the remaining negative dye solution was blotted with filter paper. The measurements were carried out after

In vitro DOX release kinetics

In vitro DOX release kinetics studies were performed by dialysis (MWCO: 10 kDa) in phosphate-buffered saline (PBS, 0.01 M, pH 7.3/6.5, 3% SDS) at various pH conditions. The prepared DOX-loaded micelles suspension was dialyzed against 5 mL of PBS under constant agitation. All of the extra dialysis solutions were taken at the scheduled sampling time, measured by fluorescence spectrum and supplemented with 5 mL of pure water as an extra dialysis solution. The cumulative release of DOX was calculated from the standard curve.

Job curve method for determining the stoichiometric ratio of DOX with BPCA4

Maintain a total concentration of 1.84 $\times 10^{-6}$ mol·L-1 constant, transfer a certain amount of DOX reserve solution and BPCA4 reserve solution, respectively in the order of 10:0, 9:1, 8.5:1.5, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9 in the concentration ratio of DOX to BPCA4. After mixing, ultrasound for 2 h and measure the fluorescence spectrum curve. The molar concentration of compound DOX, BPCA4 is represented by CDOX, CBPCA4, and the fluorescence intensity measured when the concentration ratio of DOX to the BPCA4 is 10:0 is I0. Plot Job's plot of the fluorescence emission intensity of DOX with BPCA4.

Cell culture

MCF-7, MDA-MB-231 (Human breast cancer cells), HeLa（Human cervical cancer cells, HUVEC (Human umbilical vein endothelial cells), MCF-10A(Human breast cells), 4T1 cells (Mouse breast cancer Cells), were kindly provided by KeyGEN BioTECH (Nanjing, China). All the cells were cultured at 37 ^{o}C in a 5% CO_2 humidified atmosphere. HUVEC and MCF-10A have been maintained in RPMI-1640 medium supplemented with 10% FBS, 1 % penicillin and streptomycin.

Flow cytometry

MCF-7 cells(density = 6 × 10^5 cells) were inoculated into 6-well plates 1.5 mL of cell suspension and 1 mL of complete culture medium were added into each well and cultured in a 37 ° C, 5% CO_2 incubator. After the cells adhered to the wall completely, the culture medium was discarded, followed by the addition of 1 mL of PBS buffer solution 3 times. The cells were treated with drug-loaded micelle **BPCA4** (or **PCA4**) solution (concentration of DOX: 4 µg/mL) and cultured for 0.5 h and 4 h, respectively. Then, the cells were trypsin zed, washed in PBS and stabilized in 70 % ethanol cooled. Afterward, cells were stained by PI/RNase A following the manufacturer's directions and data were measured by Modfit software.

In vitro uptake assay

To investigate if the cell penetration efficiency would be affected by biotin moiety, a fluorescence microscopy assay was performed by observing the uptake of DOX micelles by MCF-7 cancer cells. The drug-loaded **FITC-BPCA4-DOX** micelles were initially prepared by the addition of 2 mg of DSPE-PEG-FITC in 2 mL DMSO to the mixture of 2 mg of DOX and 20 mg of blank **B**P**CA4**, dialysis with 500 mL distilled water as dialysate for 24 h, collection of dialysates. In contrast to the biotin-containing **FITC-BPCA4-DOX** micelle, **FITC-PCA4-DOX** micelle was formed according to a similar procedure. Thus, the position of the micelles was marked by the green fluorescence of FITC. Then, the drug-loaded micelles were incubated with MCF-7 cells for 0.5 h and 4 h, respectively. The cells were washed three times with 0.01 M PBS buffer at pH 7.4. Subsequently, the cells were fixed with 4% paraformaldehyde for 20 min, and then the cells were washed three times with 0.01 M PBS buffer at pH 7.4. And then, the nuclei were stained with DAPI dye for 20 min in the dark, the stain was removed, and the cells were washed three times with 0.01 M PBS buffer, pH 7.4, and stored with 0.9% NaCl solution. The coverslip containing the cells was placed on a glass slide, observed under an inverted fluorescence microscope, and taken the fluorescent photograph.

Cytotoxicity assay

Cell viability was assessed by MTT cell proliferation assay with a minor modification. The cells were cultured in a logarithmic growth phase medium containing 10% FBS and seeded in a 96-well plate (Costar, Corning) culture containing 100 μL per well. The cells were preincubated for 24 h at 37 ºC in a 5% CO_2 incubator. The cells were treated with different concentrations of drug-loaded **BPCA4-DOX** (or **PCA4-DOX**) and free DOX dissolved in DMSO for 72 h. Further, 100 μL of the MTT solution (concentration: 0.5 mg/mL) was added to each well, and the cells were cultured for 4 h in the dark for the color reaction. Next, the medium was carefully discarded from each well and 100 μL DMSO was added to each well, shaken well and dissolved in the oven for 10 min so that the crystals were fully dissolved and evenly mixed. The OD (Optical Density) value of each well was measured by a microplate reader at a wavelength of 490 nm. Cell viability was defined as the ratio of the absorbance of the treated cells to the absorbance of the control groups. IC_{50} values were calculated by GraphPad Prism 6. All assays were performed in triplicate and repeated thrice.

In vivo antitumor test

Female Kunming mice (4 weeks, 18 to 20 g) were purchased from the Animal Center of Xuzhou Medical University. Animal procedures were in agreement with the approved protocols by the Institutional Animal Care and Use Committee. After 1 week, 4T1 cells (2×10^6 cells/mL) in DMEM (100 μL) were subcutaneously injected to the back of each mouse on the right side. When the tumor volume reached 100 mm^3, the 4T1 tumor-bearing mice injected via tail vein with 0.9% saline 0.1 mL were used as the control group. DOX-loaded **BPCA4** 0.1 mL with a concentration of 19 mg/mL (DOX dosage 5 mg/kg), DOX-loaded **PCA4** 0.1 mL with a concentration of 19 mg/mL (DOX dosage 5 mg/kg) and DOX·HCl 0.1mL (5 mg/kg) were injected as an experimental group, respectively. Tumor size was monitored and measured by caliper measurements over a period of 10 days. The volume was calculated using the formula: tumor volume $a\times b^2/2$ (where a is the largest length and b is the smallest width). After the mouse was sacrificed, necropsies were performed, and the tumors were removed, weighed.

Histopathological analysis

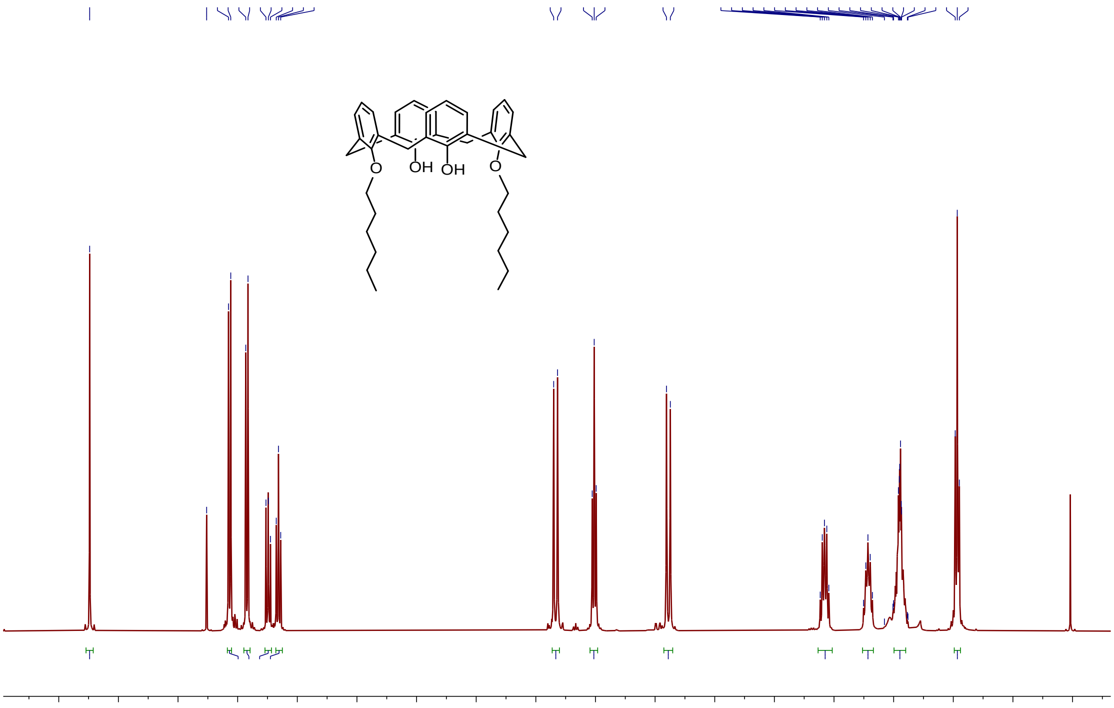
After 14 days, the treated mice were sacrificed and their major organs (heart, liver, spleen, lung and kidney) were collected.

The harvested major organs were immersed in 4% paraformaldehyde and embedded in paraffin. Then tissues were stained by H&E to evaluate the systemic toxicity and imaged with microscopy.

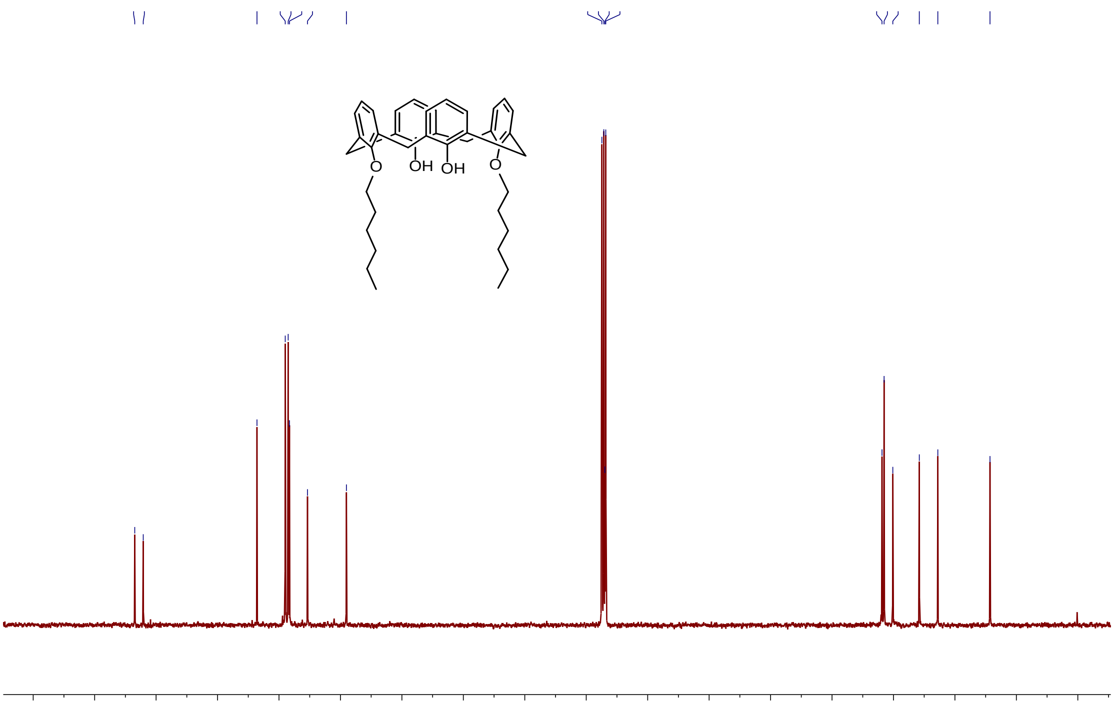
Statistical analysis

All results were expressed as mean ± standard deviation (SD) of three independent experiments performed in triplicates. Statistical analyses were carried out using SPSS 16.0 software package and One-way ANOVA was used to compare the multiple groups with a significance level of $p < 0.05$.

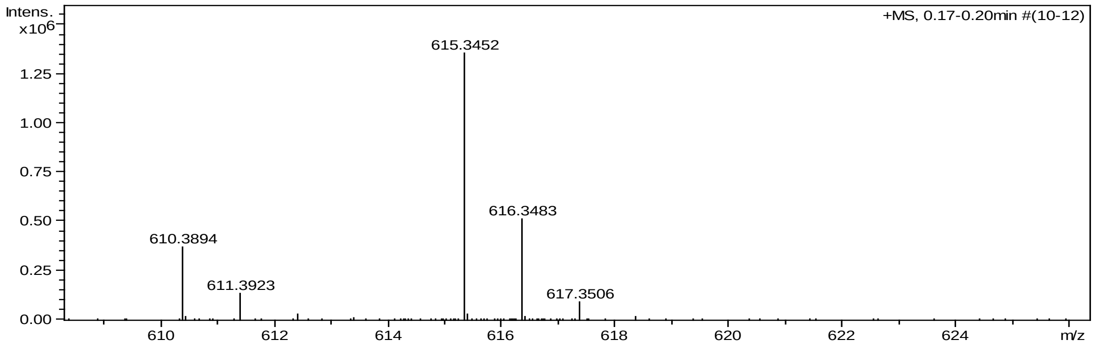
^{1}H NMR of compound **5**



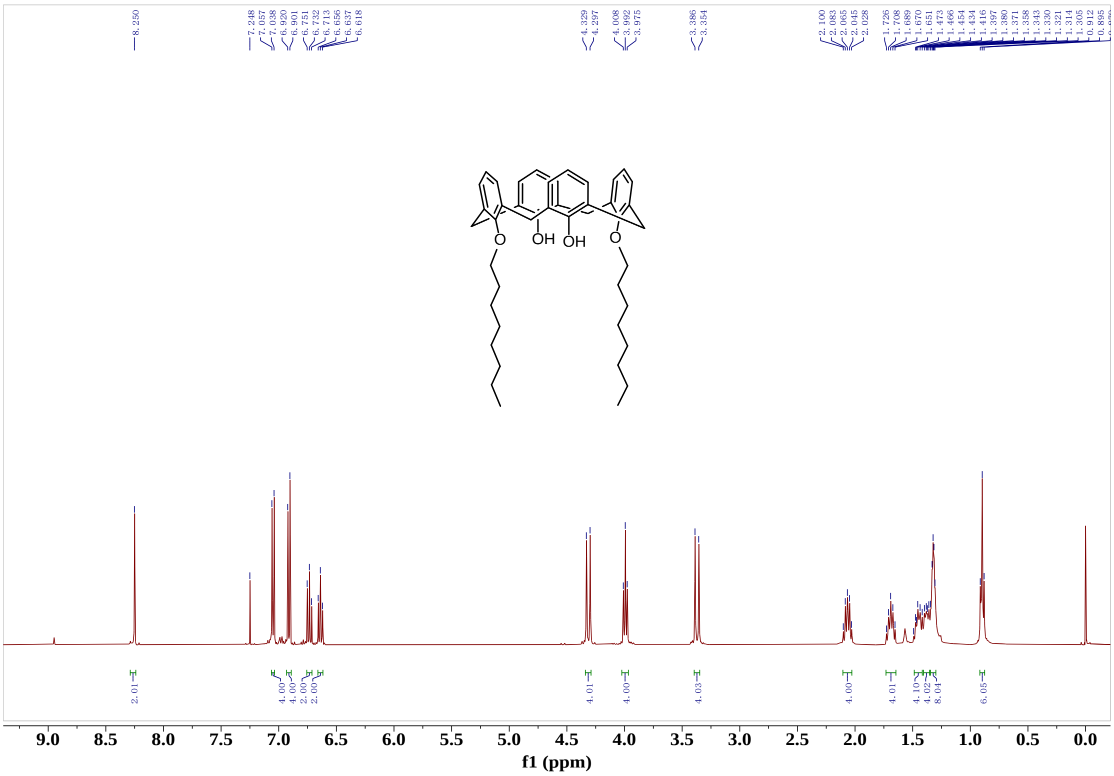
^{13}C NMR of compound **5**



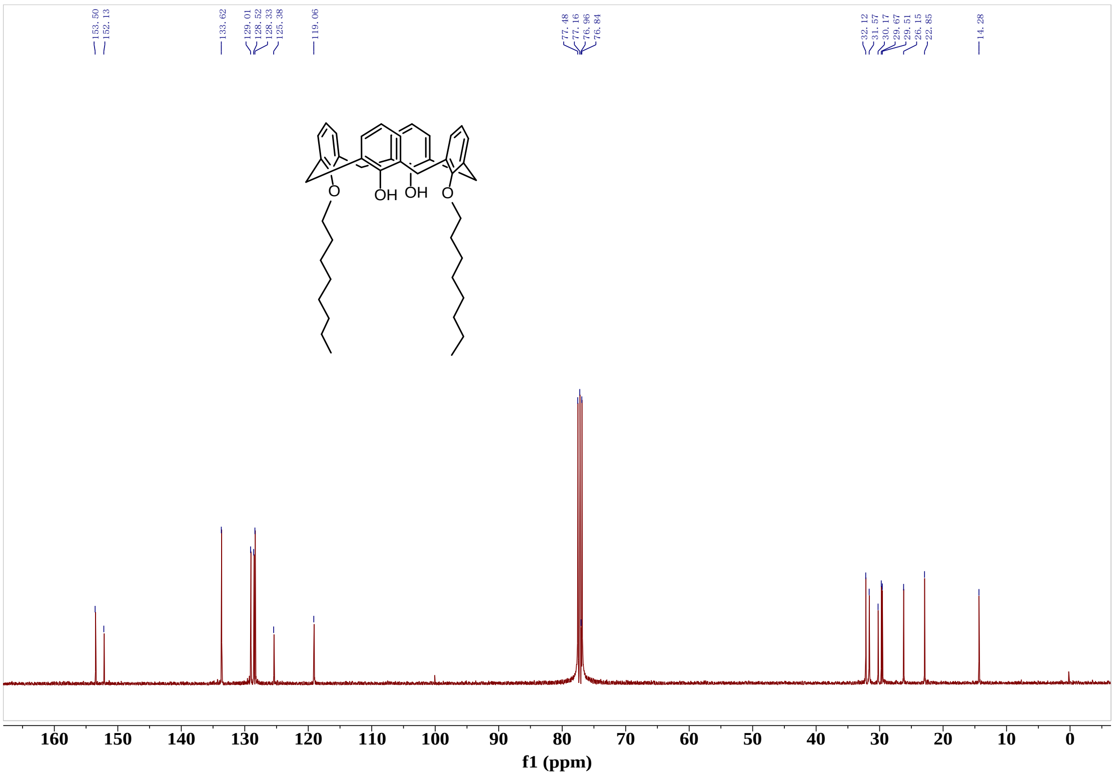
HR-MS of compound **5** ([M +Na]$^+$): 615.3452



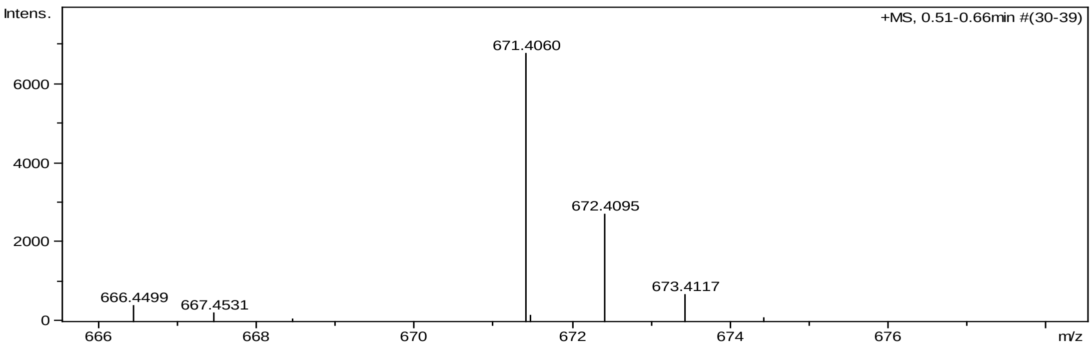
^{1}H NMR of compound **6**



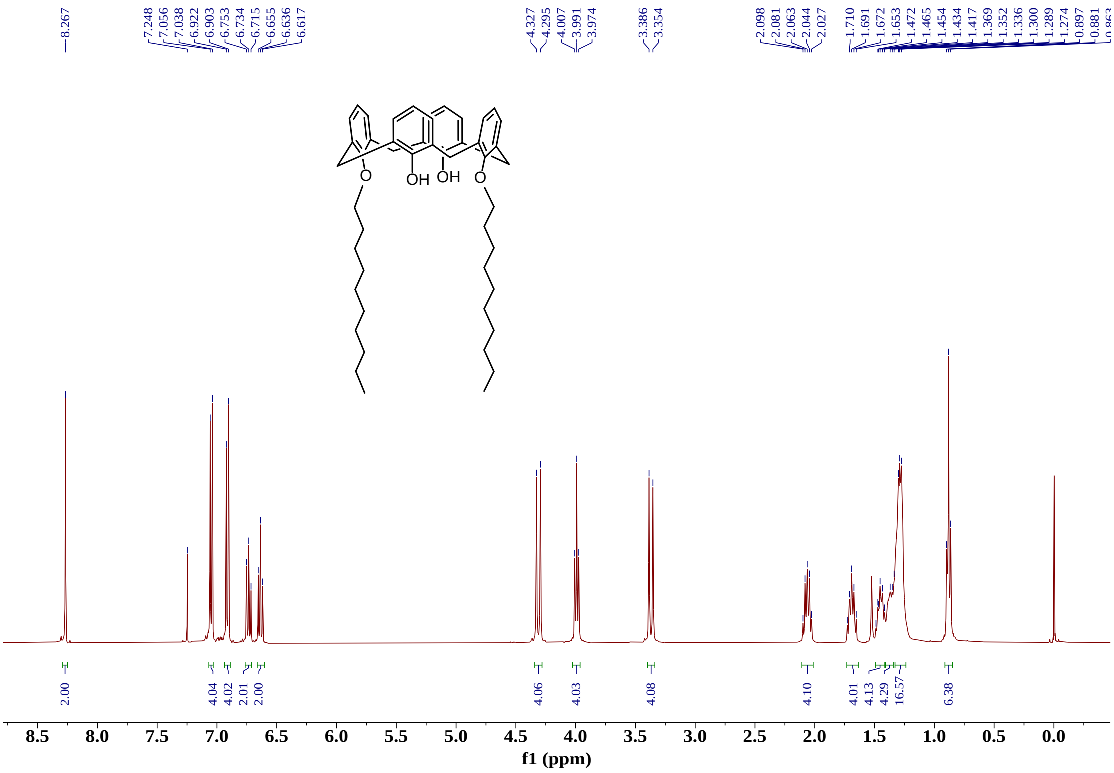
^{13}C NMR of compound **6**



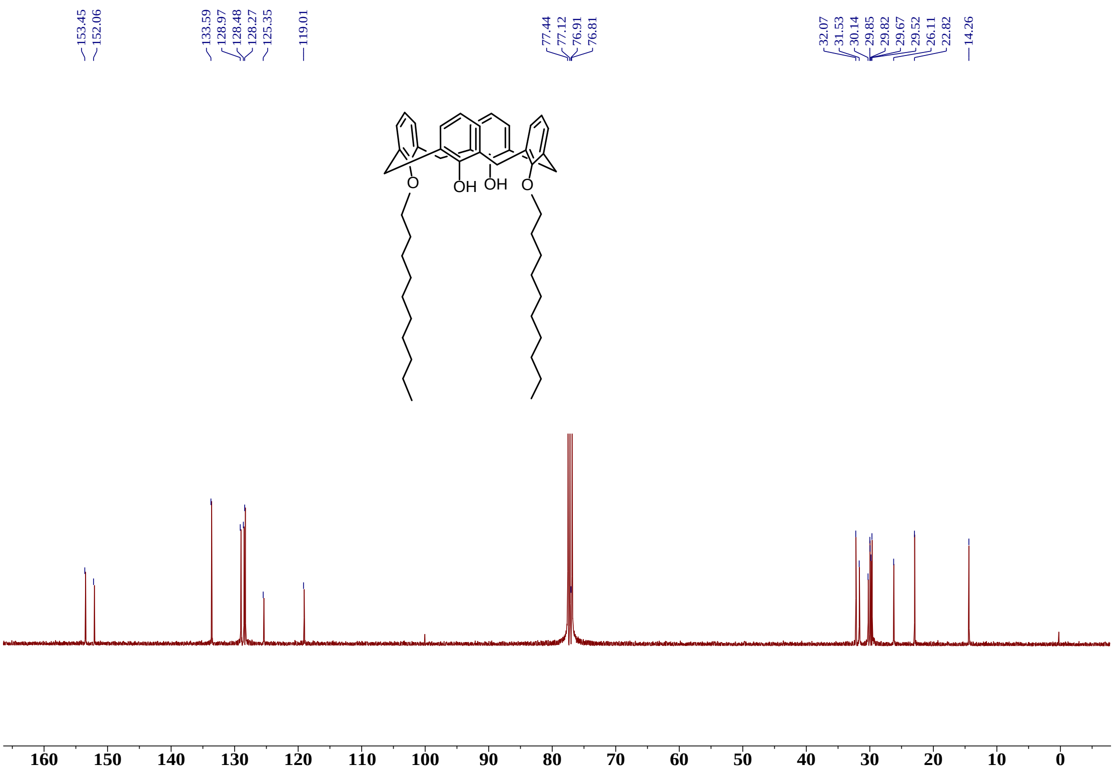
HR-MS of compound **6** ([M + Na^+]): 671.4060



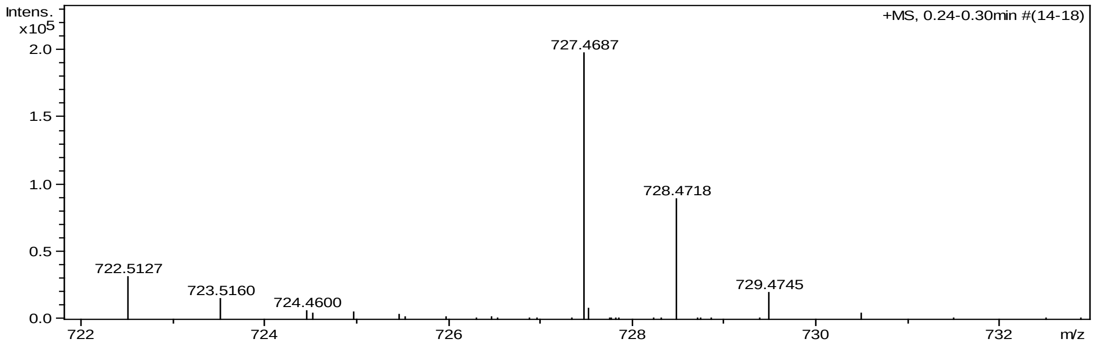
[1]H NMR of compound **7**



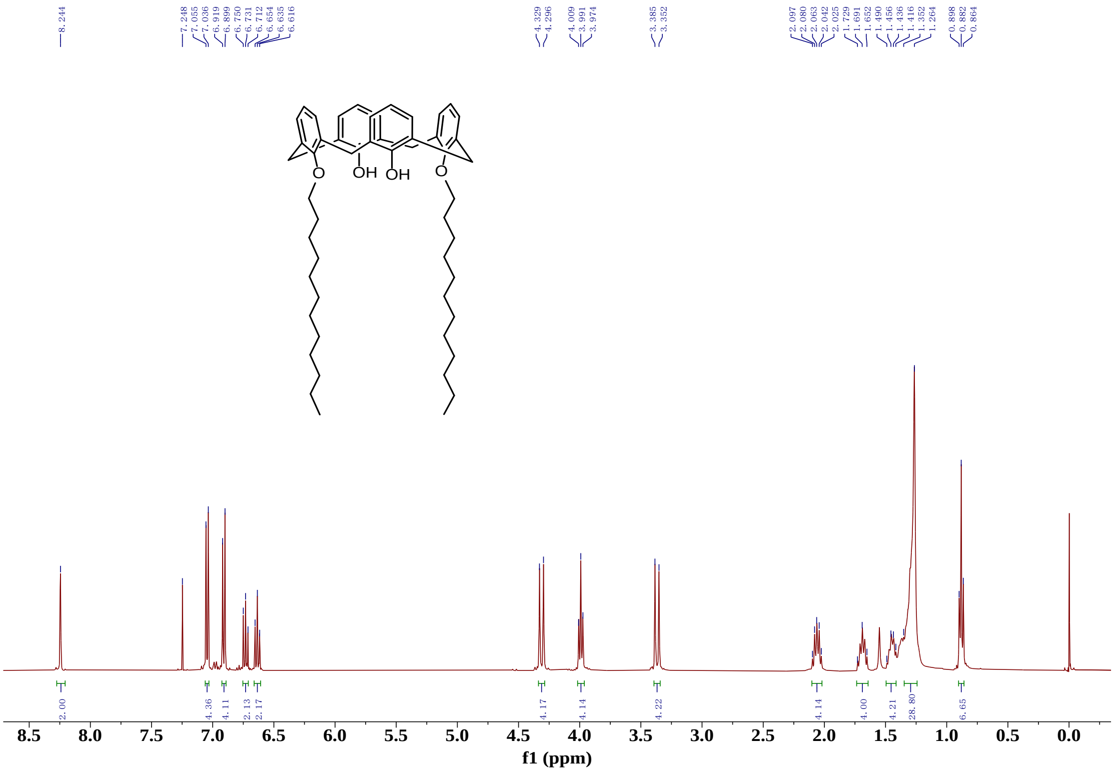
[13]C NMR of compound **7**



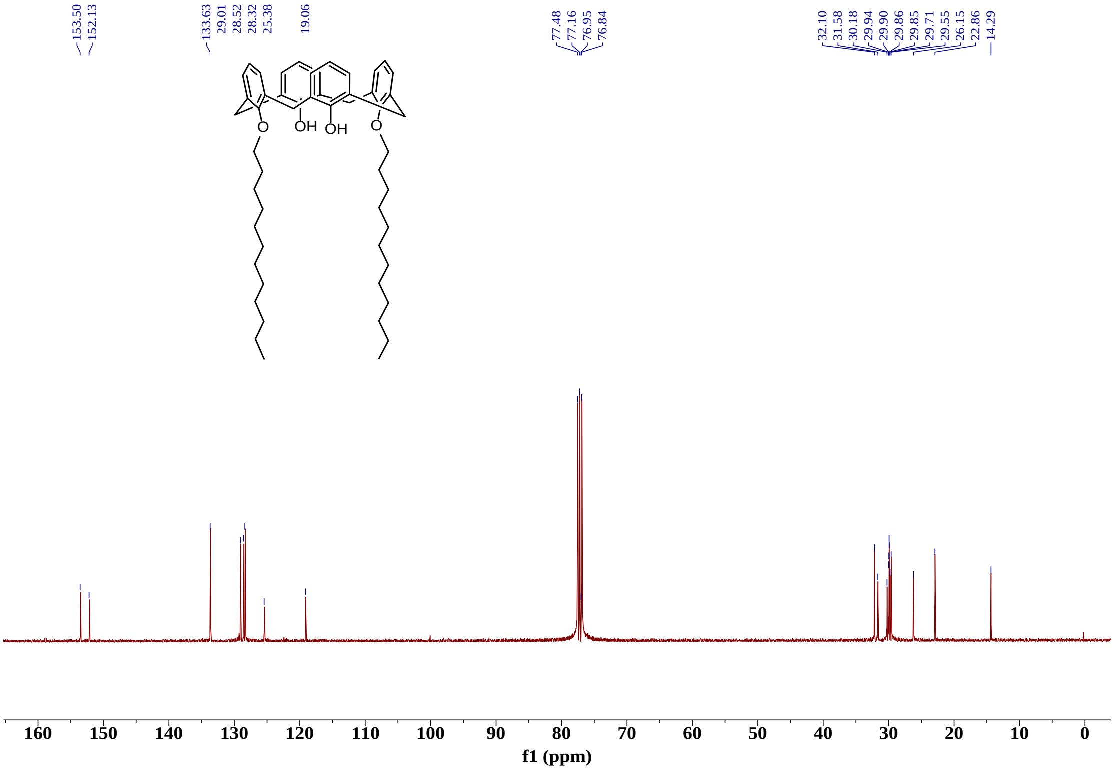
HR-MS of compound **7** ([M + Na$^+$]): 727.4687



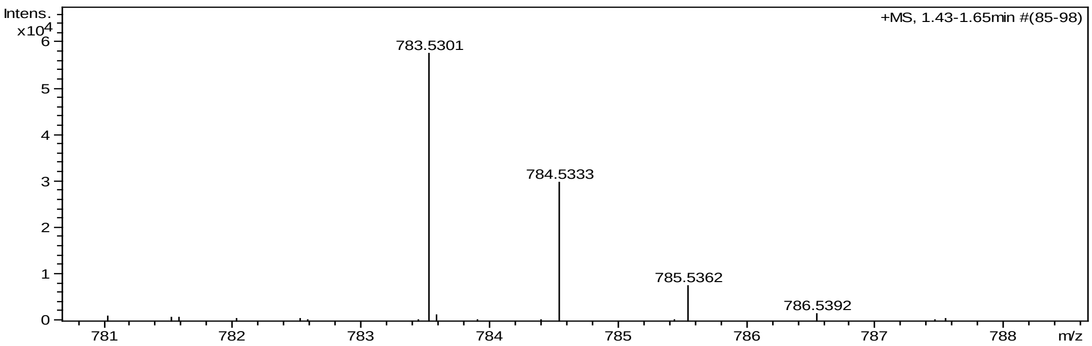
^{1}H NMR of compound **8**



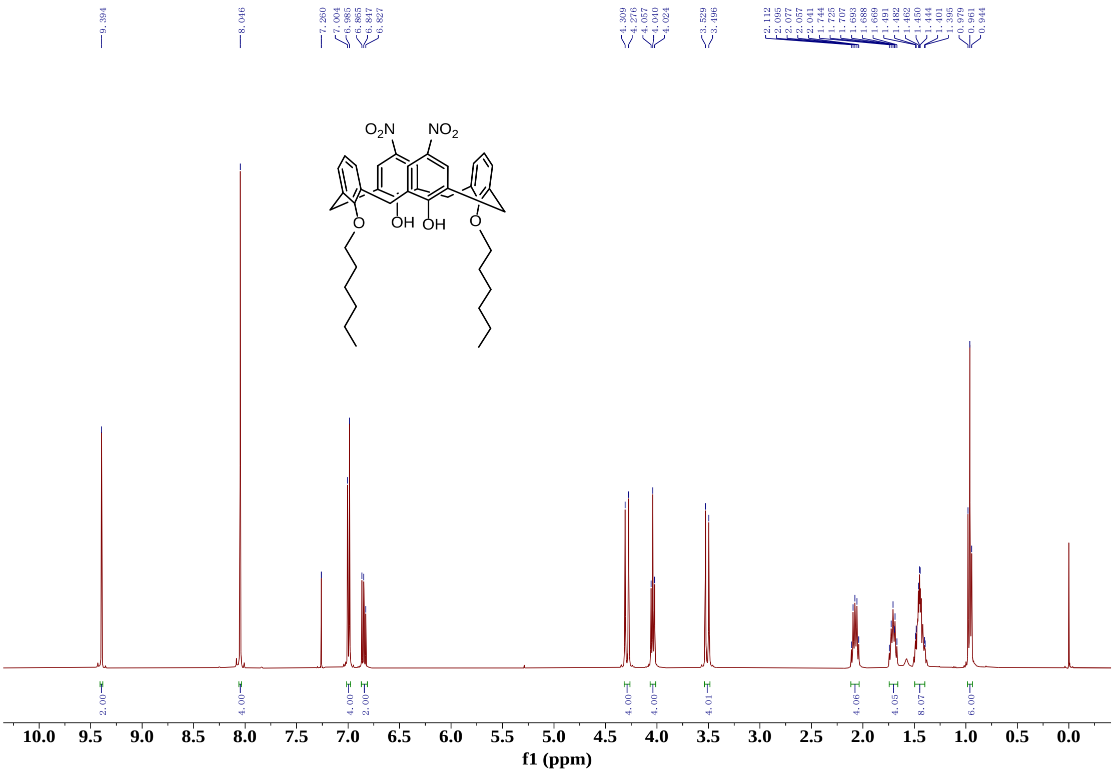
^{13}C NMR of compound **8**



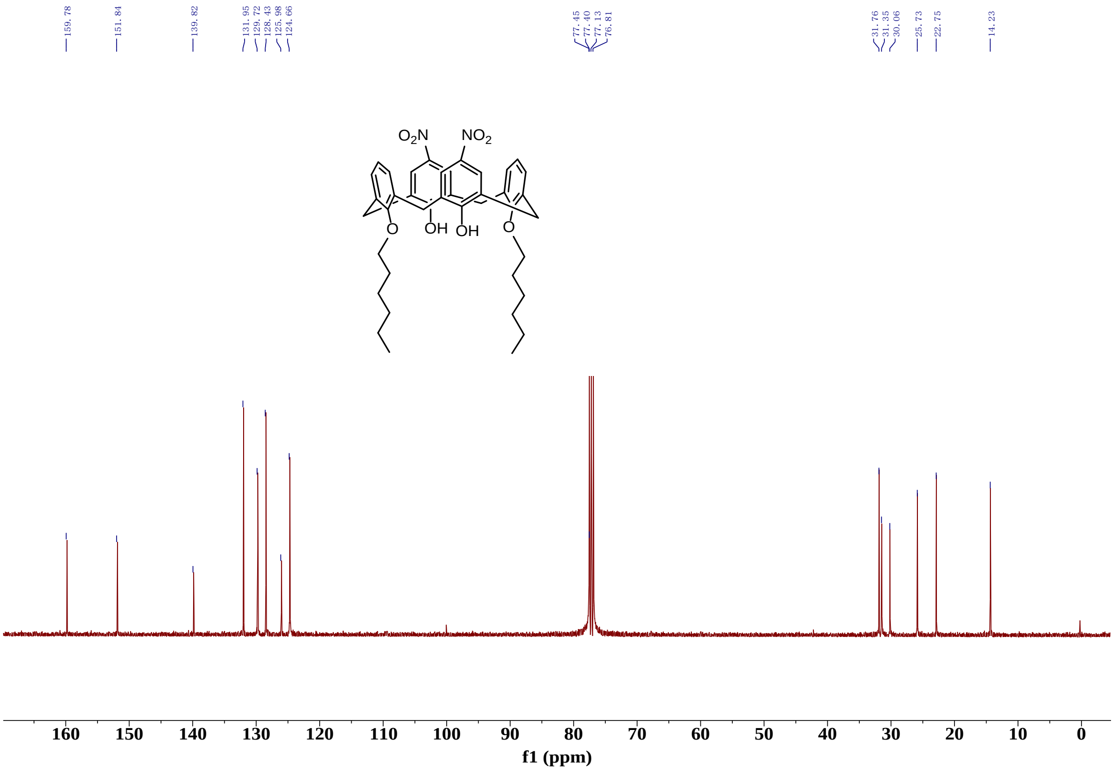
HR-MS of compound **8** ([M + Na$^+$]): 783.5301



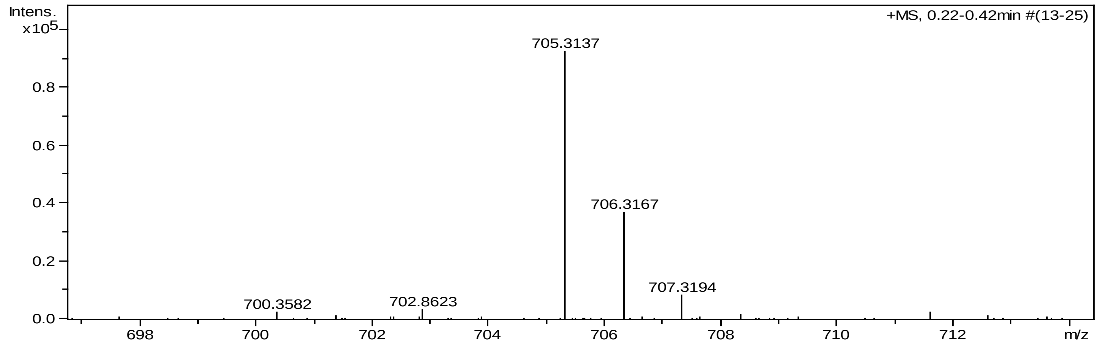
[1]H NMR of compound **9**



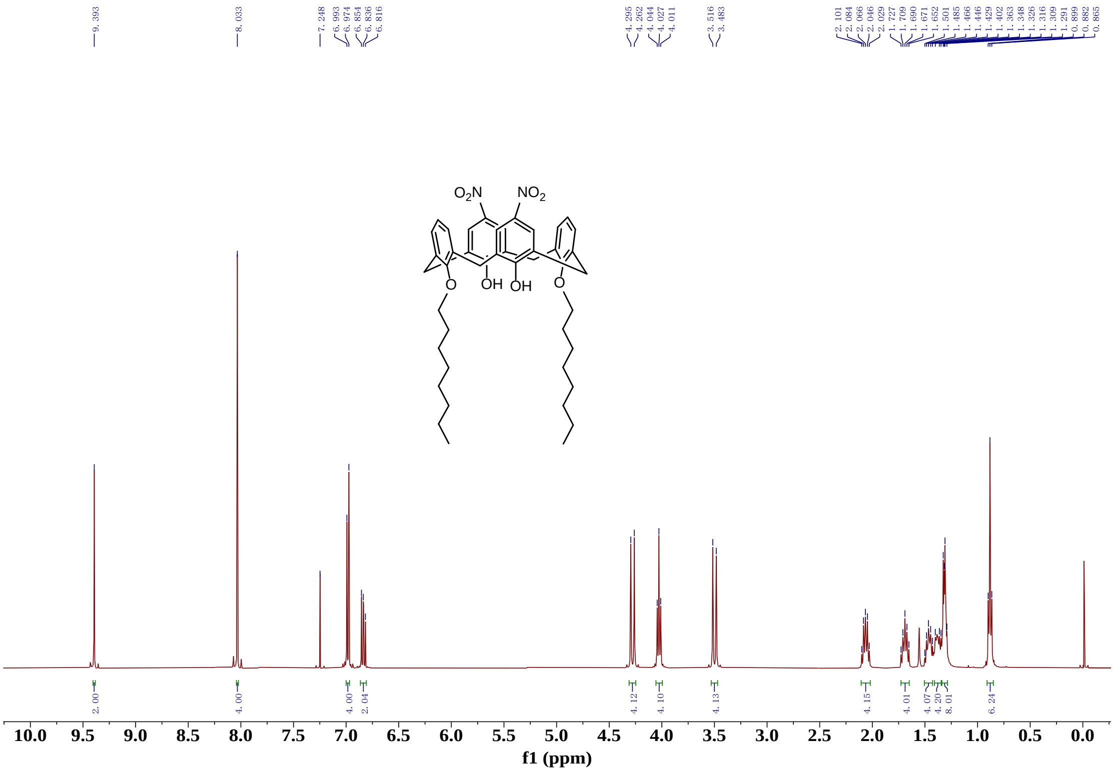
[13]C NMR of compound **9**



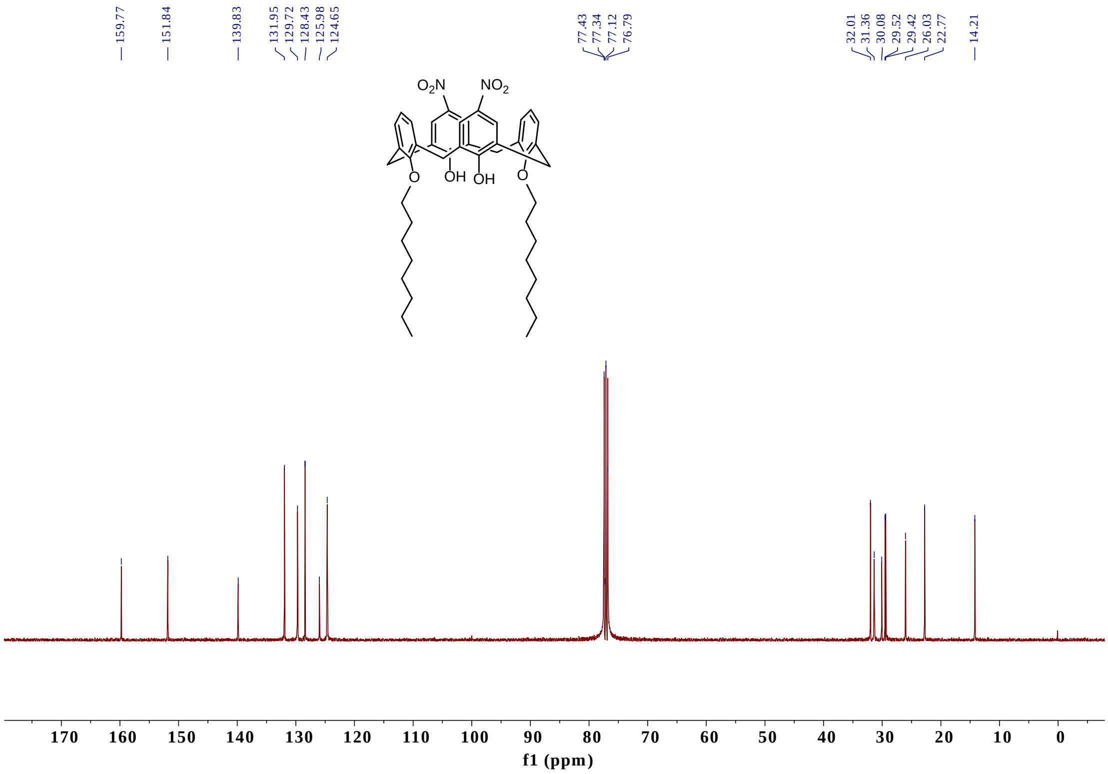
HR-MS of compound **9** ([M +Na]$^+$): 705.3137



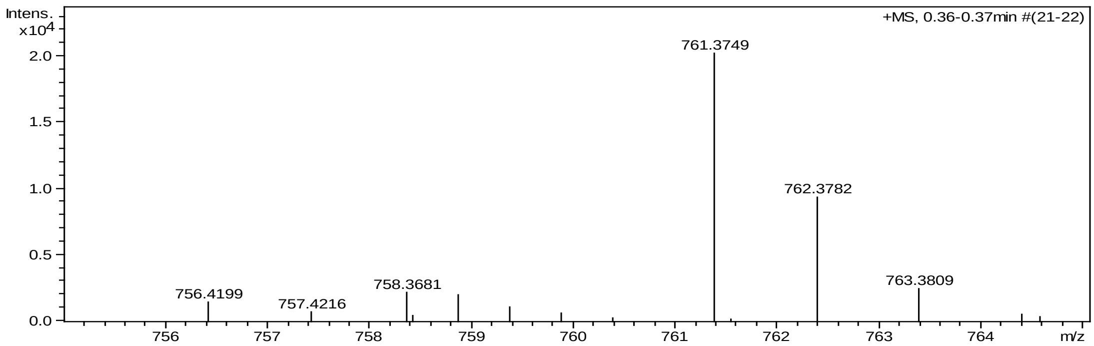
^{1}H NMR of compound **10**



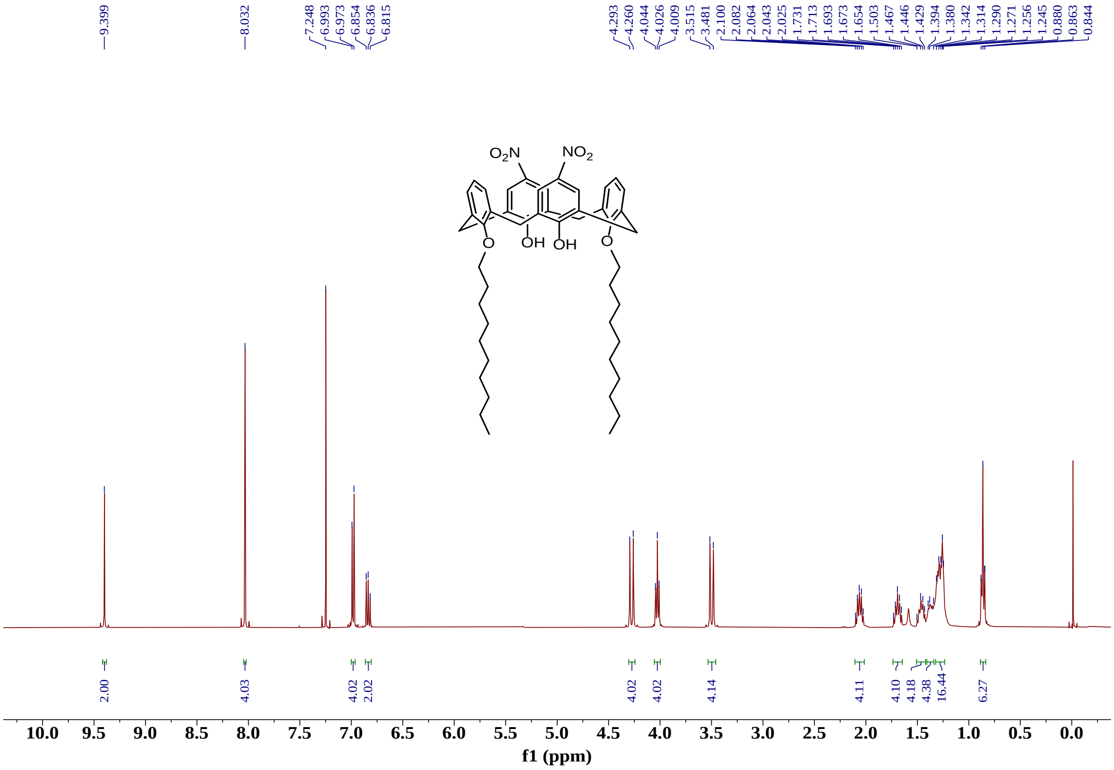
^{13}C NMR of compound **10**



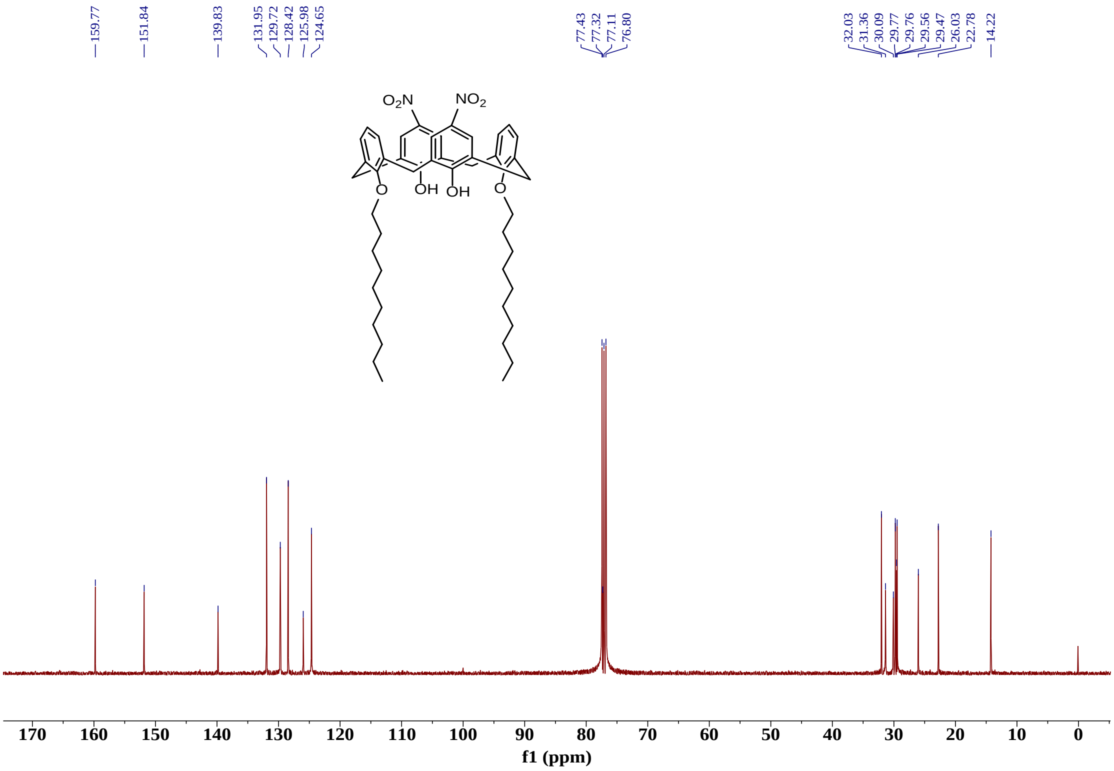
HR-MS of compound **10** ([M + Na$^+$]: 761.3749



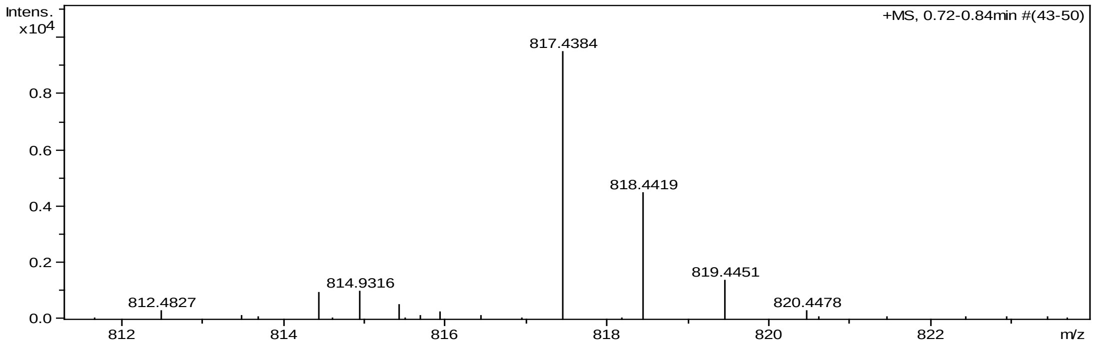
^{1}H NMR of compound **11**



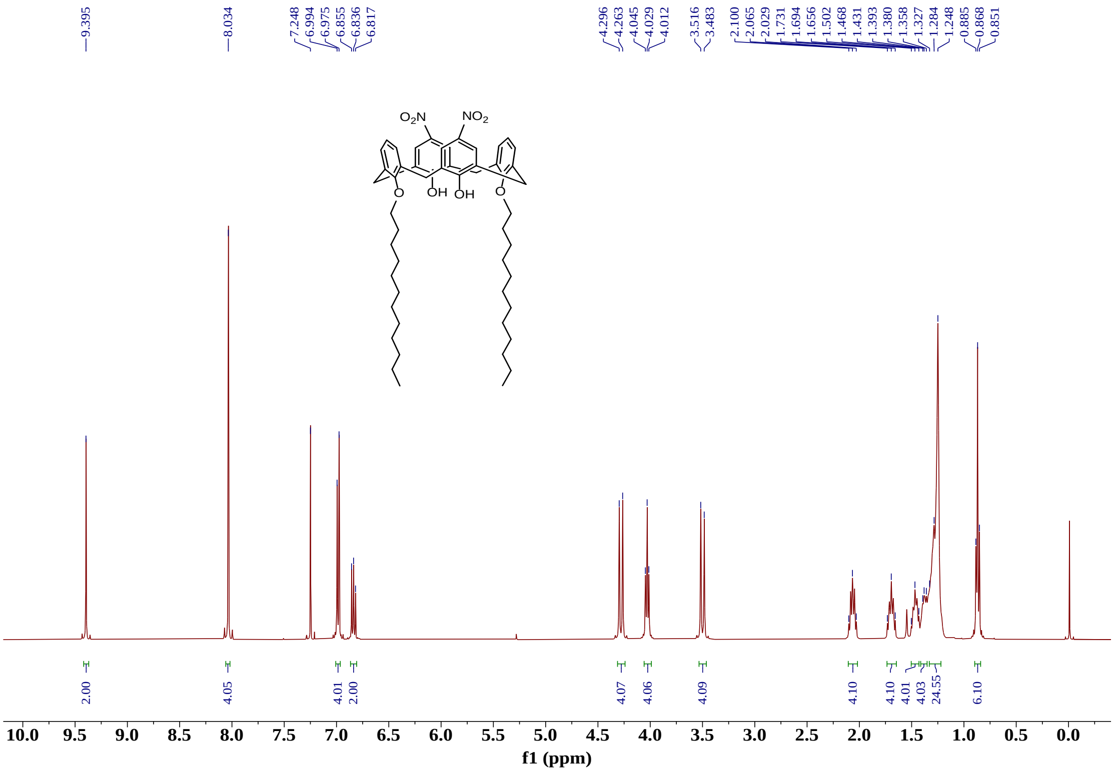
^{13}C NMR of compound **11**



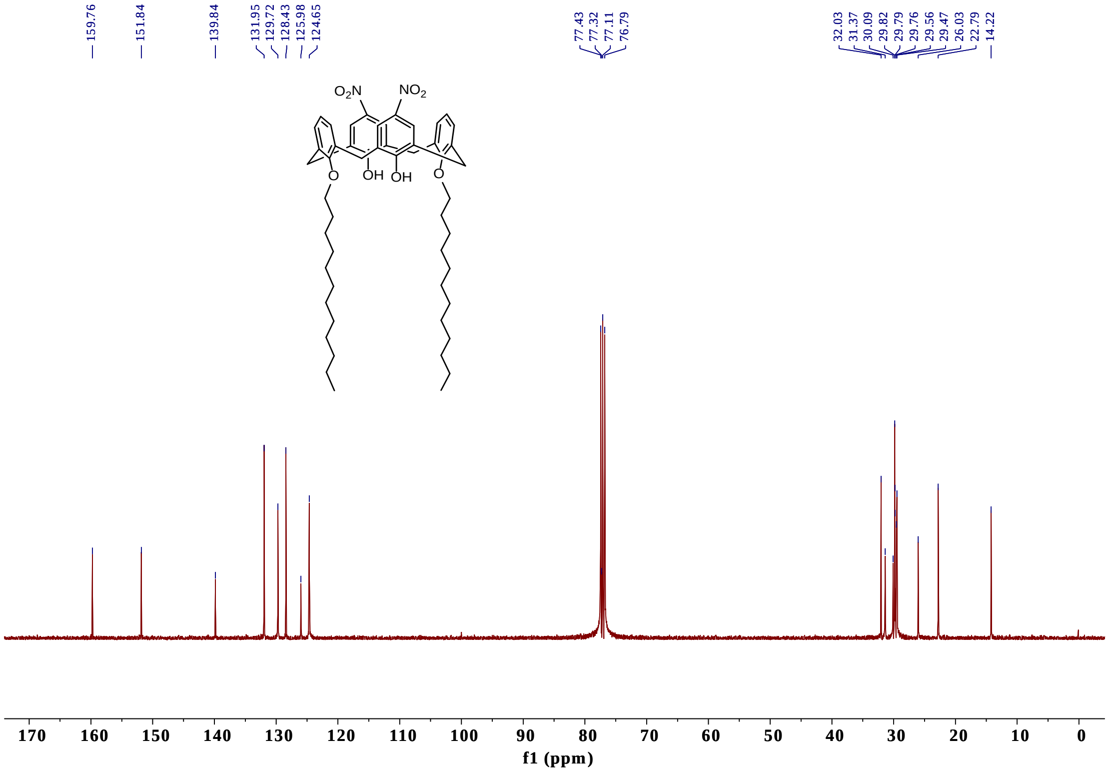
HR-MS of compound **11** ([M + Na⁺]): 817.4384



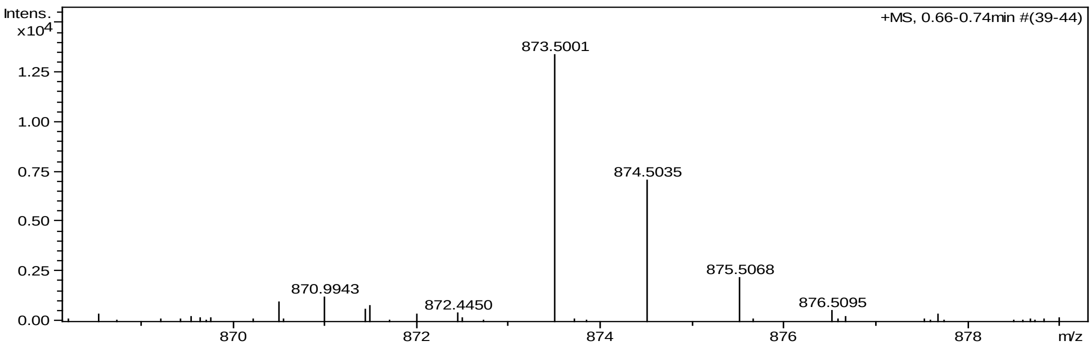
[1]H NMR of compound **12**



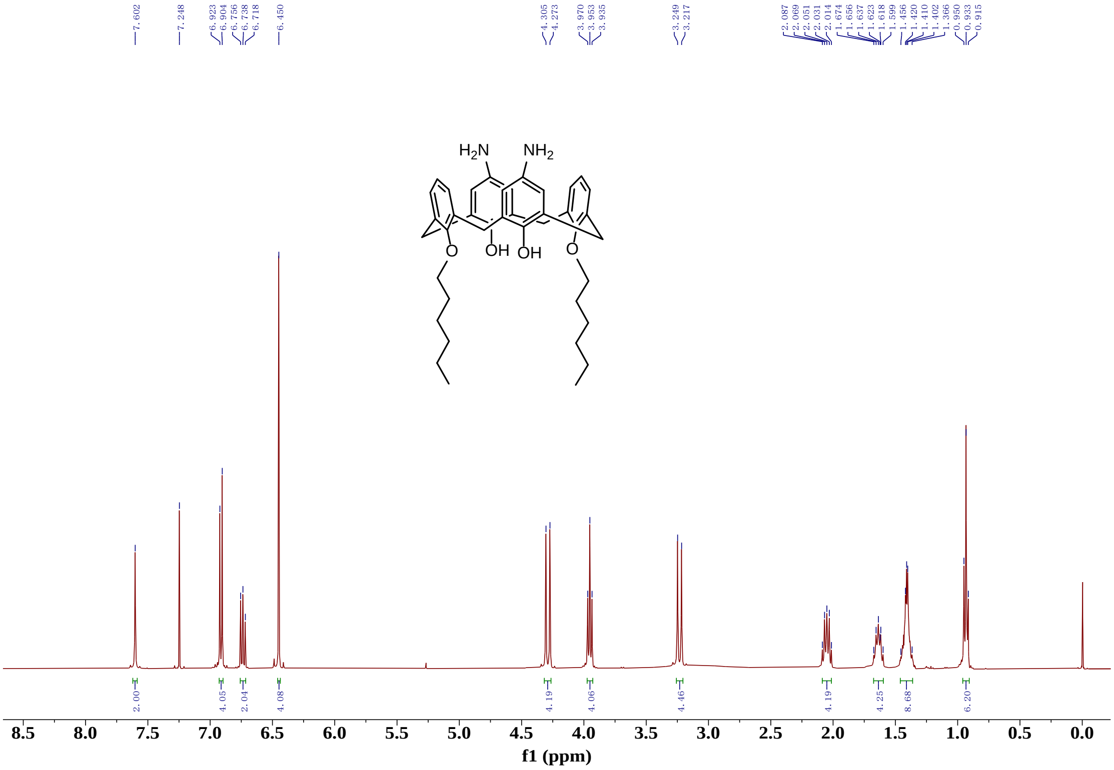
^{13}C NMR of compound **12**



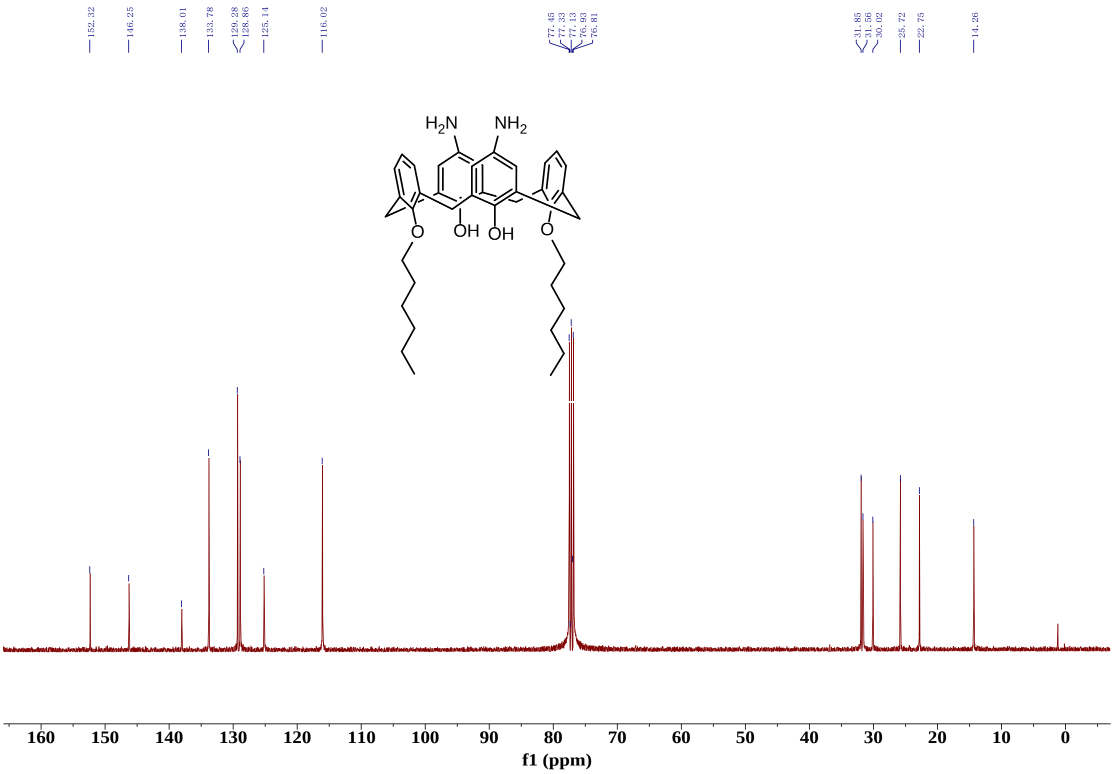
HR-MS of compound **12** ([M + Na$^+$]):: 873.5001



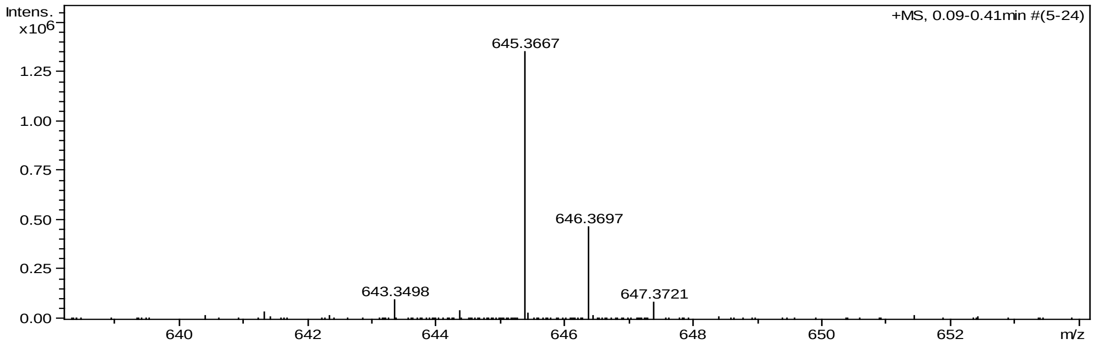
[1]H NMR of compound **13**



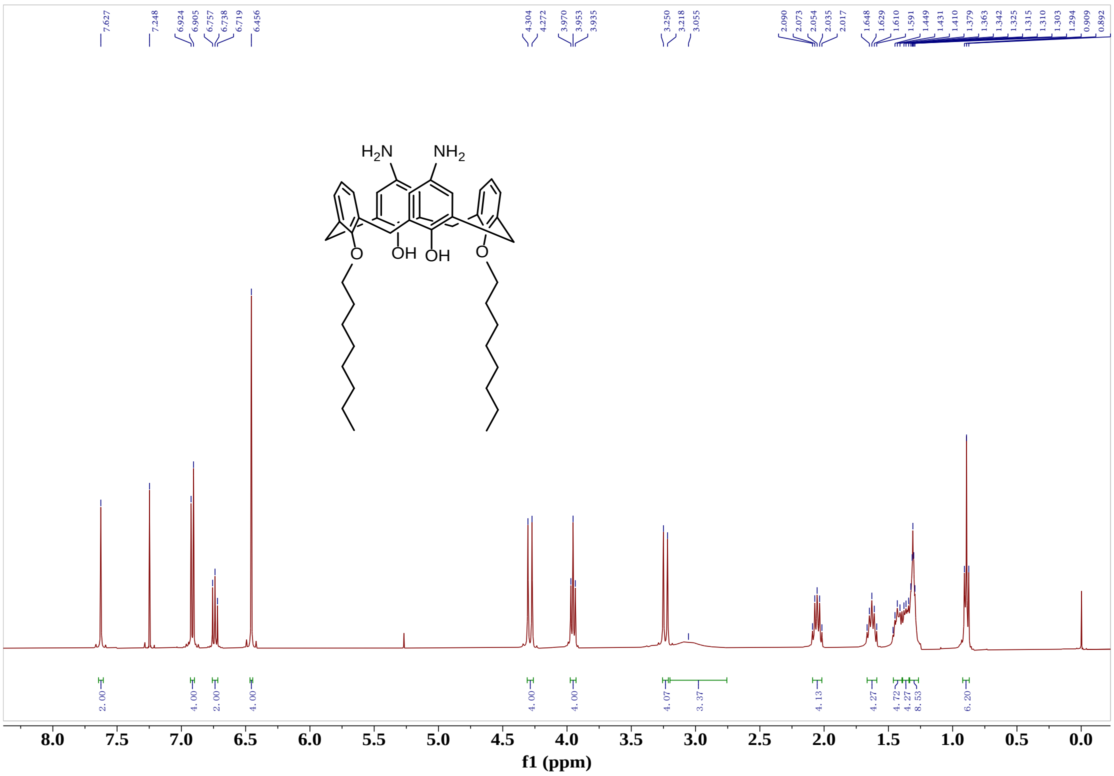
[13]C NMR of compound **13**



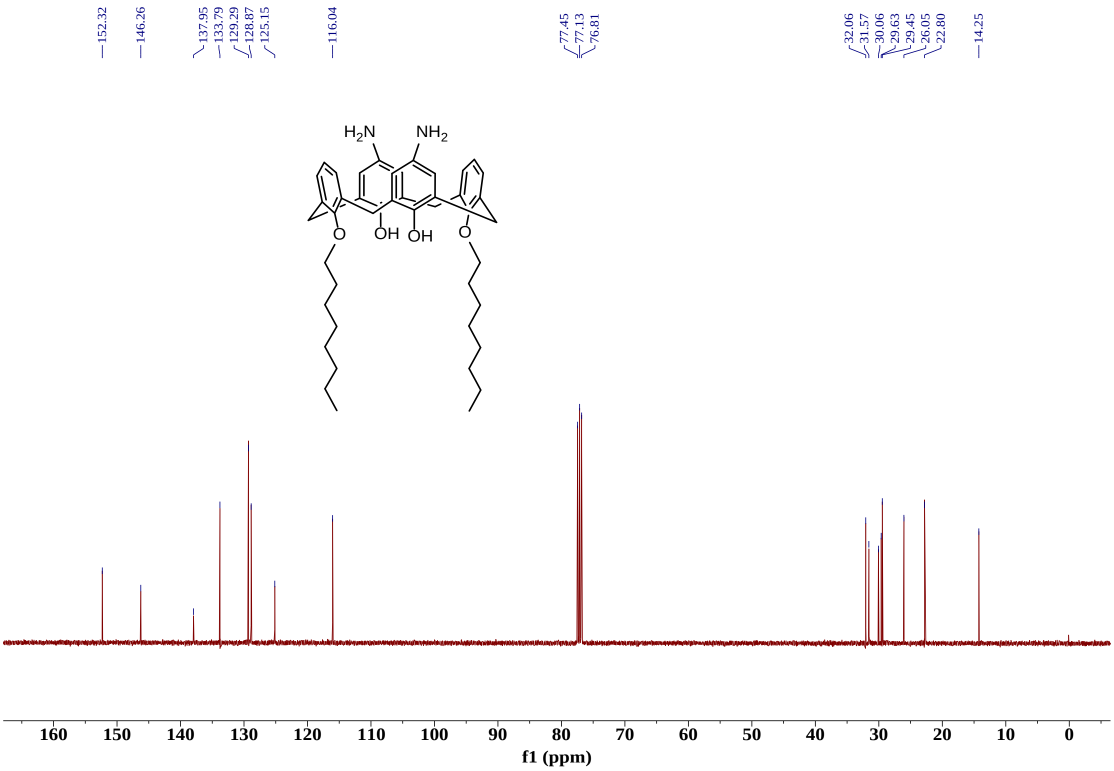
HR-MS of compound **13** ([M +Na]+): 645.3667



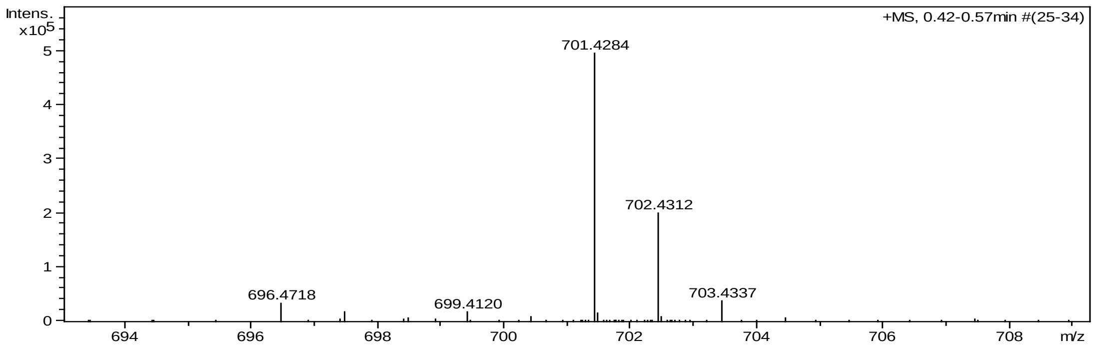
^{1}H NMR of compound **14**



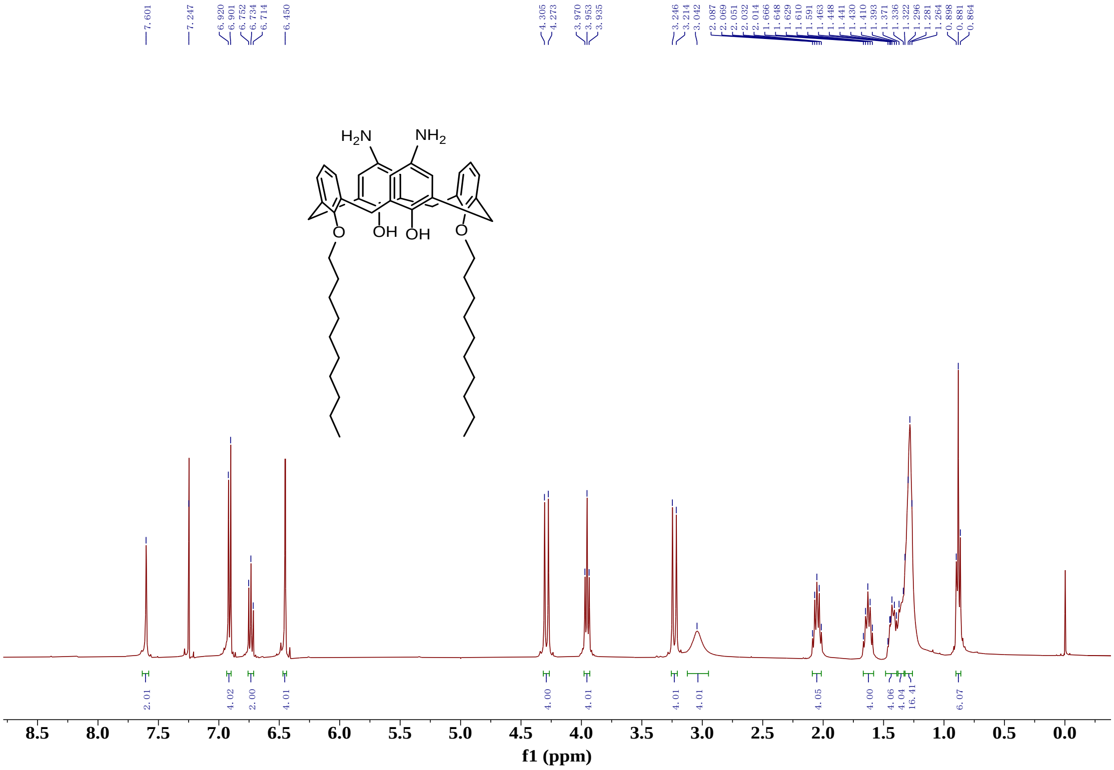
^{13}C NMR of compound **14**



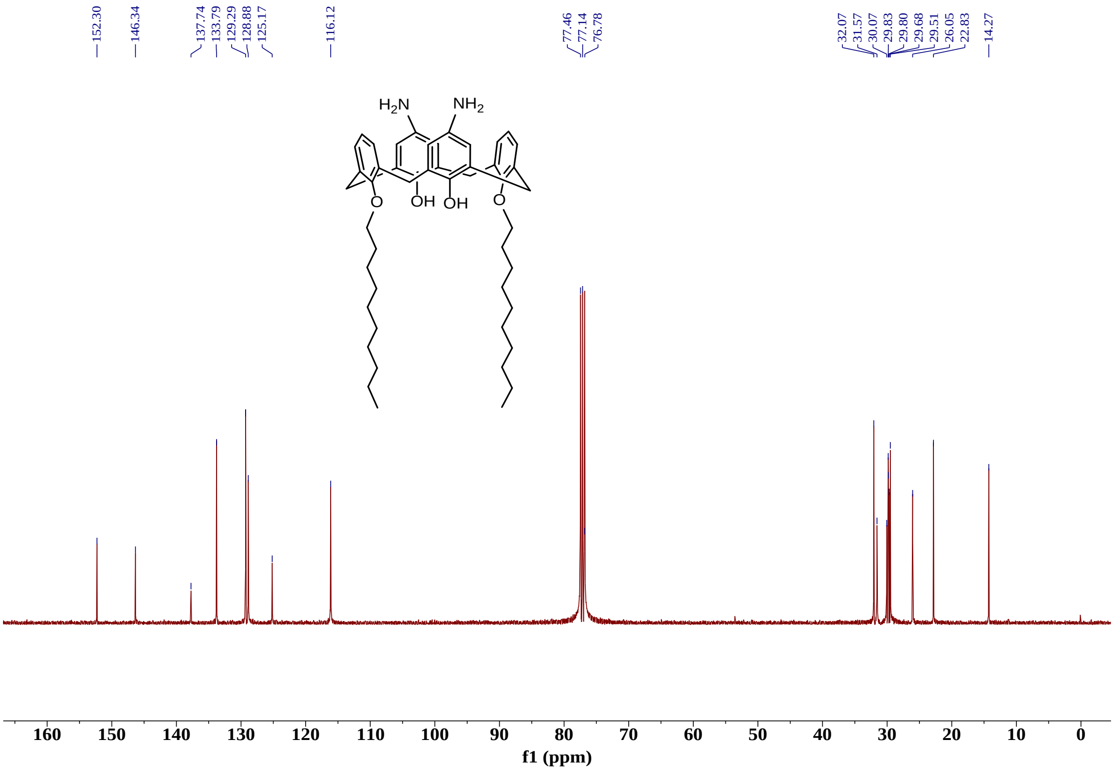
HR-MS of compound **14** ([M + Na^+]): 701.4284



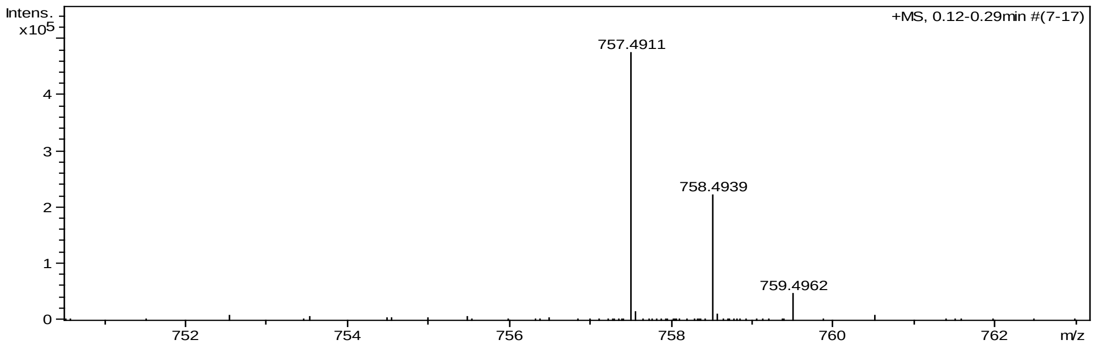
[1]H NMR of compound **15**



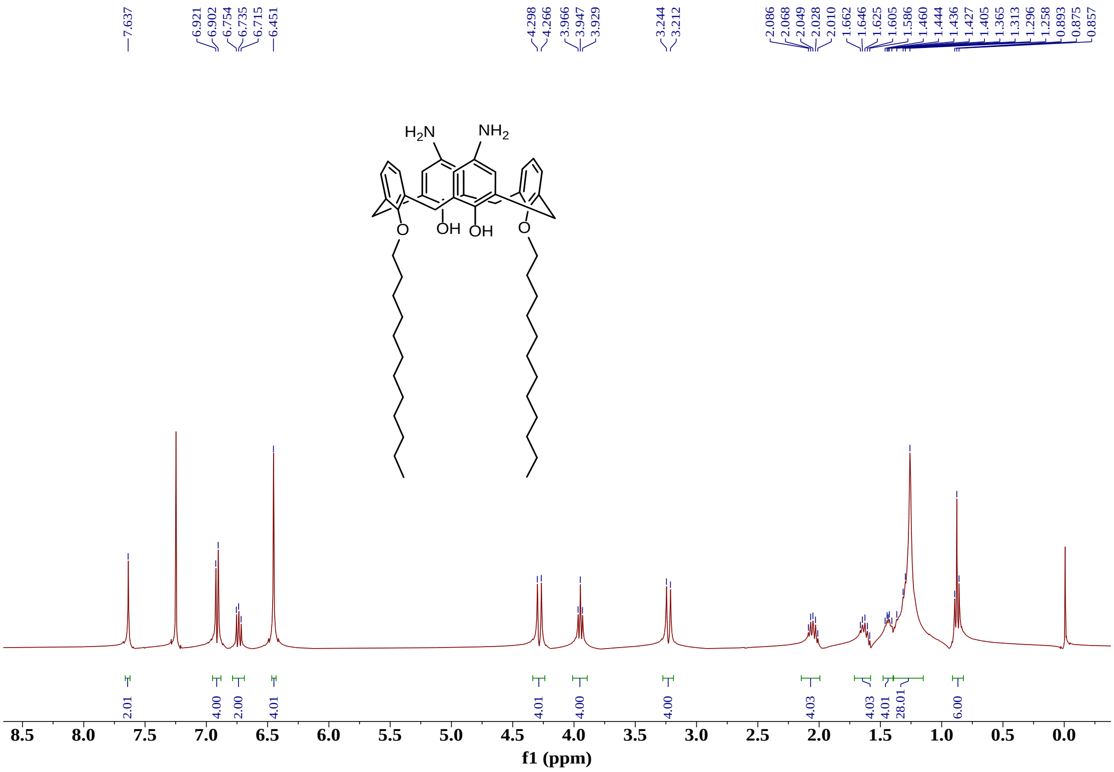
[13]C NMR of compound **15**



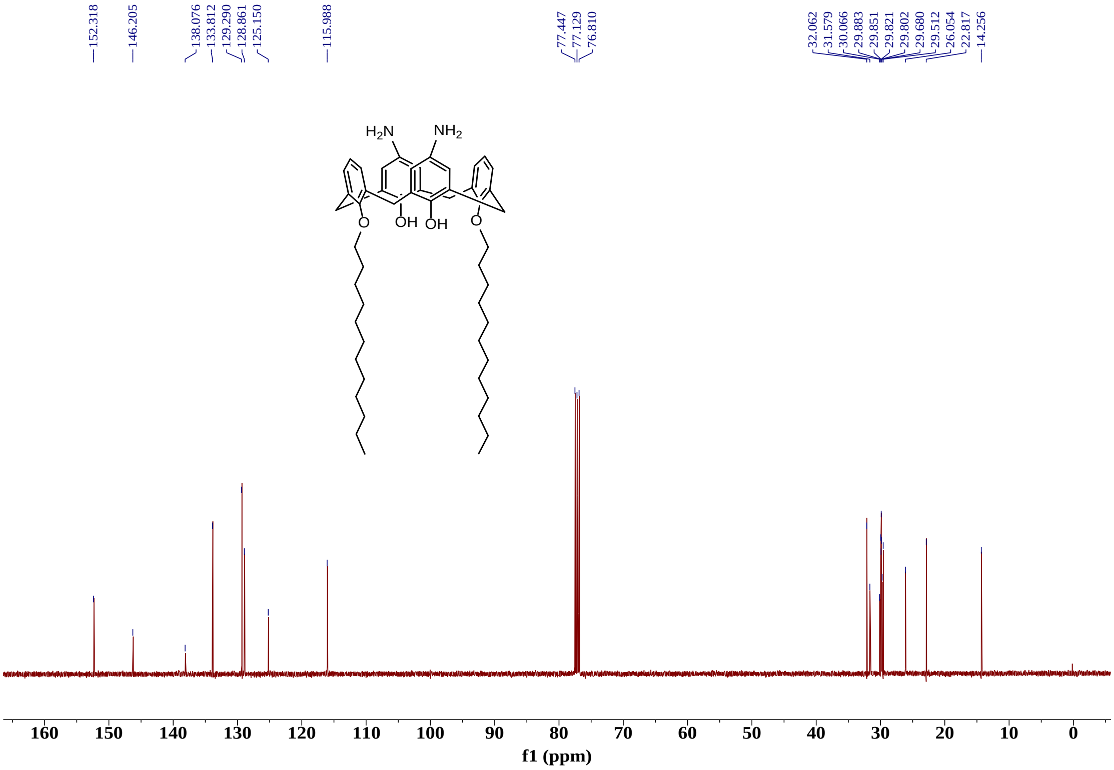
HR-MS of compound **15** ([M + Na$^+$]):757.4911



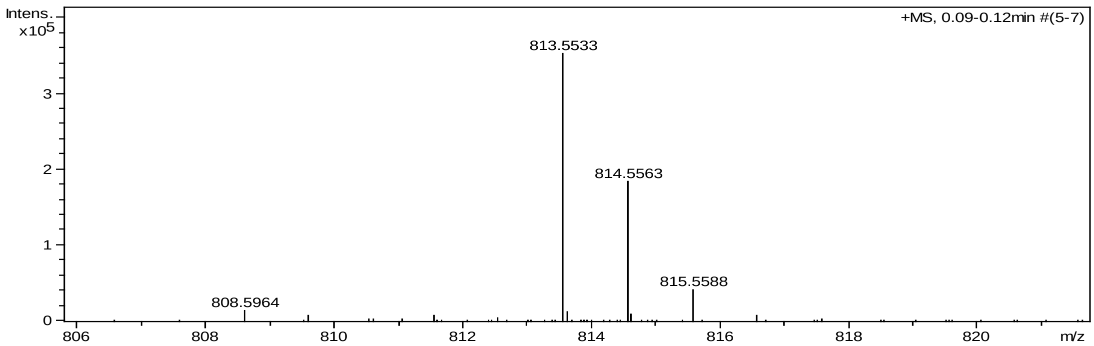
^{1}H NMR of compound **16**



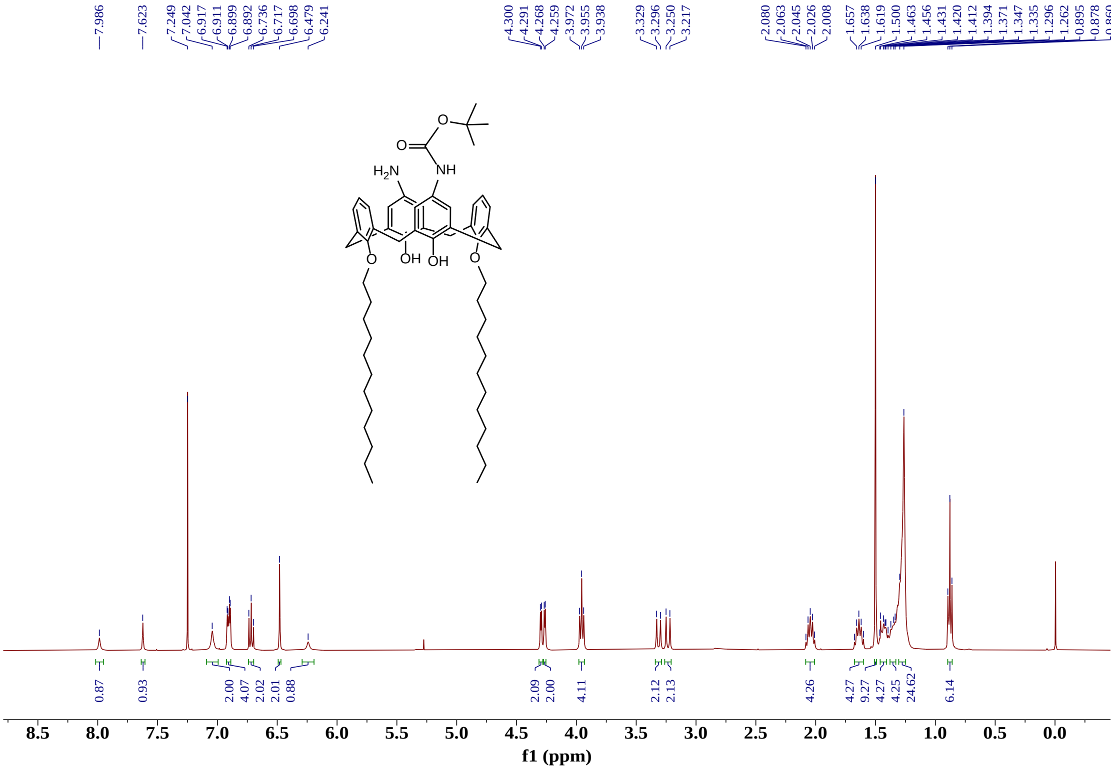
^{13}C NMR of compound **16**



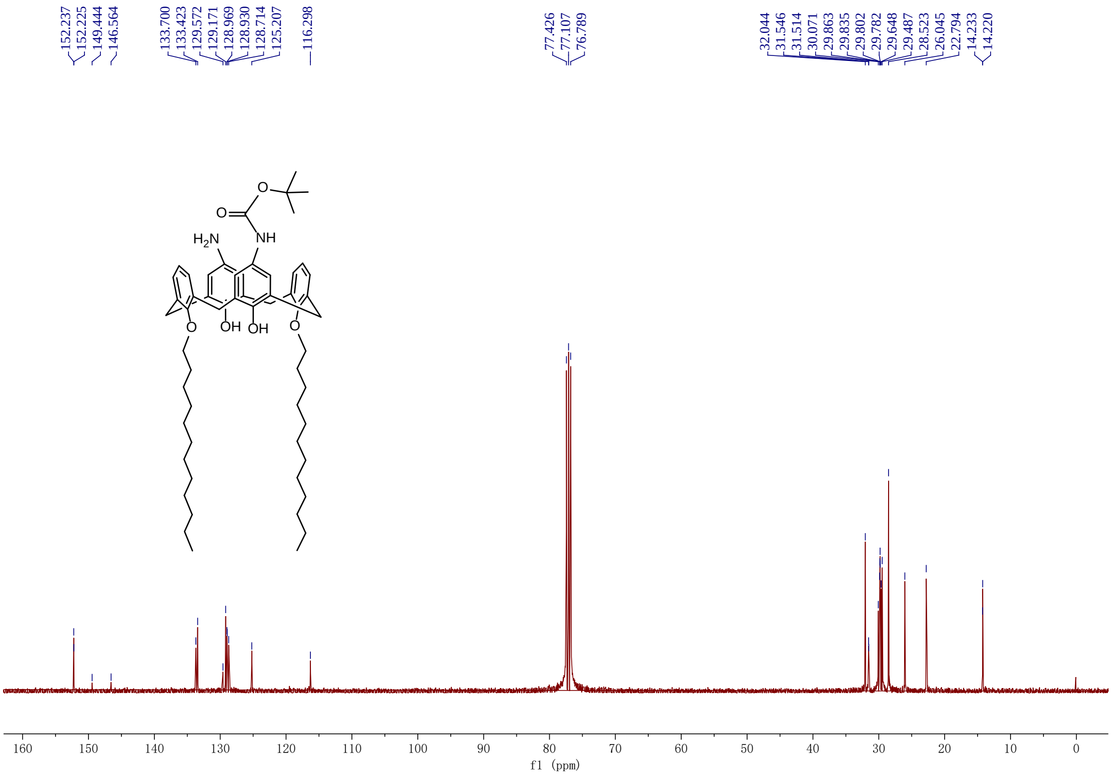
HR-MS of compound **16** ([M + Na$^+$]): 813.5533



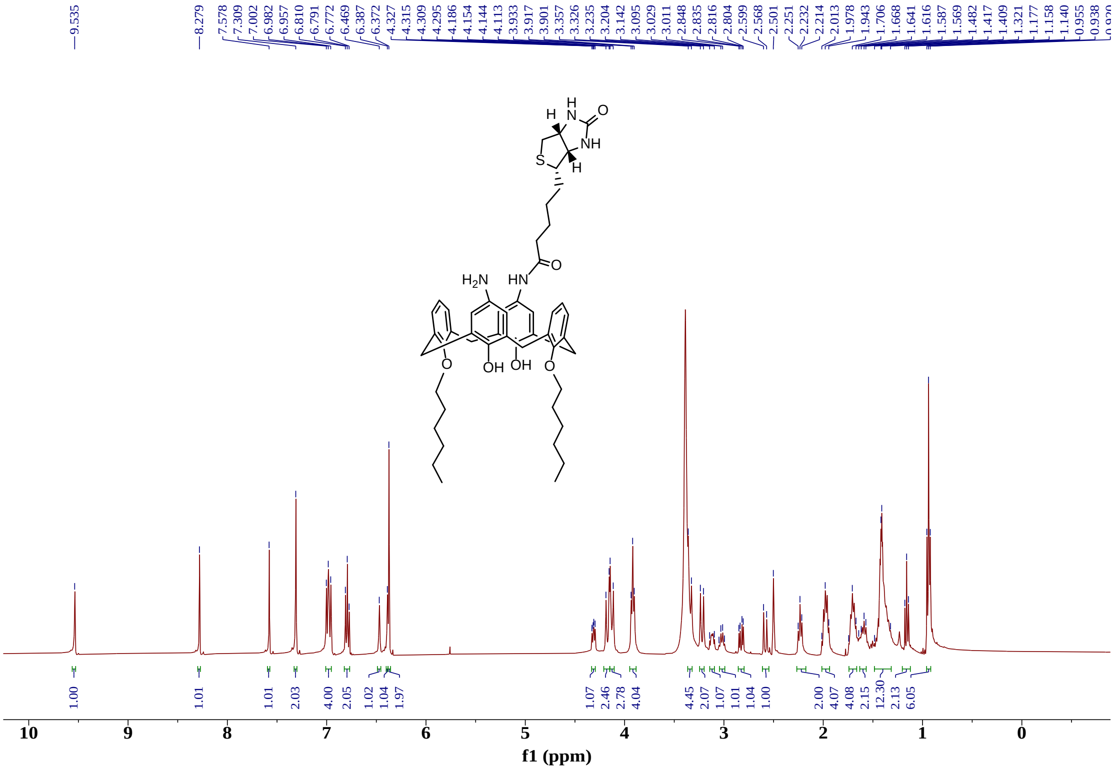
^{1}H NMR of compound **16a**



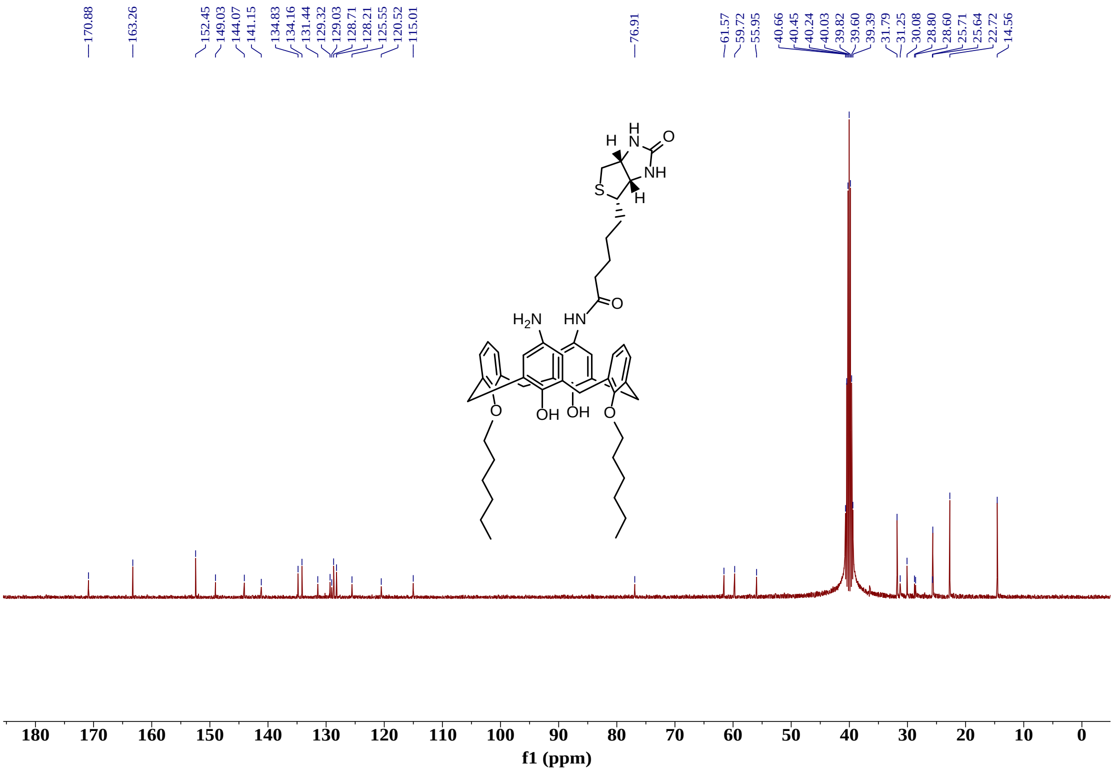
^{13}C NMR of compound **16a**



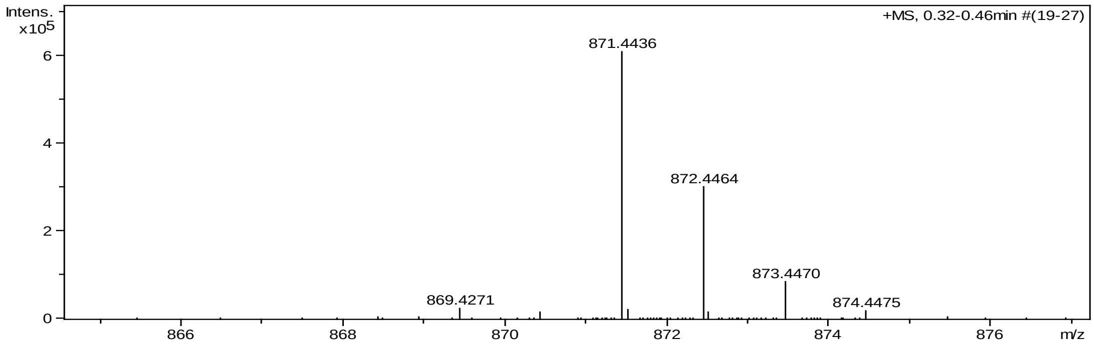
^{1}H NMR of compound **17**



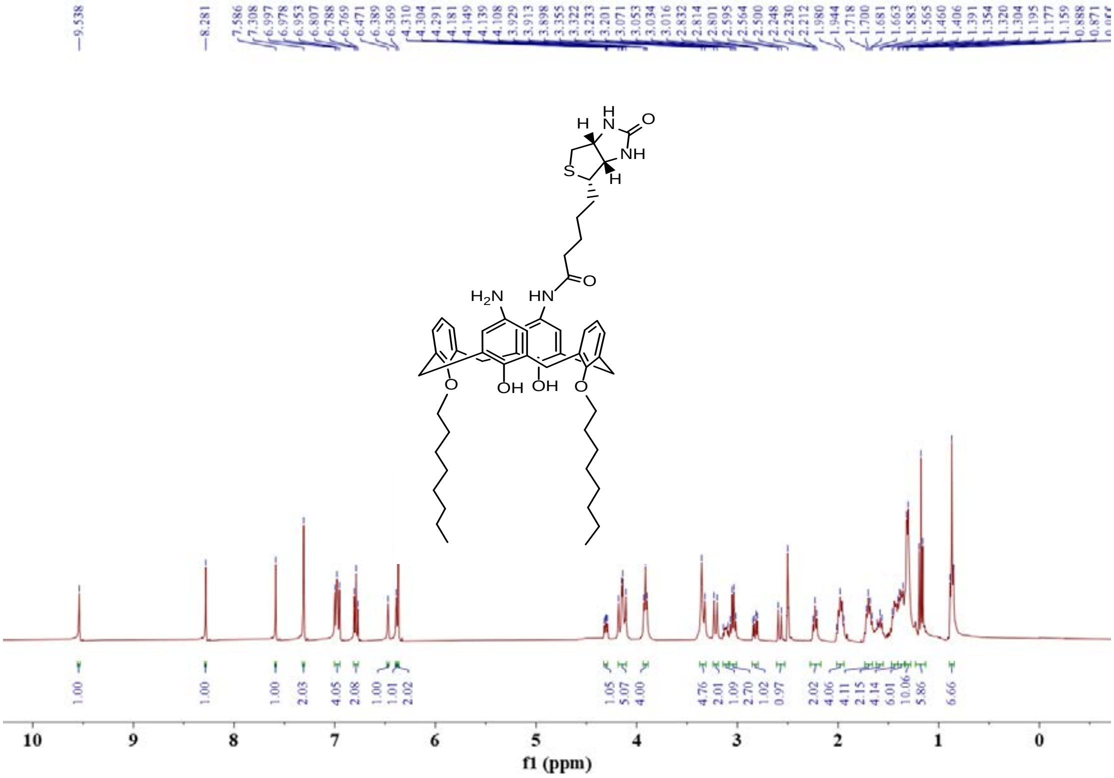
^{13}C NMR of compound **17**



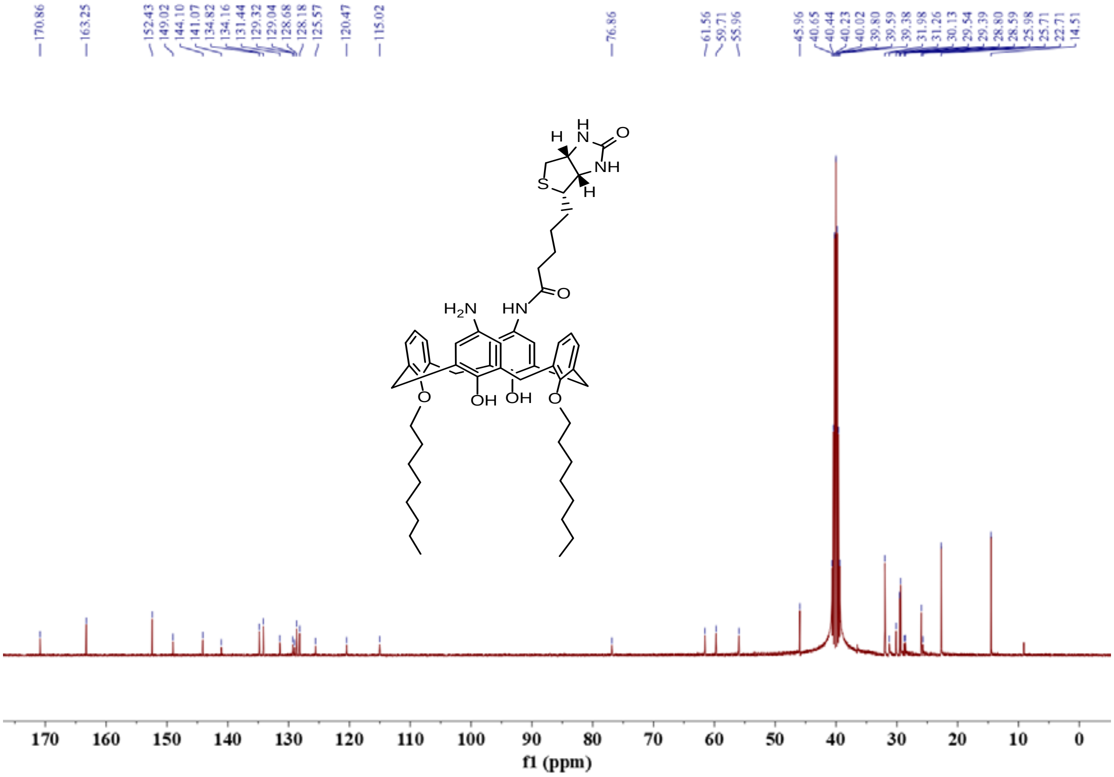
HRMS of compound **17** ([M +Na]$^+$): 871.4436



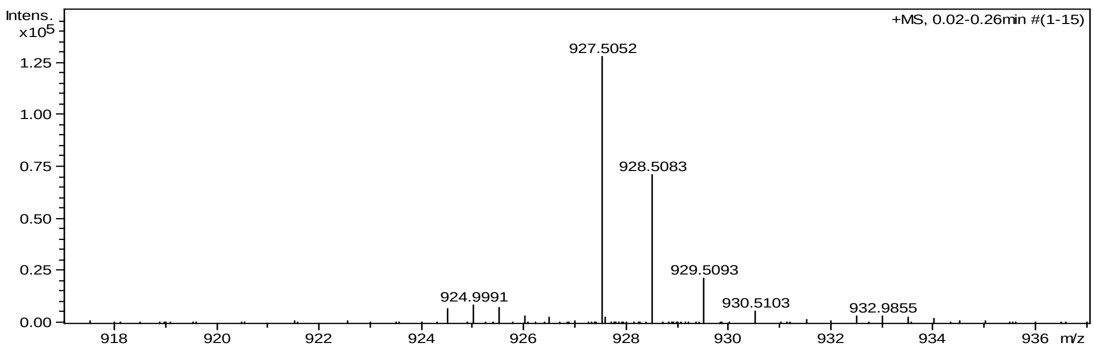
^{1}H NMR of compound **18**



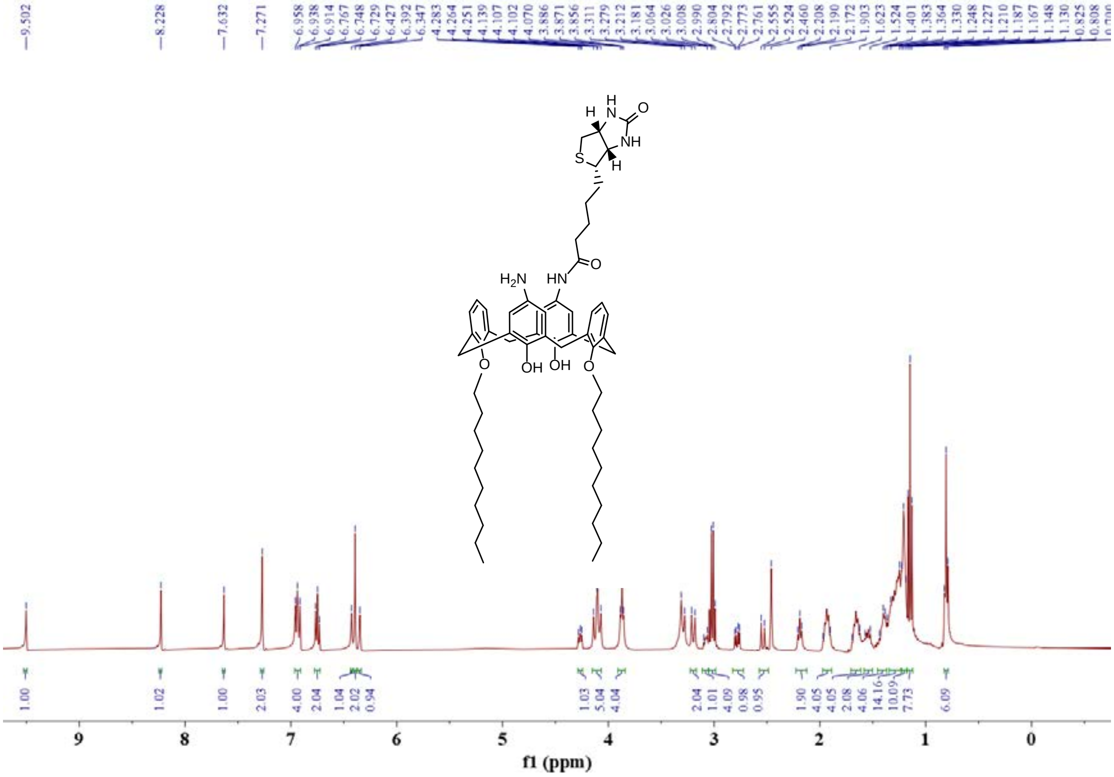
^{13}C NMR of compound **18**



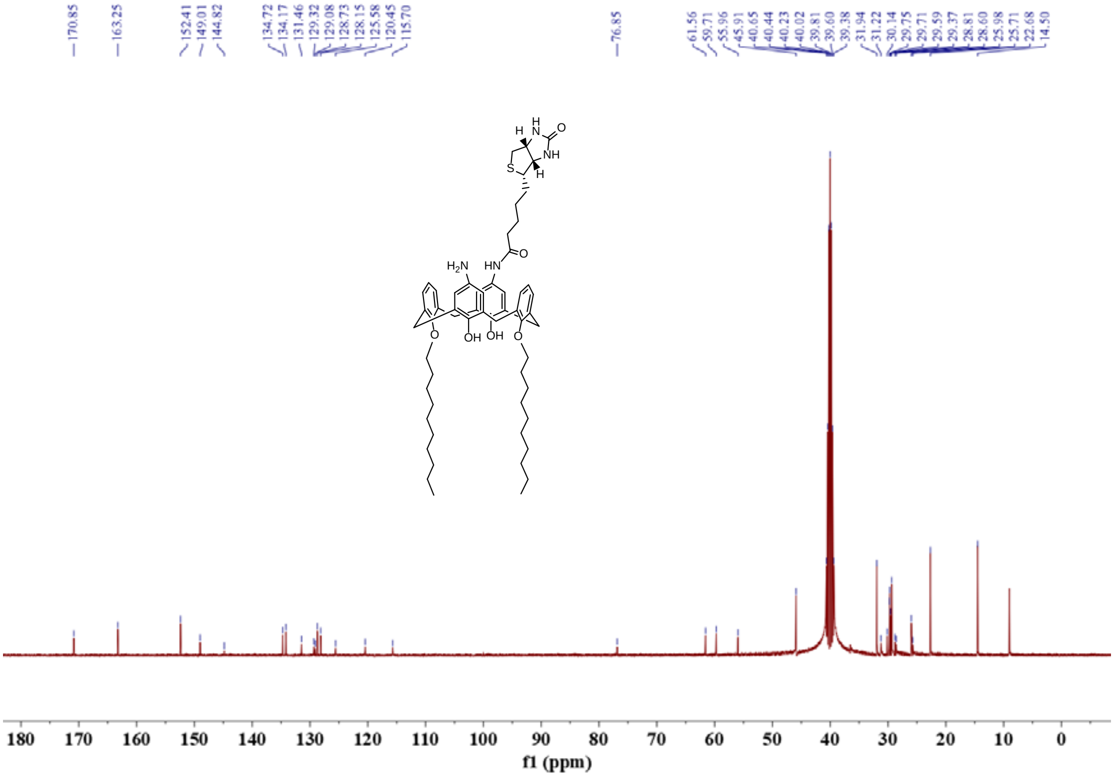
HR-MS of compound **18** ([M⁺ + Na⁺])：927.5052



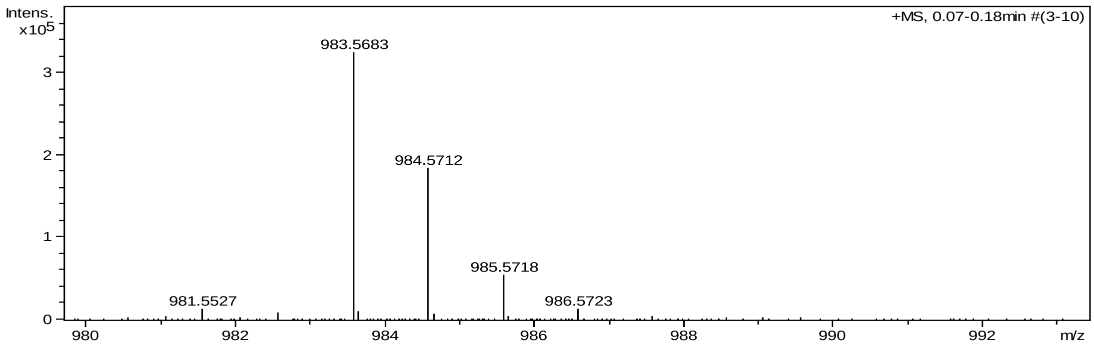
^{1}H NMR of compound **19**



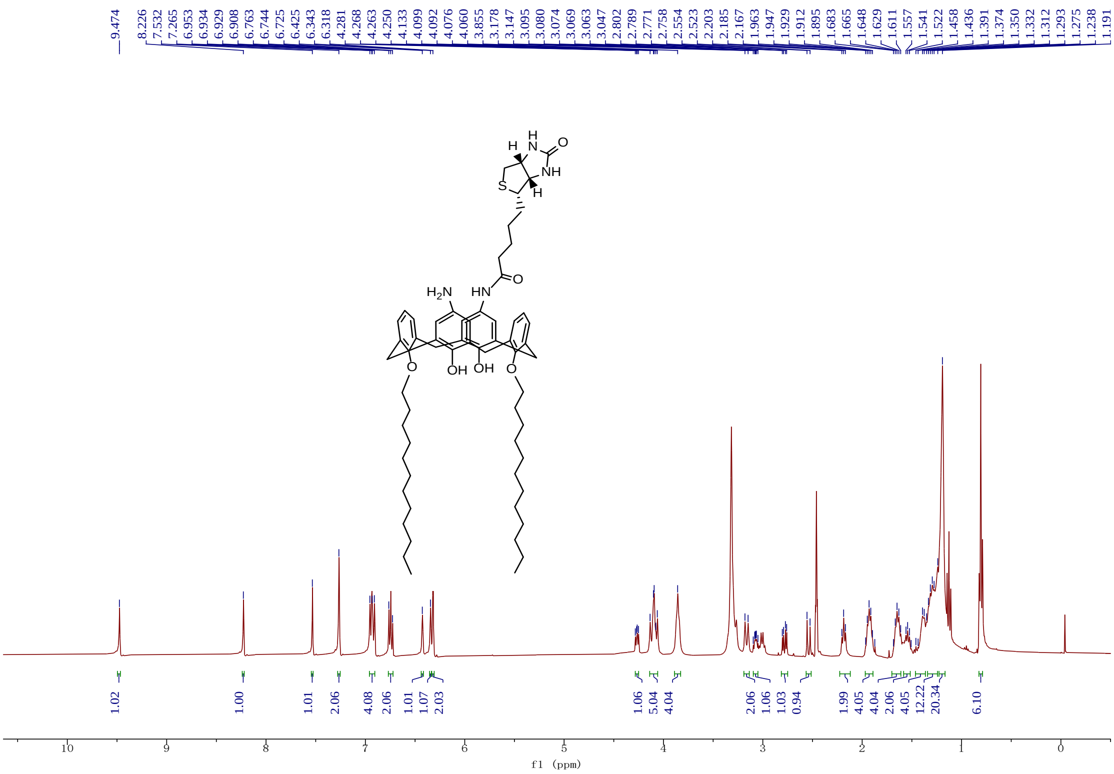
^{13}C NMR of compound **19**



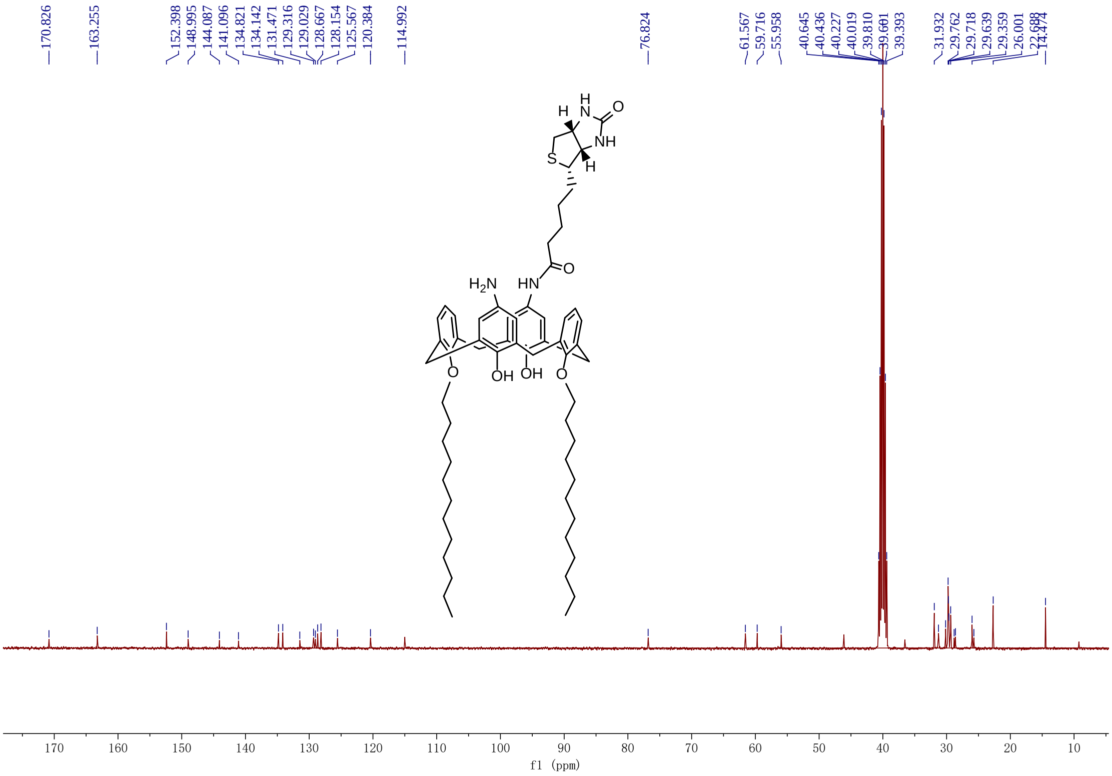
HR-MS of compound **19** ([M + Na$^+$]): 983.5683



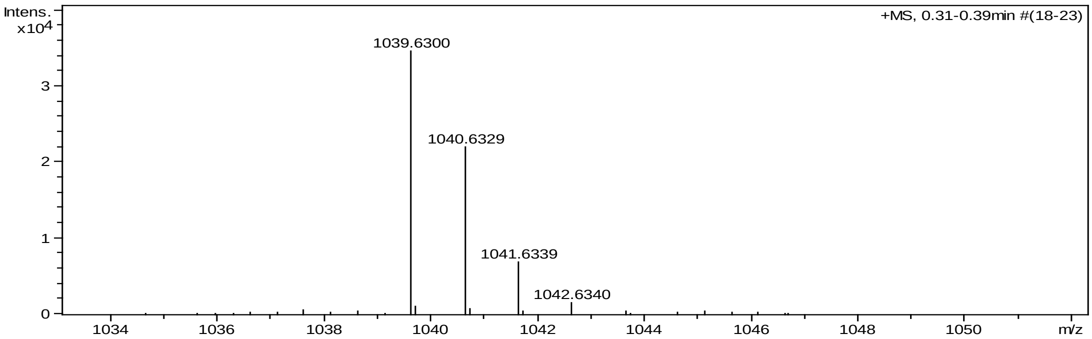
^{1}H NMR of compound **20**



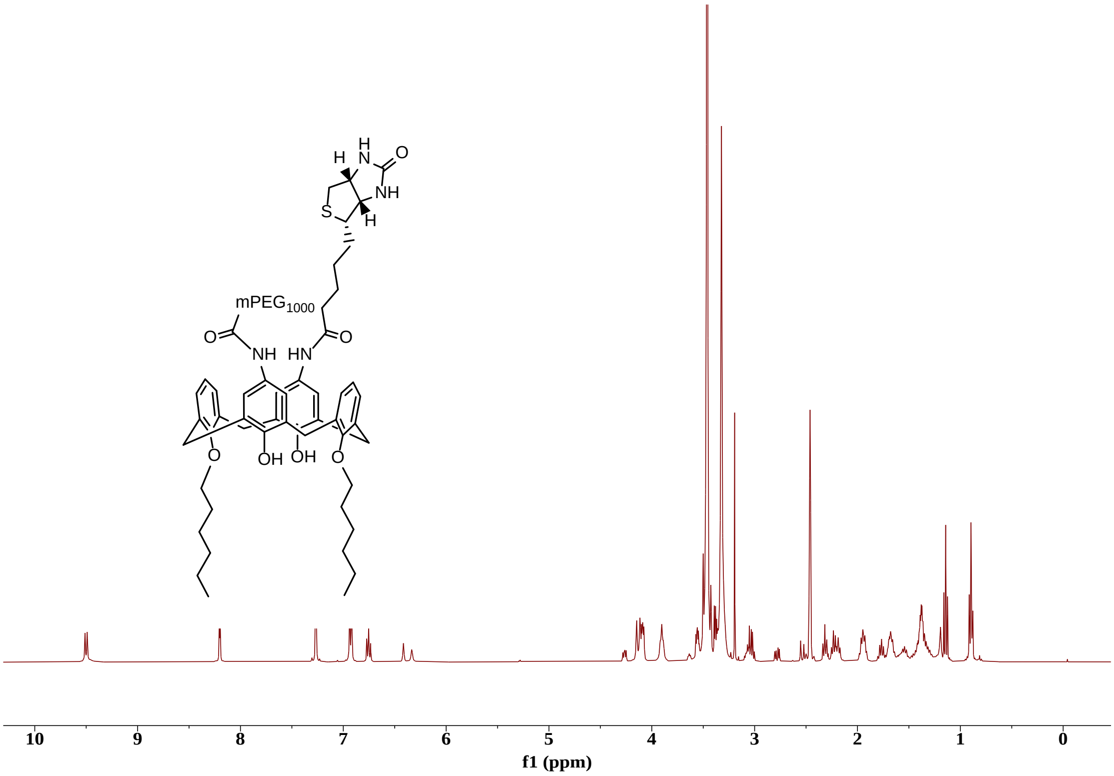
^{13}C NMR of compound **20**



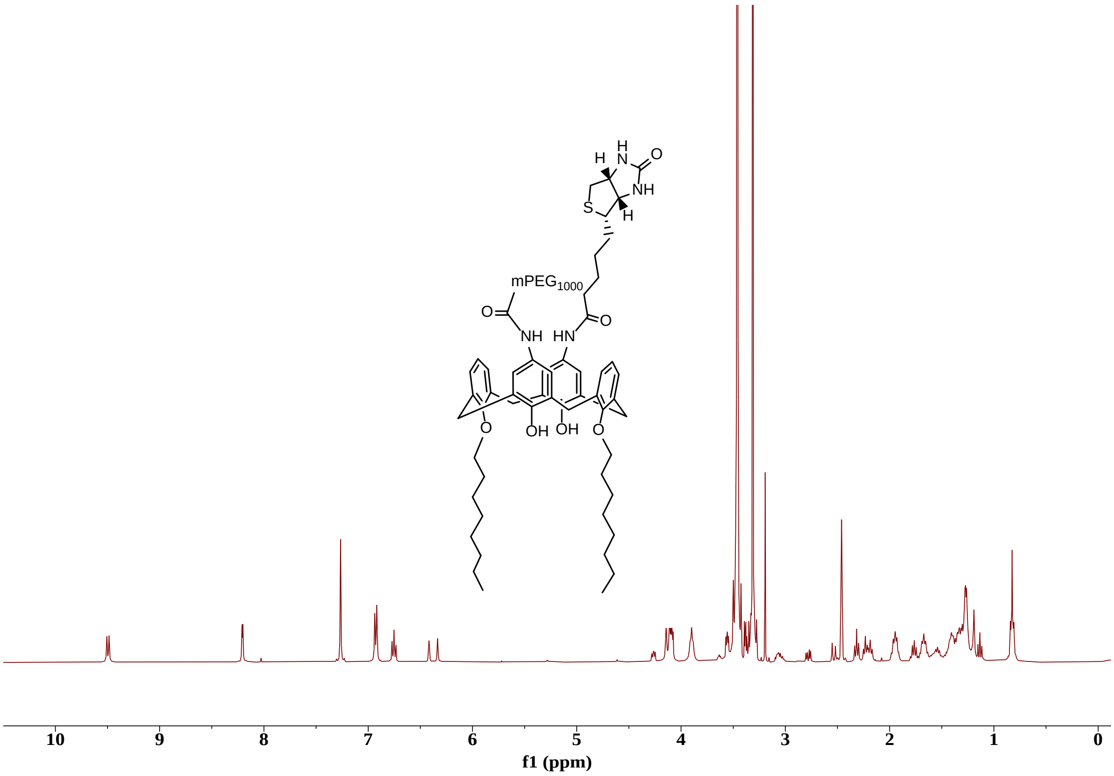
HR-MS of compound **20** ([M + Na^{+}]): 1039.6300



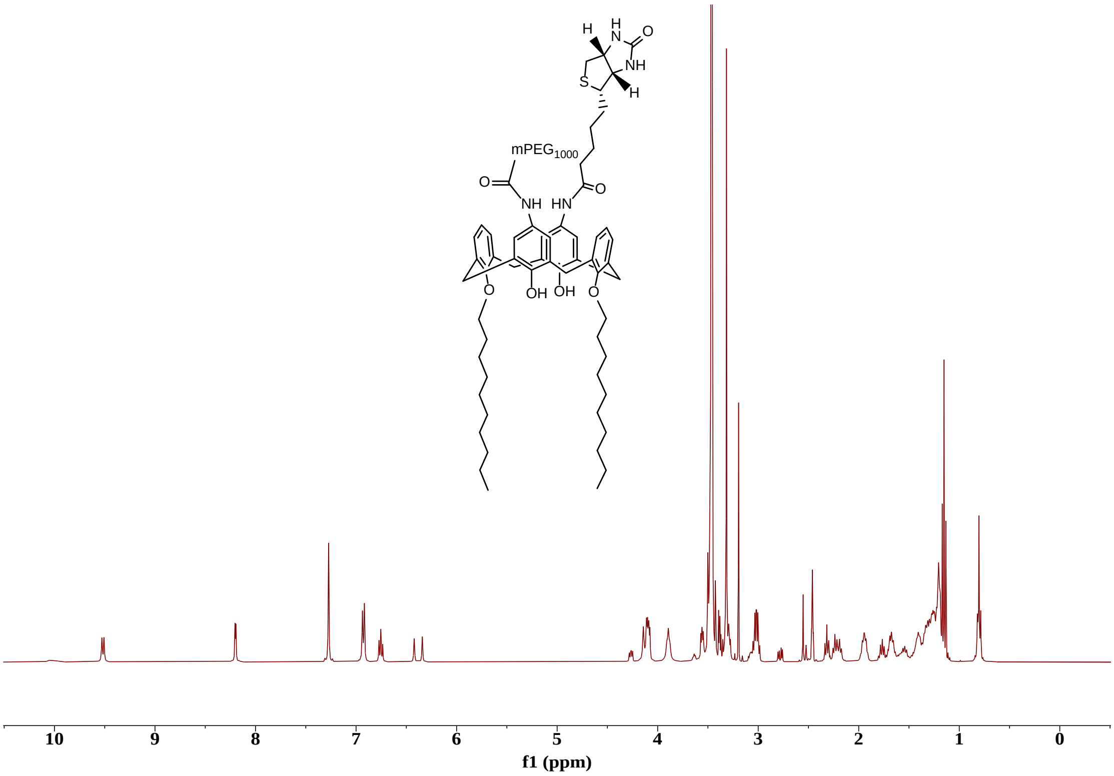
^{1}H NMR of compound **BPCA1**



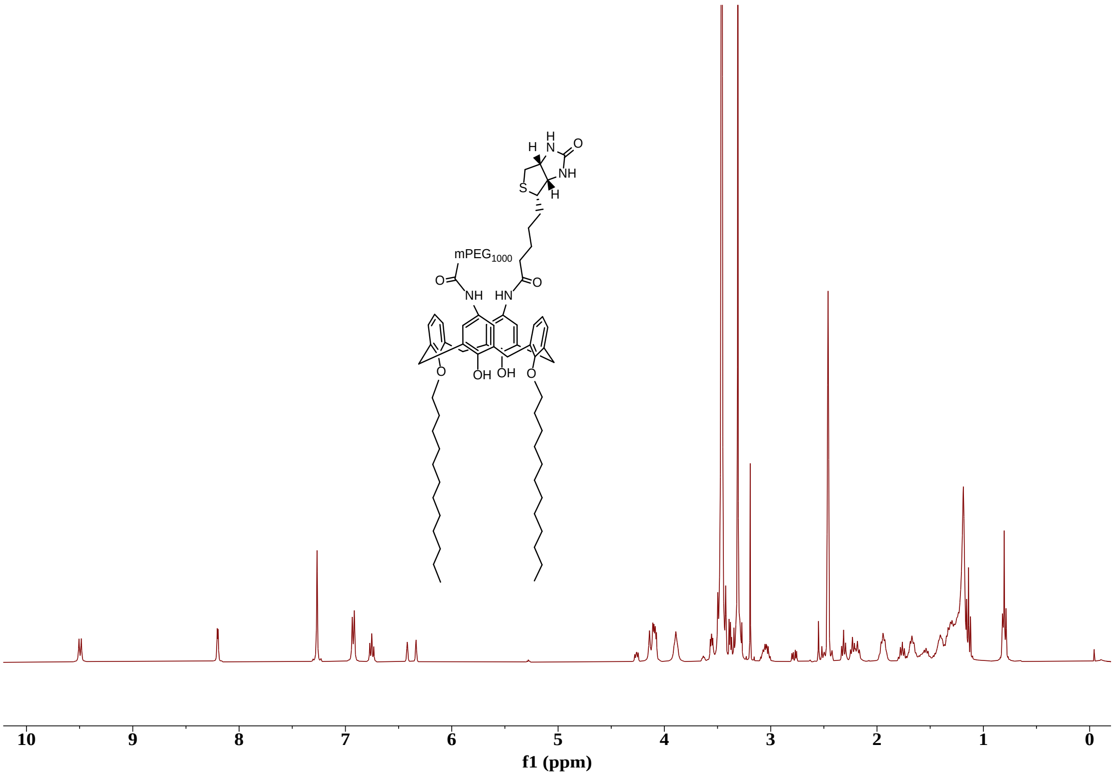
^{1}H NMR of compound **BPCA2**



^{1}H NMR of compound **BPCA3**



^{1}H NMR of compound **BPCA4**



[1]H NMR of compound **PCA4**