Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Regulated Assemblies and Anion Responsive Vesicles Based on 1,3-Alternate Oxacalix[2]arene[2]triazene Amphiphiles

Qing He, Zhi-Tang Huang, De-Xian Wang*

Beijing National Laboratory for Molecular Sciences, CAS Key Laboratory of Molecular Recognition and Function, Institute of Chemistry, Chinese Academy of Sciences, Beijing, 100190, China

1. General information 2
2. Experimental details 2
3. Synthesis 3
4. SEM images of **3a** and **3b** 7
5. TEM images of **3a-d** 9
6. LSCM images of **3a** and **3b** 13
7. DLS and Zeta potential results 14
8. Calculated molecular models of **3a-d** 16
9. Determination of CAC values of **3a-d** 17
10. XRD results for determination of the membrane thicknesses of **3a** and **3b** 19
11. Vesicular size responses of **3b** to anions 20
12. NMR titrations 21
13. ^{1}H NMR spectra and ^{13}C NMR spectra of the compounds 23

1. General information

^{1}H and ^{13}C NMR spectra were recorded on 300 MHz NMR spectrometer. Chemical shifts are reported in ppm versus tetramethylsilane or the residual solvent resonance used as an internal standard. Melting points are uncorrected. All chemicals, except if stated otherwise, were obtained from commercial sources and used without further purification. Elemental analyses, mass spectrometry, SEM, TEM, DLS, LSCM and X-ray diffraction analysis were performed at the Institute of Chemistry. The fluorescence spectra were measured with a Hitachi F-4500 fluorescence spectrophotometer. Synthesis of macrocyclic compound **1** and **4**, intermediates **2c′**, **2d″** and **2d** followed the reported methods. [1-4]

2. Experimental details

Preparation of vesicles or micelle: A small volume (100 ul) of the stock solution of **3** (**3a-d**) in THF (5×10^{-4} M) was transferred to a sample bottle. Double distilled water (1 ml) was injected quickly, giving a mixture solution of 5×10^{-5} M. The mixture solution was put in the ultrasonic bath for 30 minutes, incubated at 60 ℃ for 30 minutes to remove the organic solvent. Solutions of **3a** and **3b** hence yielded as opalescent, whereas **3c** and **3d** as clear dispersions.

Laser scanning confocal microscopy (LSCM): Laser scanning confocal microscopic experiments were carried out by preparing the vesicles in a solution containing lucigenin. The fluorescence agent outside the vesicles was then removed through repeated dialysis. The resulting vesicle solution were deposited on a glass surface, covered with a glass slide, and then visualized using a confocal laser scanning microscope (LSCM, Olympus FV1000, Olympus FV1000 with a 100×oil-immersion objective and a numerical aperture of 1.4. The excitation wavelength was set at 405nm).

Dynamic light scattering (DLS): The DLS measurements were carried out at 25℃ using an LLS spectrometer (ALV/SP-125) with a multi-τ digital time correlater (ALV-5000). Light of λ=632.8nm from a solid-state He-Ne laser (22 mW) was used as the incident beam. The measurement was performed at a scattering angle of 90°. The correlation function was analyzed from the scattering data via the CONTIN method to obtain the distribution of diffusion coefficients (D) of the solutes. The apparent hydrodynamic radius R_h was deduced from D by the Stokes-Einstein equation $R_h=k_BT/(6\pi\eta D)$ for spherical particles, where k_B is the Boltzmann constant, T is the Kelvin temperature, and η is viscosity of solvent. For the effect of salts on

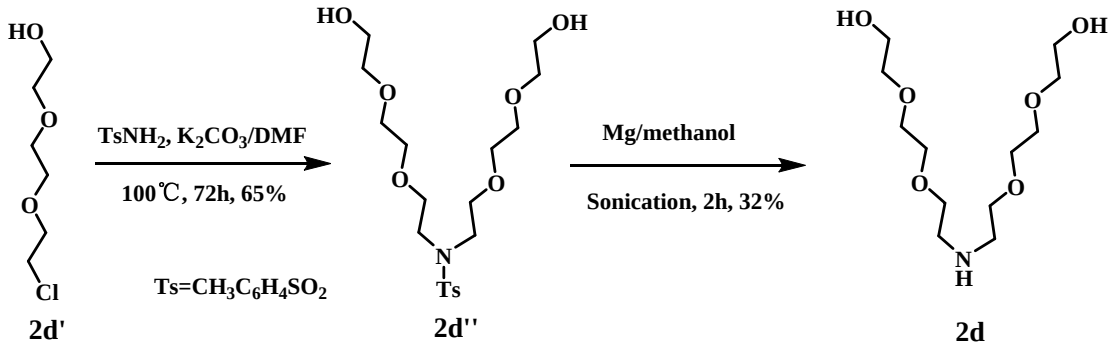
vesicle sizes, small volume of salt solutions (2.5 M) was added to the vesicle solutions (2 mL) assembled from **3a** or **3b**, the mixture was then balanced for 1 minute and the size distribution was recorded by DLS equipment.

Zeta potential measurement: The zeta potential measurements were conducted at 25 °C, using a Malvern Zetasizer Nano-ZS instrument (ZEN3600, Malvern Instruments, Worcestershire, UK) equipped with a 4 mW He−Ne laser at a wavelength of 633 nm. A clear disposable capillary cell (DTS1060C) was used. All the measurements were performed at θ=173°. The zeta potential was calculated using the Helmholtz-Smoluchowski relationship from the mobility measured in an electrophoretic light-scattering (ELS) experiment.

X-ray diffraction (XRD) analysis: Samples for XRD measurement were prepared through directly casting vesicle solution on the glass slide for 4 times and the solvent was evaporated at room temperature. XRD analysis was performed on an X-ray diffracometer (SW, X'PERT) with nickel filtered Cu Kα radiation (λ=1.54060 Å) operated at 40 kV and 10 mA.

3. Synthesis

Synthesis of 2d



Scheme S1. Synthesis of **2d**.

1.32 g (3.03 mmol) of **2d''** was dissolved in methanol (50 ml) and magnesium powders (0.42 g, 30 mmol) were added. The resulting suspension was sonicated for 2 h when TLC on silica (ethyl acetate–methanol, 6 : 1) indicated that all starting material had been consumed. After filtration, the filtrate was evaporated under reduced pressure to afford oily residue, which was further purified by column chromatography using silica gel with acetone/methanol (v/v, 20/1) as eluent to give **2d** as oil (0.27 g, 32%): ^{1}H NMR (300 MHz, CD_3Cl, ppm): δ 4.46 (br. s, 3H), 3.57-3.72 (m, 20H), 2.89 (t, J=5.3 Hz, 4H); MS (ESI): m/z 282.16 $[M+H]^+$, 304.06 $[M+Na]^+$.

General procedures for the synthesis of 3a, 3b, 3c′ and 3d

A mixture of **1** (0.46 g, 0.5 mmol for **3a**, 0.30 g, 0.3 mol for **3b**, 0.13 g, 0.14 mmol for **3c′**, 0.18 g, 0.2 mmol for **3d**), **2** (**2a:** 0.16 g, 1.5 mmol, **2b**: 0.10 g, 1.0 mmol, **2c′:** 0.13 g, 0.4 mmol, **2d:** 0.23 g, 0.8 mmol) and DIPEA (0.52 g, 4.0 mmol for **3a**, 0.34 g, 2.6 mmol for **3b**, 0.14 g, 1.1 mmol for **3c′**, 0.21 g, 1.6 mmol for **3d**) in THF (30 ml) was stirring at room temperature for 5 h. After removal of the solvent, the residue was subjected to silica gel chromatography to get pure products of **3** as white solids (0.42 g, 81% for **3a**, 0.32 g, 92% for **3b**, 0.22 g, 92% for **3c`** and 0.12 g, 46% for **3d**).

3a: mp 159~160℃; IR (KBr) ν 3335, 3081, 2923, 2853, 1645, 1603, 1583, 1525, 1392, 1304, 1172; ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 8.36 (t, J=5.4Hz, 2H), 7.34 (d, J=2.0Hz, 4H), 7.20 (t, J=2.0Hz, 2H), 4.85 (t, J=5.3Hz, 4H), 3.70~3.72 (m, 8H), 3.63~3.66 (m, 8H), 3.13~3.19 (m, 4H), 1.41~1.46 (m, 4H), 1.20~1.24 (m, 44H), 0.84 (t, J=6.5Hz, 6H). ^{13}C NMR (300 MHz, DMSO-d_6, ppm): δ 170.92, 167.47, 163.81, 151.78, 136.53, 119.29, 117.02, 58.32, 50.52, 31.29, 29.05, 29.02, 28.96, 28.84, 28.71, 26.45, 22.09, 13.92. MS (MALDI-TOF): m/z 1081.3 $[M+Na]^+$. Calcd for $C_{56}H_{86}N_{10}O_{10}$: C, 63.49; H, 8.18; N, 13.22. Found: C, 63.55; H, 8.37; N, 13.19.

3b: mp 86~87℃; IR (KBr) ν 3078, 2964, 2934, 2874, 1589, 1523, 1391, 1265, 1201, 1141; ^{1}H NMR (300 MHz, CD_3Cl, ppm): δ 7.13 (d, J=2.1Hz, 4H), 6.78 (t, J=2.1Hz, 2H), 6.06 (t, J=5.6Hz, 2H), 3.57 (t, J=7.7Hz, 8H), 3.34 (q, J=6.8Hz, 4H), 1.64~1.74 (m, 8H), 1.52~1.59 (m, 4H), 1.23~1.32 (m, 44H), 0.96 (t, J=7.35Hz, 12H), 0.88 (J=6.6Hz, 6H). ^{13}C NMR (300 MHz, CD3Cl, ppm): δ 171.88, 167.83, 166.22, 152.58, 137.36, 120.03, 118.01, 49.47, 40.43, 32.07, 29.81, 29.78, 29.73, 29.66, 29.50, 27.20, 22.83, 20.97, 14.25, 11.44. MS (MALDI-TOF): m/z 1073.7 $[M+Na]^+$. Calcd for $C_{60}H_{94}N_{10}O_6$: C, 68.54; H, 9.01; N, 13.32. Found: C, 68.64; H, 9.06; N, 13.32.

3c`: mp 116~117℃; IR (KBr) ν 3305, 3066, 3034, 2924, 2853, 1750, 1645, 1579, 1538, 1386, 1300, 1168, 1030; ^{1}H NMR (300 MHz, CD_3Cl, ppm): δ 7.32-7.37 (m, 20H), 7.13 (d, J=2.0 Hz, 4H), 6.71 (t, J=2.0 Hz, 2H), 6.08 (t, J=2.3 Hz, 2H), 5.22 (s, 8H), 4.58 (s, 8H), 3.36 (q, J=5.0 Hz, 4H), 1.59 (m, 4H), 1.21-1.32 (m, 44H), 0.88 (t, J=6.9 Hz, 6H). ^{13}C NMR (300 MHz, CD_3Cl, ppm): δ 172.06, 169.18, 168.98, 165.95, 152.37, 137.76, 135.34, 128.80, 128.64, 128.42, 119.67, 118.20, 67.37, 49.65, 40.48, 32.07, 29.84, 29.80, 29.73, 29.64, 29.50, 27.20, 22.83, 14.26. MS (MALDI-TOF): m/z 1497.5 $[M+Na]^+$. Calcd for $C_{84}H_{102}N_{10}O_{14}$: C, 68.36; H, 6.97; N, 9.49.

3d: mp 72~73℃; IR (KBr) ν 3342, 3079, 2924, 2853, 1644, 1581, 1525, 1386, 1305, 1138, 1078,

811; ^{1}H NMR (300 MHz, CD_3Cl, ppm): δ 7.16 (d, J=2.1Hz, 4H), 6.76 (t, J=2.1Hz, 2H), 6.37 (s, 2H), 3.96 (t, J=4.7Hz, 8H), 3.80 (t, J=5.0Hz, 8H), 3.67-3.71 (m, 24H), 3.59 (t, J= 4.7Hz, 8H) 0-3.37 (m, 8H), 1.21-1.57 (m, 4H), 1.22-1.32 (m, 44H), 0.88 (t, J=6.6Hz, 6H). ^{13}C NMR (300 MHz, CD_3Cl, ppm): δ 171.69, 168.07, 166.23, 152.18, 137.30, 119.67, 118.11, 72.71, 70.47, 69.11, 61.71, 48.32, 40.36, 31.99, 29.77, 29.73, 29.69, 29.49, 29.43, 27.13, 22.76, 14.21. MS (MALDI-TOF): m/z 1434.0 $[M+Na]^+$. Calcd for $C_{72}H_{118}N_{10}O_{18}$: C, 61.25; H, 8.42; N, 9.92. Found: C, 60.95; H, 8.42; N, 9.80.

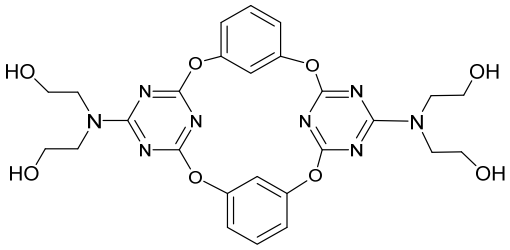
Procedures for synthesis of 3c

0.22 g of **3c′** was dissolved in THF, to which Pd/C (10%, 0.04 g) was added. The resulting mixture was stirred at room temperature overnight under hydrogen atmosphere. The resulting solution was filtrated through celite. After removal of organic solvent, the residues were recrystallized with a mixture of chloroform and acetonitrile to afford **3c** as white solid (0.15 g, 88%).

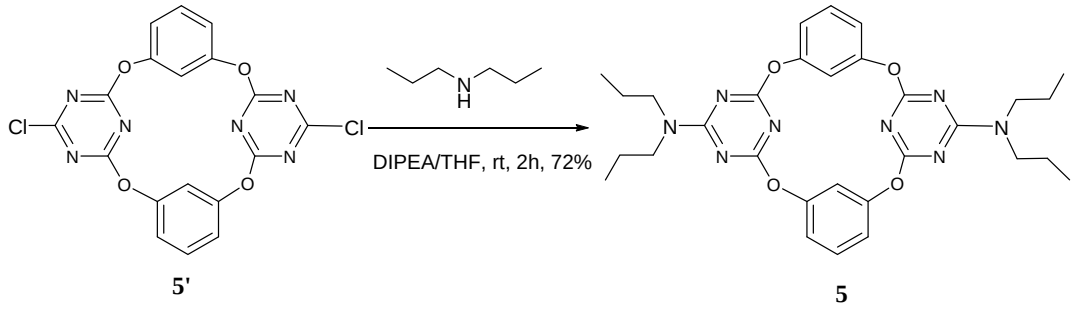
3c: mp 212~213℃; IR (KBr) ν 3325, 3084, 2924, 2853, 2543, 1732, 1578, 1541, 1387, 1300, 1170; ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 12.88 (br, 4H), 8.32 (t, J=5.4Hz, 2H), 7.37 (d, J=2.1Hz, 4H), 7.27 (t, J=2.1Hz, 2H), 4.37 (s, 8H), 3.12-3.18 (m, 4H), 1.42-1.46 (m, 4H), 1.20-1.25 (m, 44H), 0.84 (t, J=6.6Hz, 6H). ^{13}C NMR (300 MHz, DMSO-d_6, ppm): δ 170.98, 170.25, 168.22, 163.61, 151.59, 136.56, 119.31, 117.29, 49.86, 31.27, 29.03, 28.99, 28.82, 28.74, 28.69, 26.47, 22.06, 13.85. MS (MALDI-TOF): m/z 1137.4 $[M+Na]^+$. HRMS (APCI): Calcd for $C_{56}H_{78}N_{10}O_{14}$ $[M+H]^+$: 1115.5772. Found: 1115.5770.

Procedures for syntheses of compound 4 and 5

Compound **4** was prepared according to the reported methods.[5]



4



5' **5**

Scheme S2. Synthesis of compound **5**.

Synthesis of **5**

Tetraoxacalix[2]arene[2]triazene (**5′**) (1 mmol, 442 mg) was dissolved in THF (30 ml), to which n-dipropylamine (3 mmol, 303 mg) and DIPEA (8 mmol, 1.032 g) were added. The resulting mixture was stirring for 2 hours at room temperature. After removal of the organic solvent, the residue was subjected to silica gel chromatography with a mixture of dichloride methane and n-hexane as an eluent, giving pure **5** as white solids (0.411 g, 72%): mp 167-168℃; IR (KBr) ν 3084, 2964, 2934, 2874, 1589, 1523, 1464, 1391, 1322, 1266, 1201, 1141; ^{1}H NMR (300 MHz, CD_3Cl, ppm): δ 7.14 (t, J=8.1 Hz, 2H), 6.78 (dd, J=2.1 Hz, J=8.1 Hz, 4H), 6.67 (t, J=2.1 Hz, 2H), 3.57 (t, J=7.7 Hz, 8 H), 1.64-1.76 (m, 8H), 0.96 (t, J=7.4 Hz, 12H); ^{13}C NMR (300 MHz, CD_3Cl, ppm): δ 172.14, 167.88, 152.52, 129.47, 118.96, 117.42, 49.32, 20.98, 11.44; HRMS: m/z 573.2932 $[M+H]^+$ (Calcd.: 573.2938); Anal. Calcd for $C_{30}H_{36}N_8O_4$: C, 62.92; H, 6.34; N, 19.57. Found: C, 63.08; H, 6.50; N, 19.13.

4. SEM images of 3a and 3b

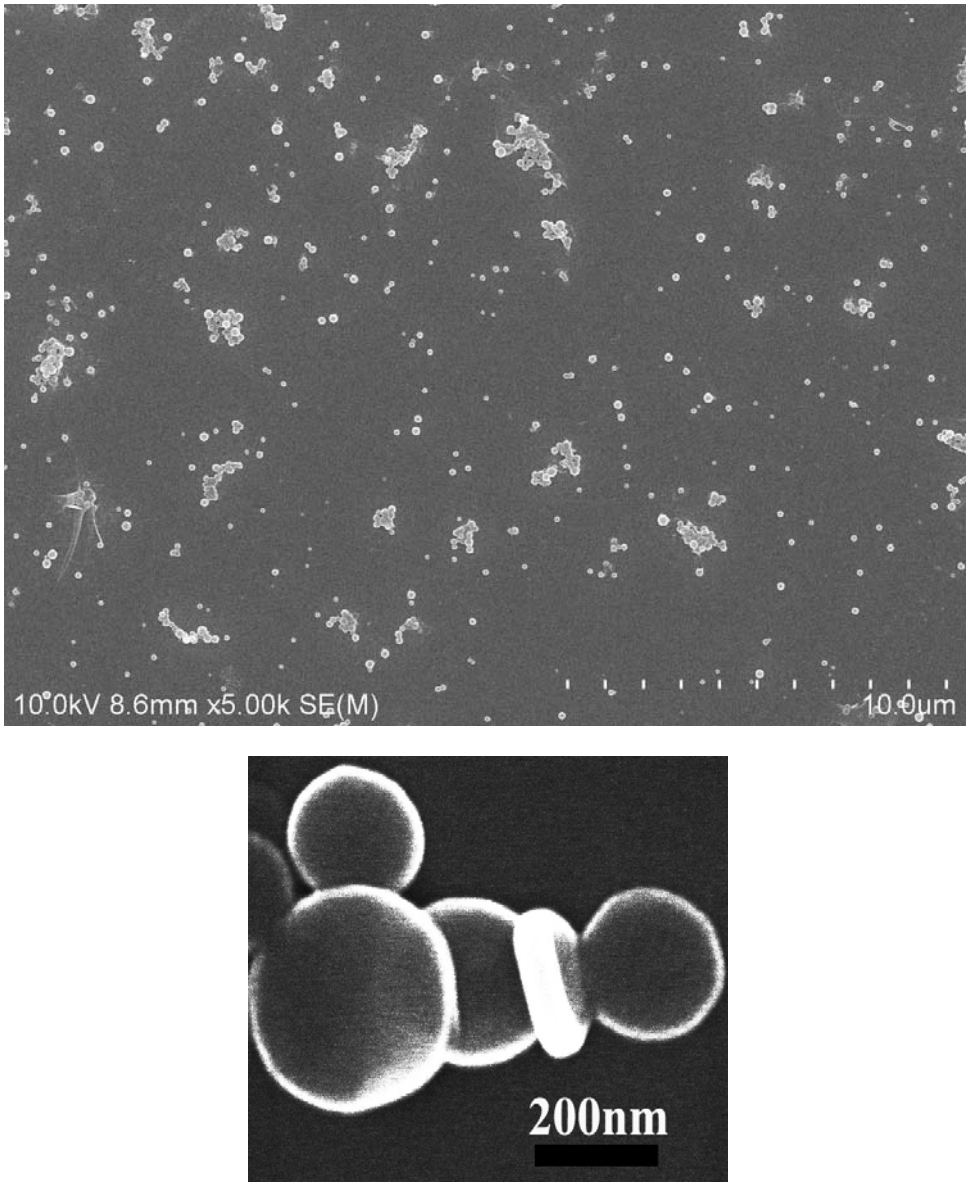


Fig. S1 SEM images of vesicles formed with **3a** in water.

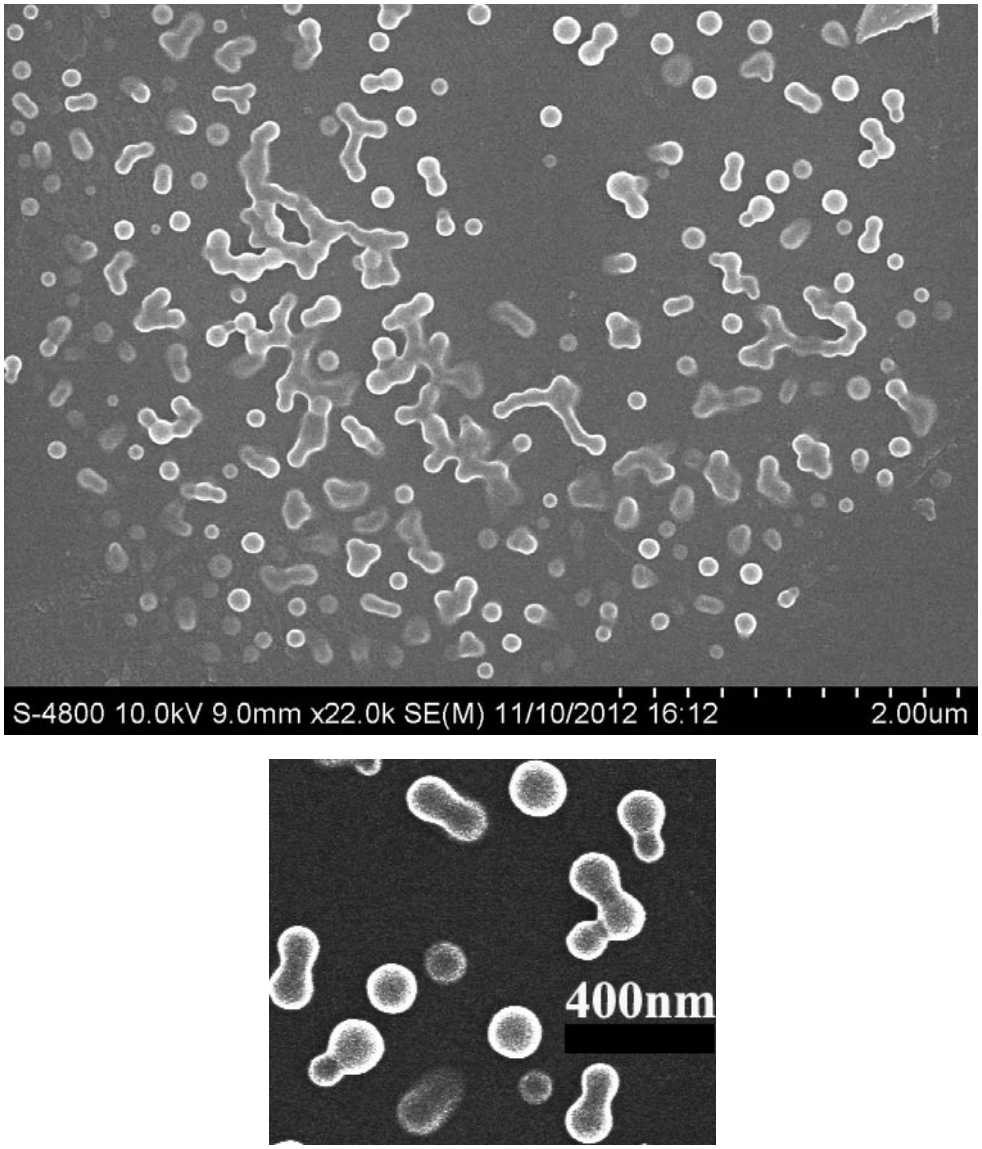


Fig. S2 SEM images of vesicles formed with **3b** in water.

5. TEM images of 3a-d

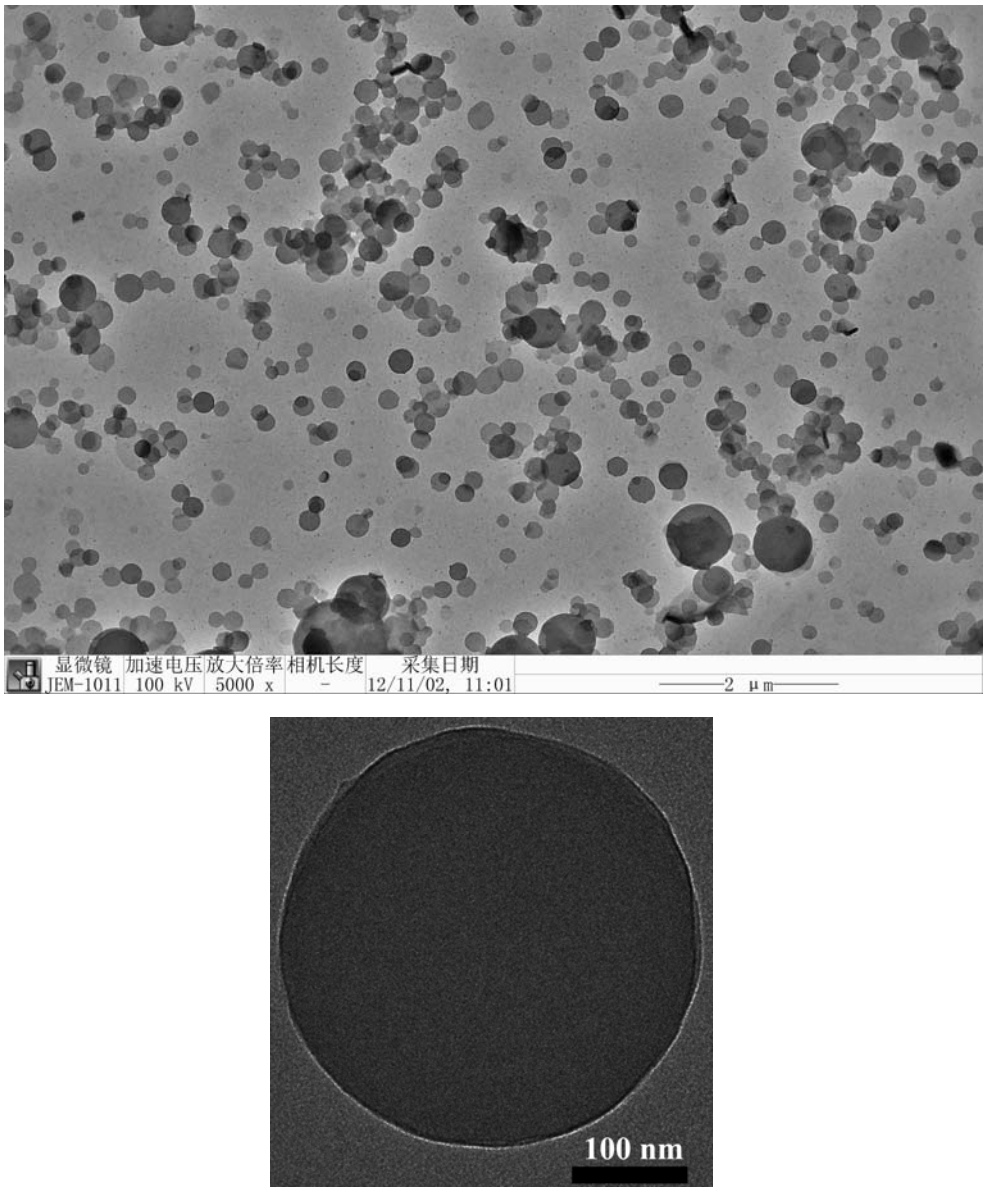


Fig. S3 TEM images of assemblies formed with **3a** in water.

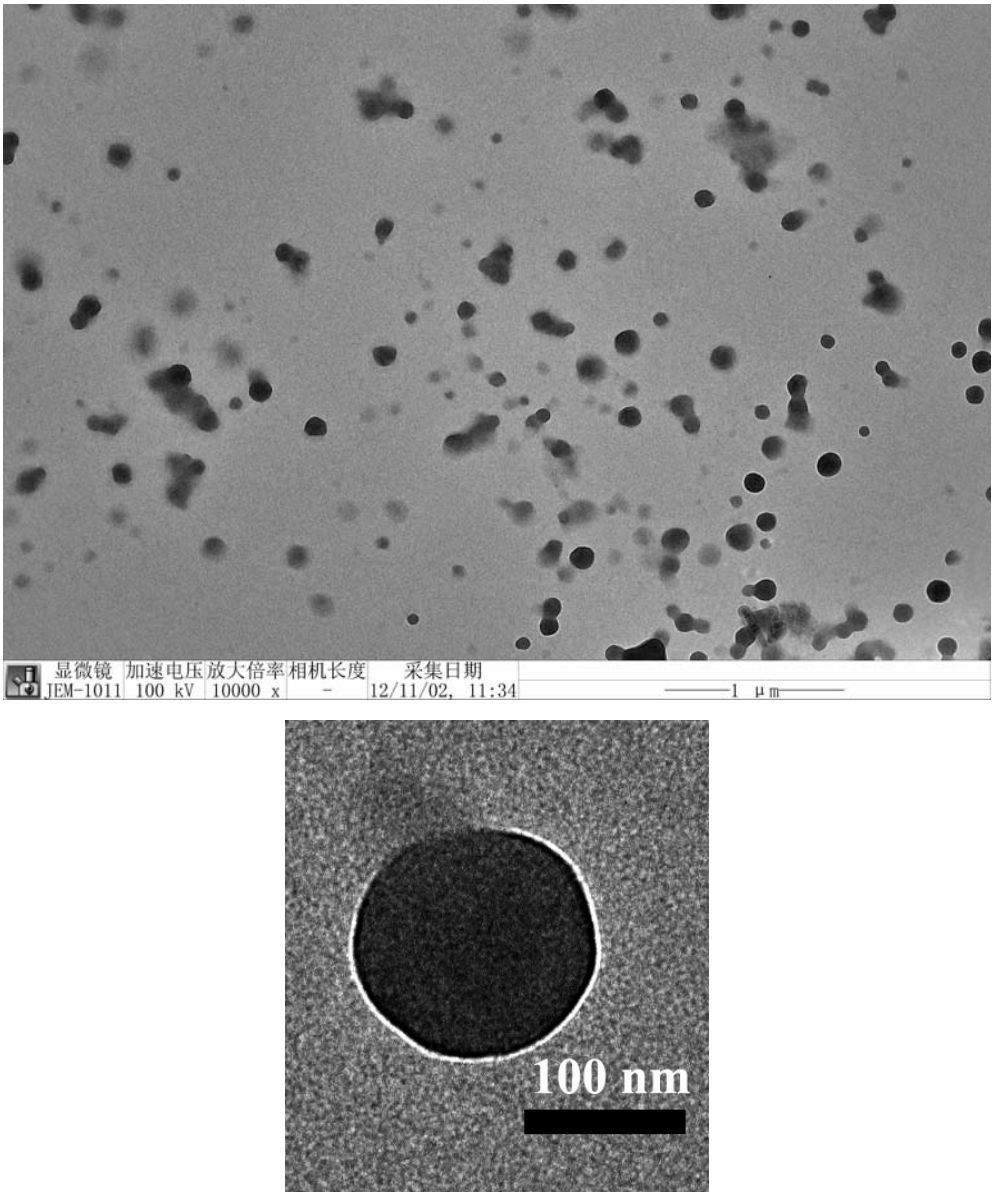


Fig. S4 TEM images of assemblies formed with **3b** in water.

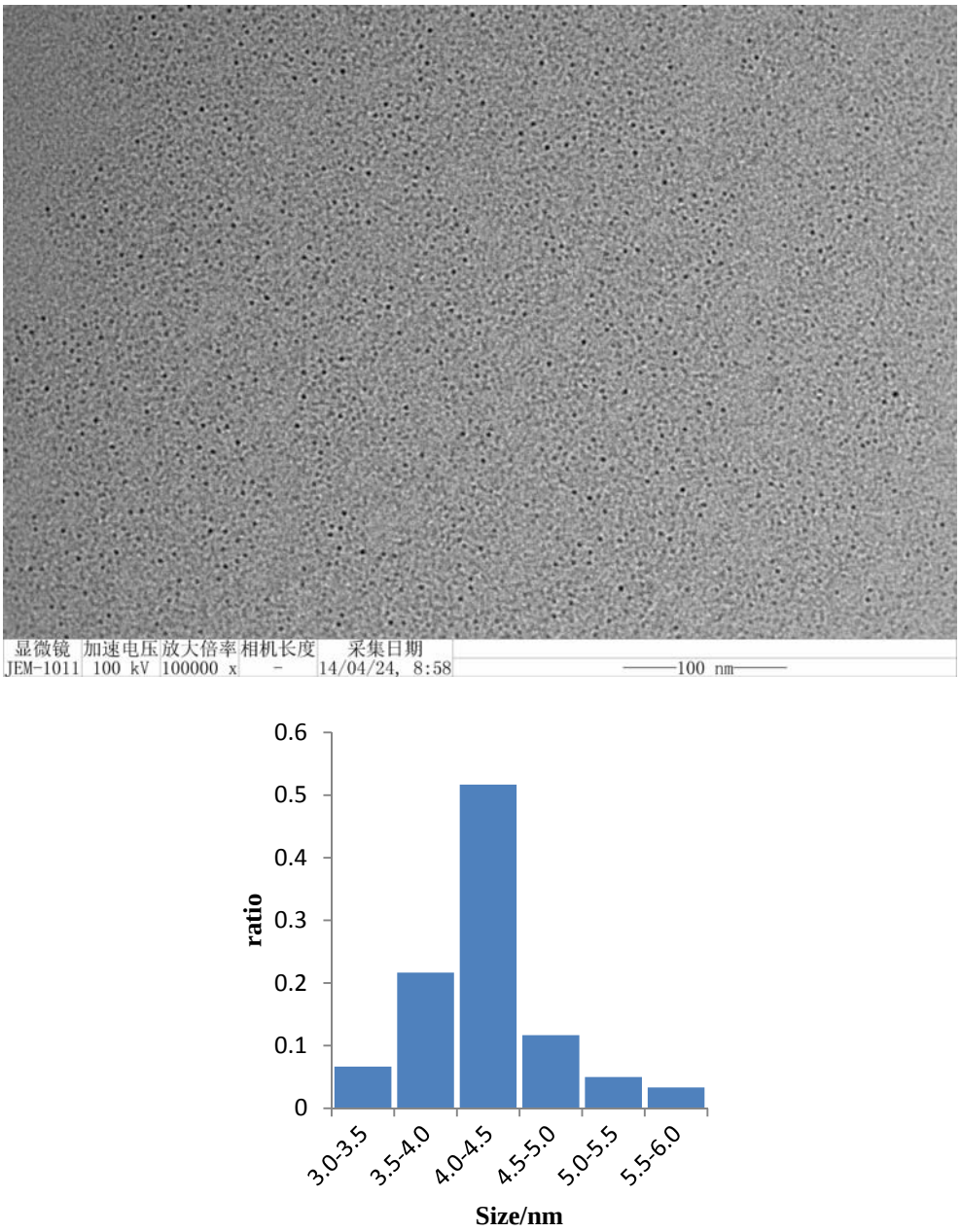
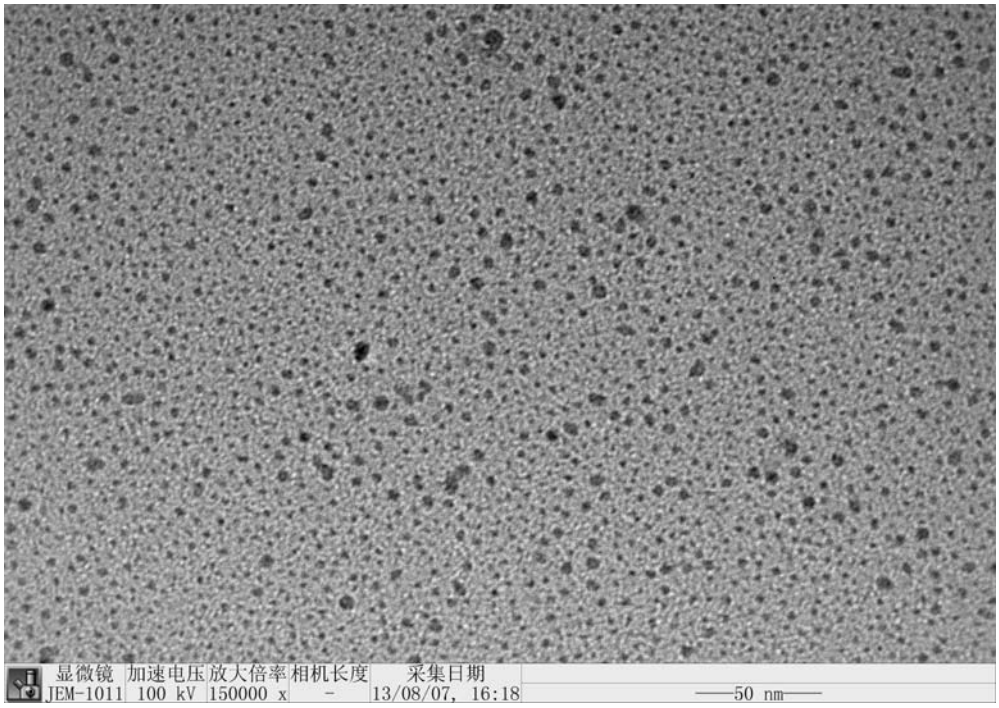


Fig. S5 TEM images of assemblies formed with **3c** in water (top) and the statistic size distribution from TEM images (bottom).



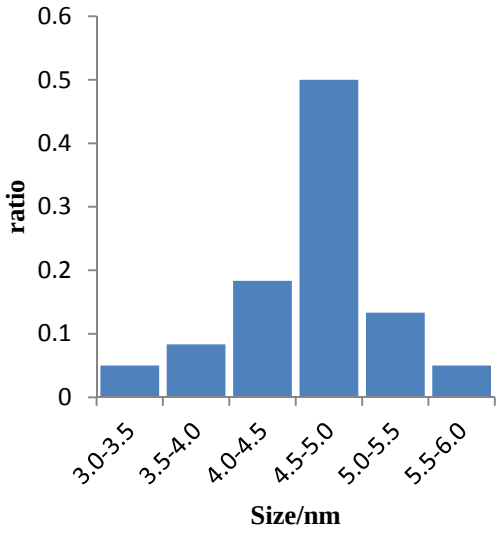


Fig. S6 TEM images of assemblies formed with **3d** in water (top) and statistic size distribution from TEM images (bottom)

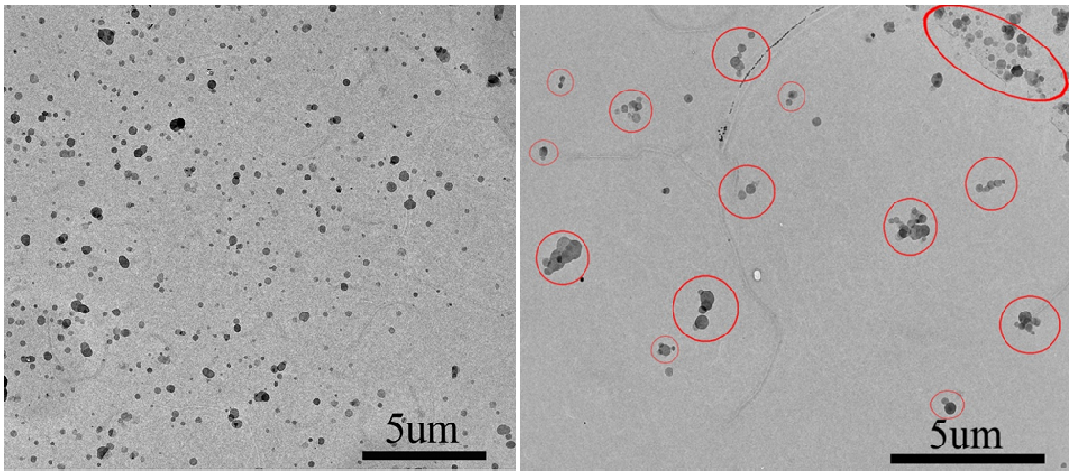


Fig. S7 TEM images of vesicles of **3a** before (left) and after (right) treated with NaCl.

6. LSCM images of 3a and 3b

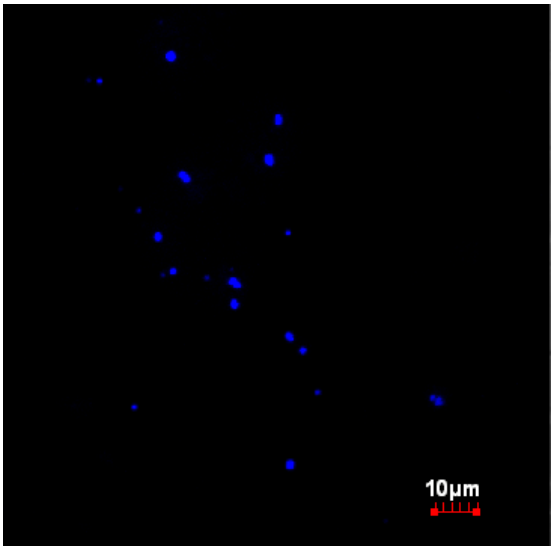


Fig. S8 LSCM images of vesicles formed with **3a**.

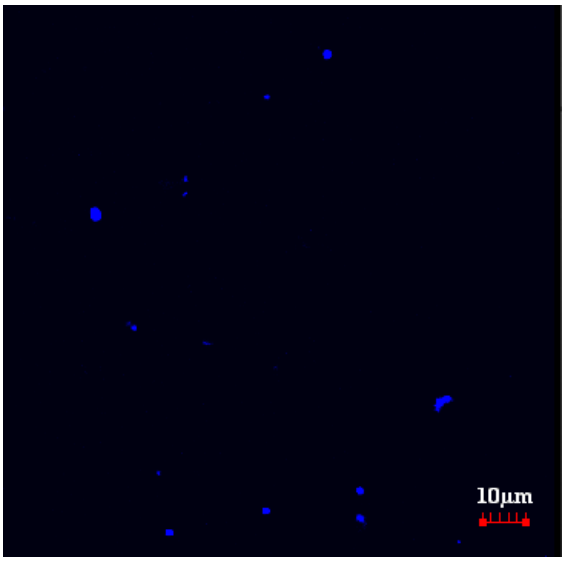


Fig. S9 LSCM images of vesicles formed with **3b**.

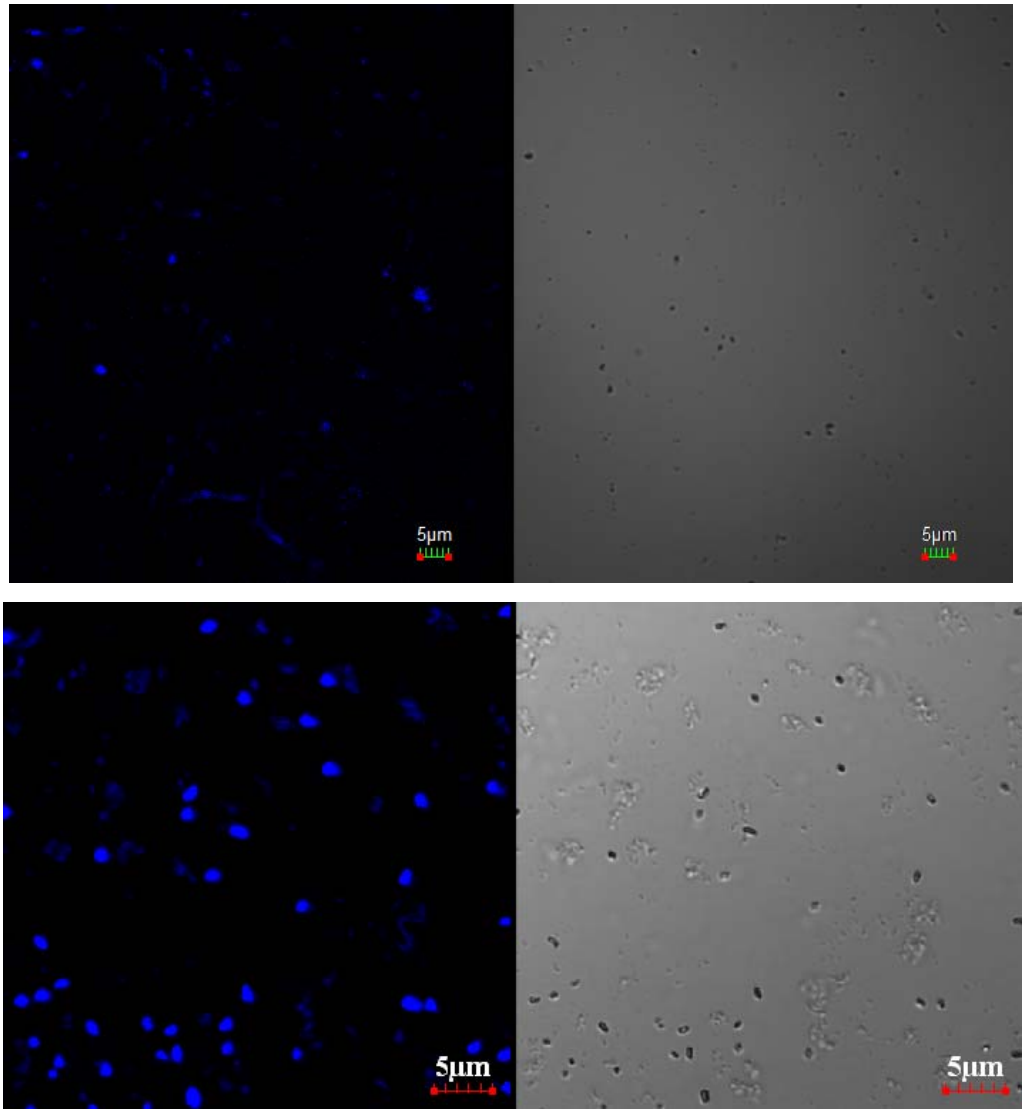


Fig. S10 LSCM images of vesicles of **3a** before (top) and after (bottom) treatment with NaCl.

7. DLS results of 3a-d

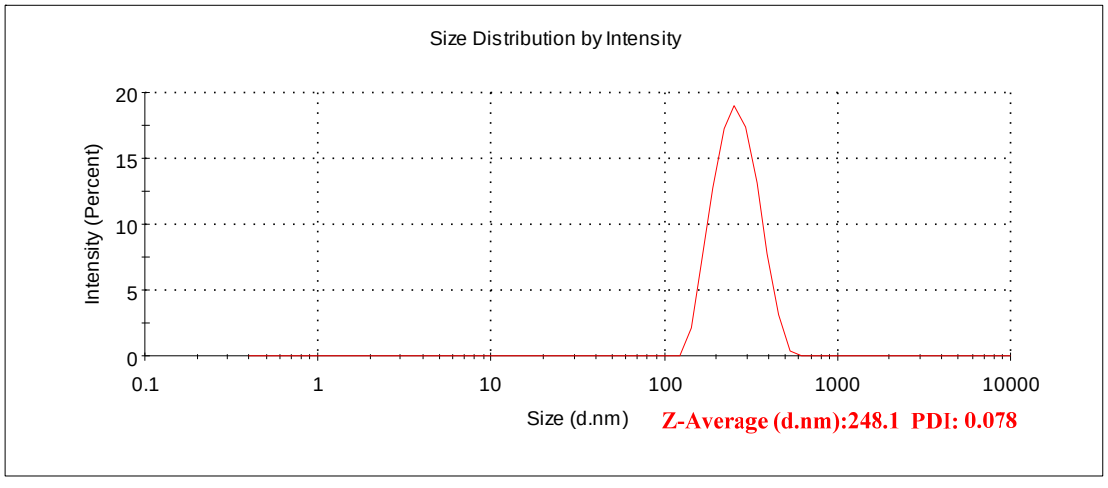


Fig. S11 Size distribution of assemblies formed with **3a**.

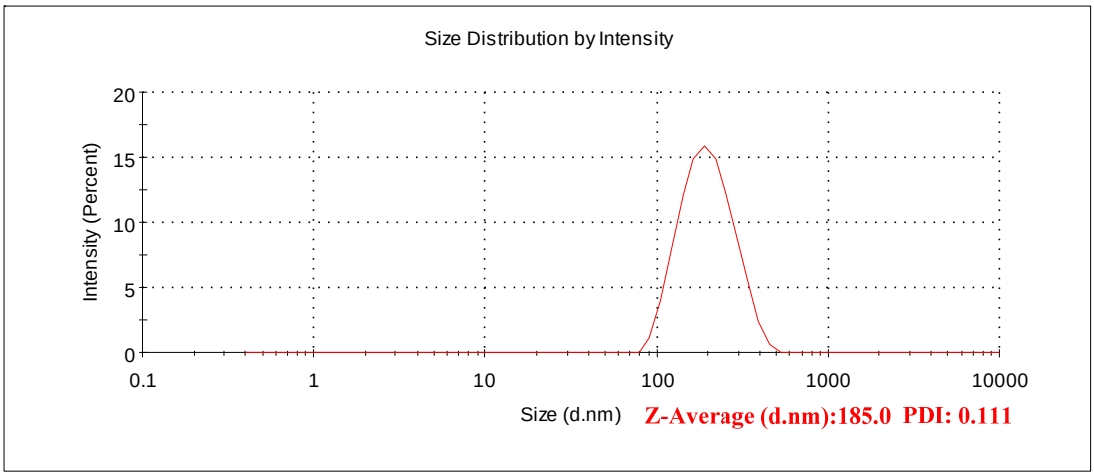


Fig. S12 Size distribution of assemblies formed with **3b**.

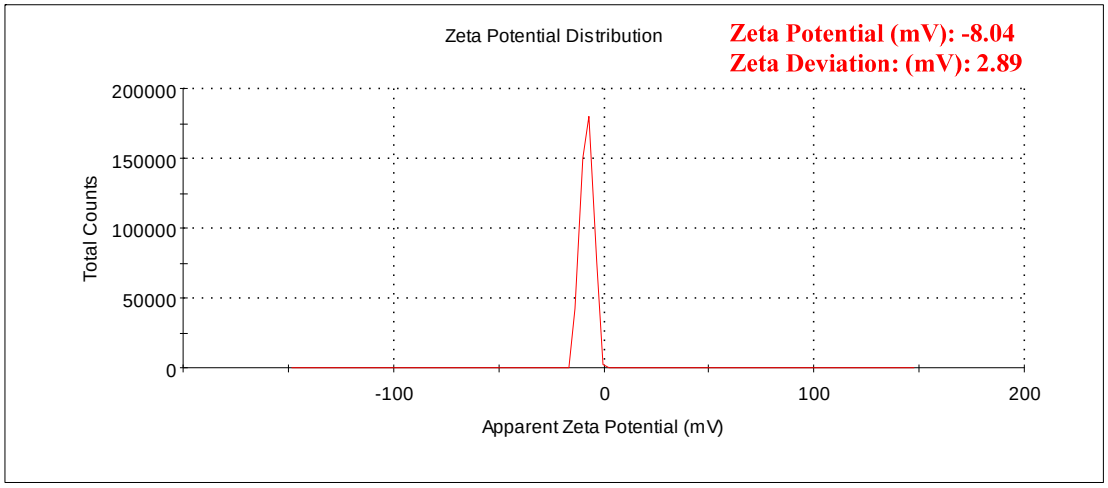


Fig. S13 Zeta potential of vesicles formed with **3a** in the presence of NaCl (0.63 mM)

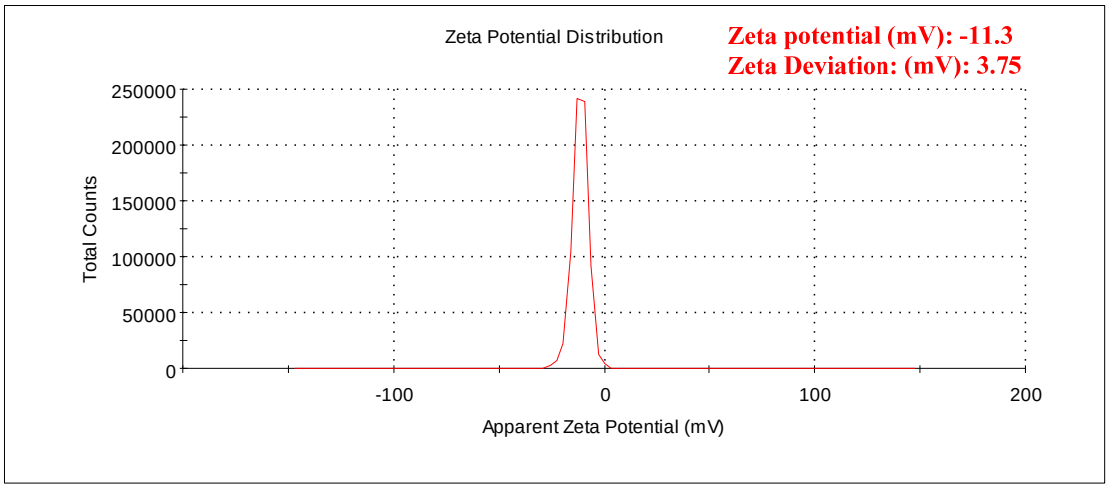


Fig. S14 Zeta potential of vesicles formed with **3b** in the presence of NaCl (6.3 mM)

8. Calculated molecular models of 3a-d

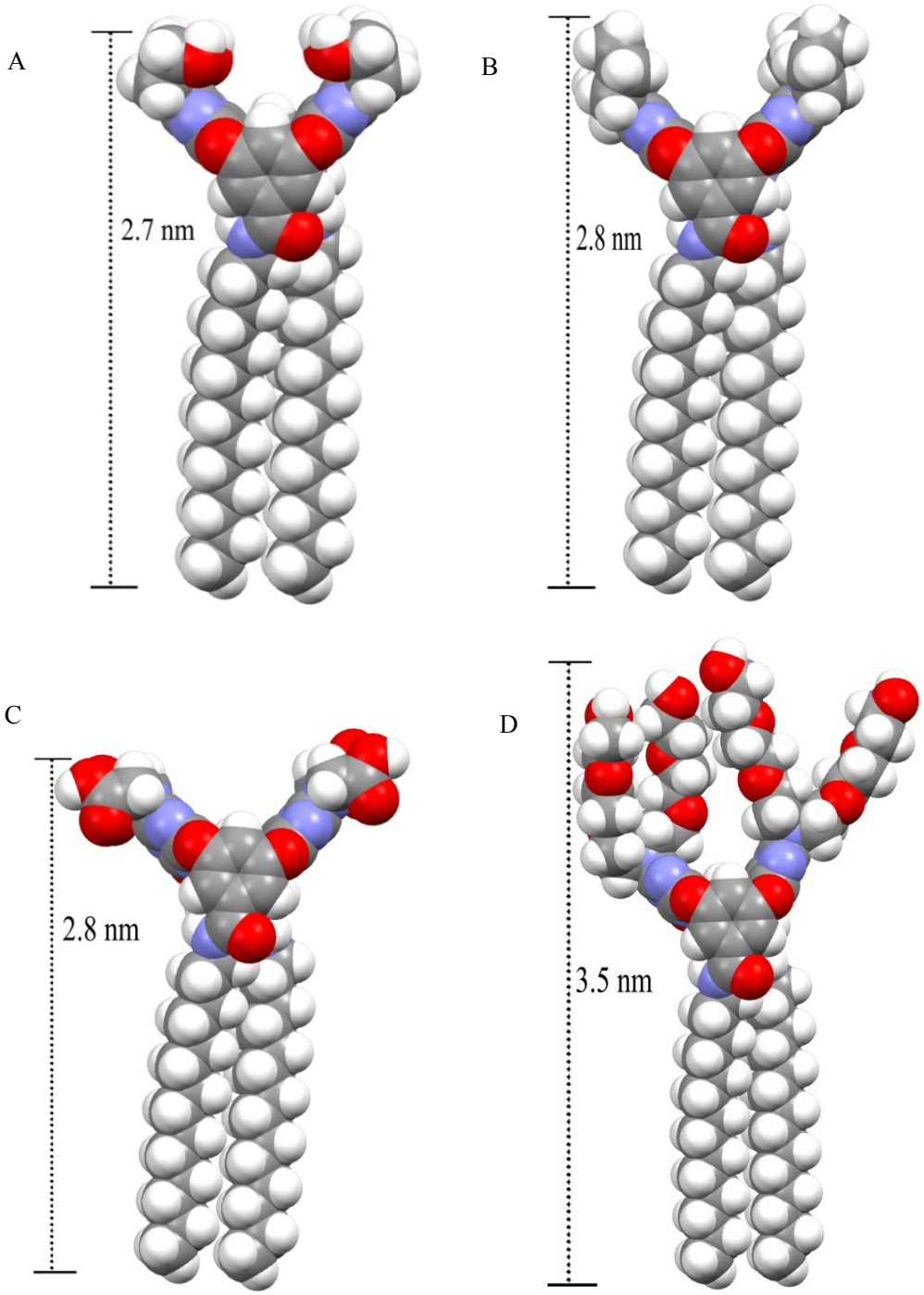


Fig. S15 Molecular model of **3a** (A), **3b** (B), **3c** (C) and **3d** (D). Optimized at b3lyp/6-31g* level

9. Determination of CAC values of 3a-d

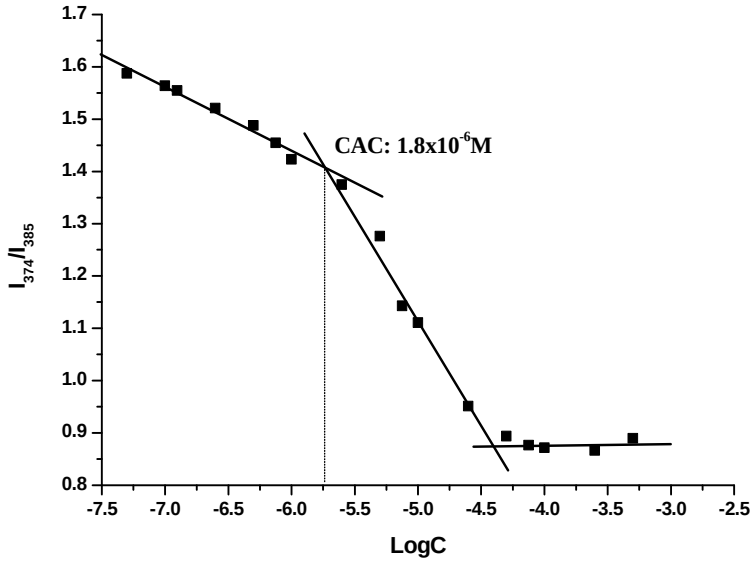


Fig. S16 Plot of I_1/I_3 ratio of pyrene vs concentration of **3a**. [pyrene]=5×10^{-7} M, excitation 335 nm.

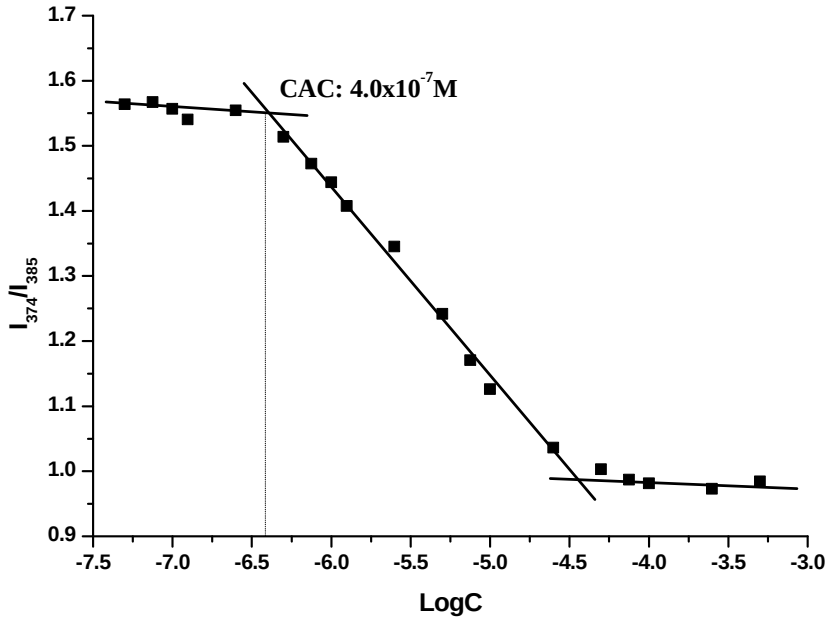


Fig. S17 Plot of I_1/I_3 ratio of pyrene vs concentration of **3b**. [pyrene]=5×10^{-7} M, excitation 335 nm.

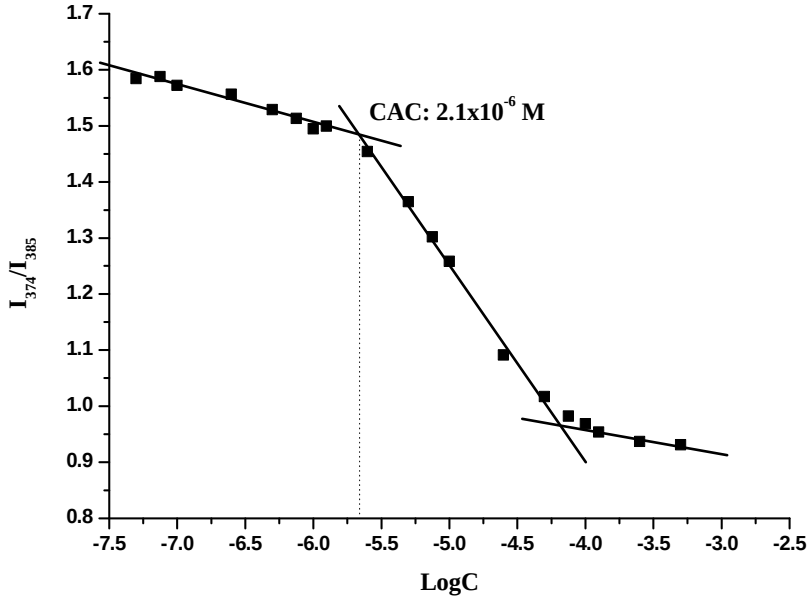


Fig. S18 Plot of I_1/I_3 ratio of pyrene vs concentration of **3c**. [pyrene]=5×10^{-7} M, excitation 335 nm.

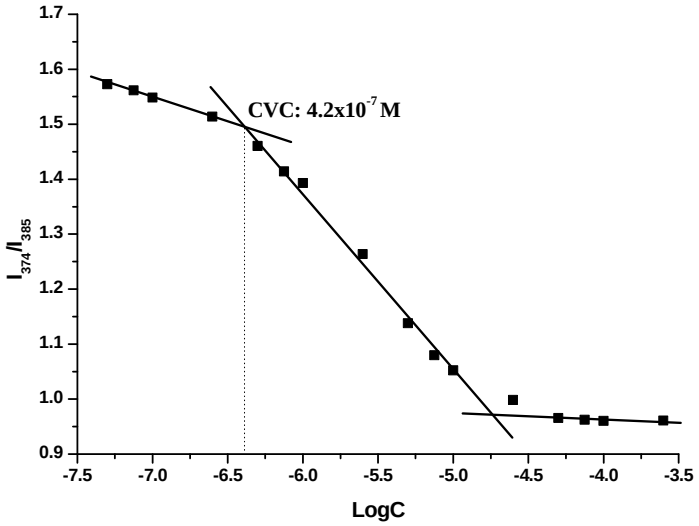


Fig. S19 Plot of I_1/I_3 ratio of pyrene vs concentration of **3d**. [pyrene]=5×10^{-7} M, excitation 335 nm.

10. XRD results for determination of the membrane thicknesses of 3a and 3b

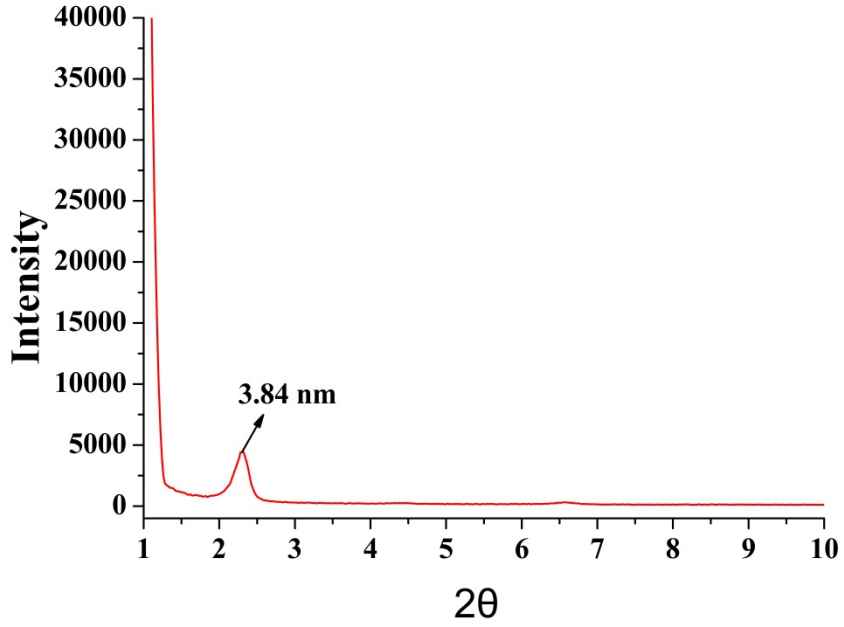


Fig. S20 XRD result of **3a**.

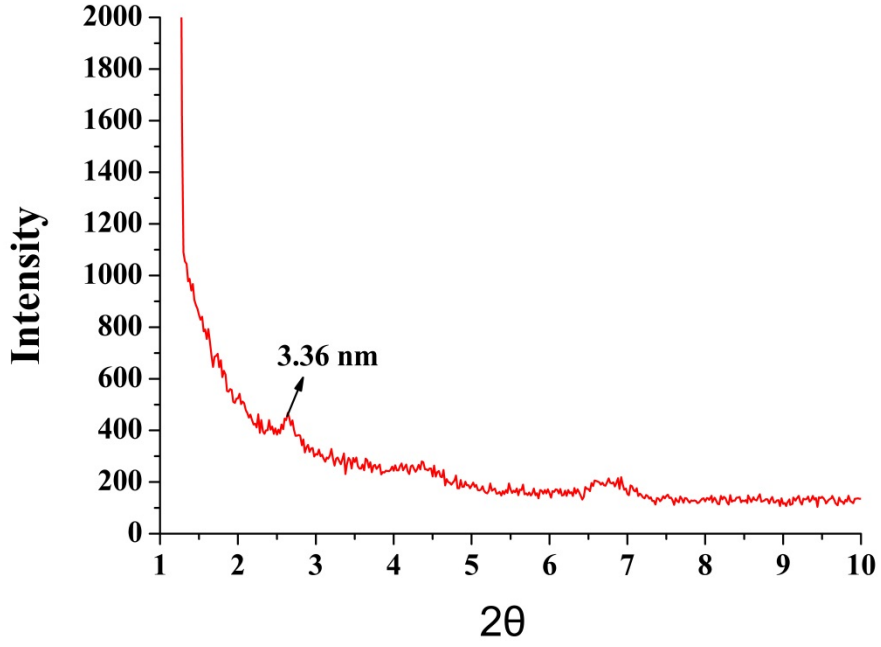


Fig. S21 XRD result of **3b**.

11. Vesicular size responses of 3b to anions

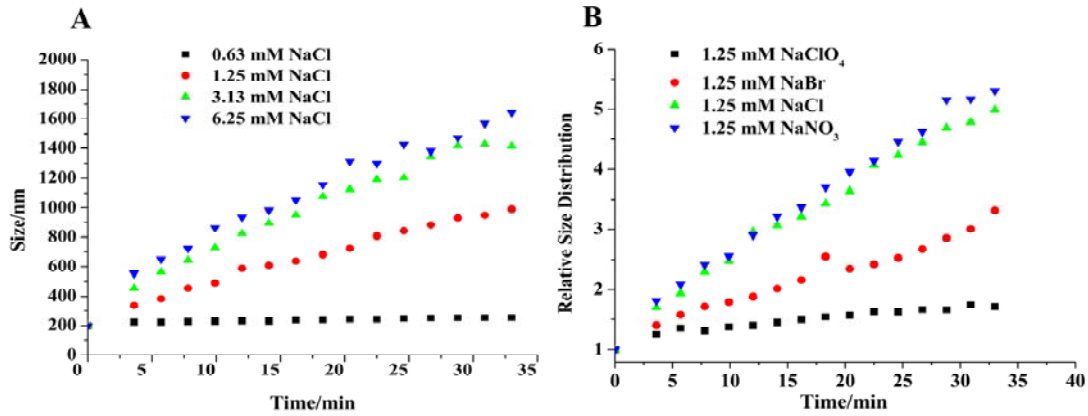


Fig. S22 Vesicular size response of **3b** to (A) NaCl of different concentrations and various anions.

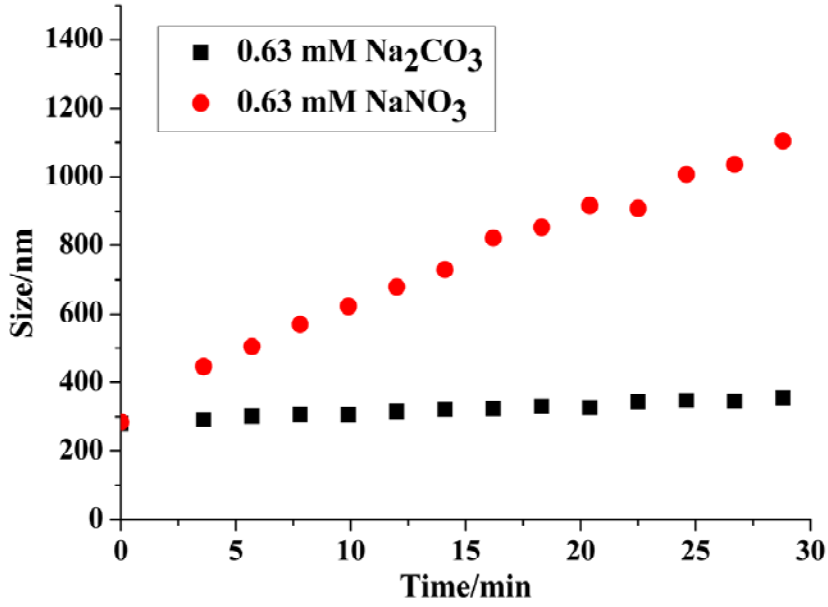


Fig.S23 Comparison of effect nitrate and carbonate on the size of **3a**.

12. NMR titrations

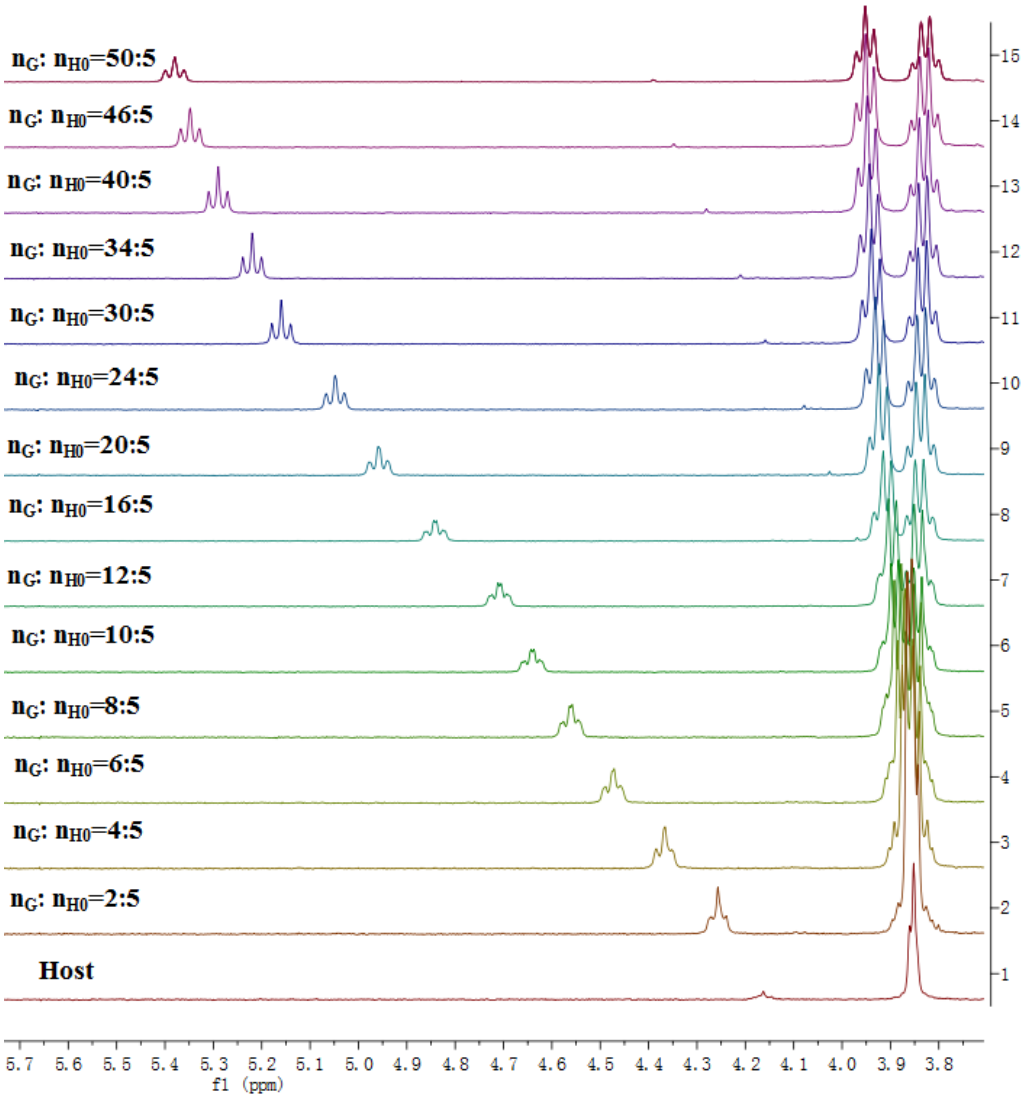


Fig. S24 ^{1}H NMR titrations of **4** in presence of n-Bu_4NCl in acetone-d_6.

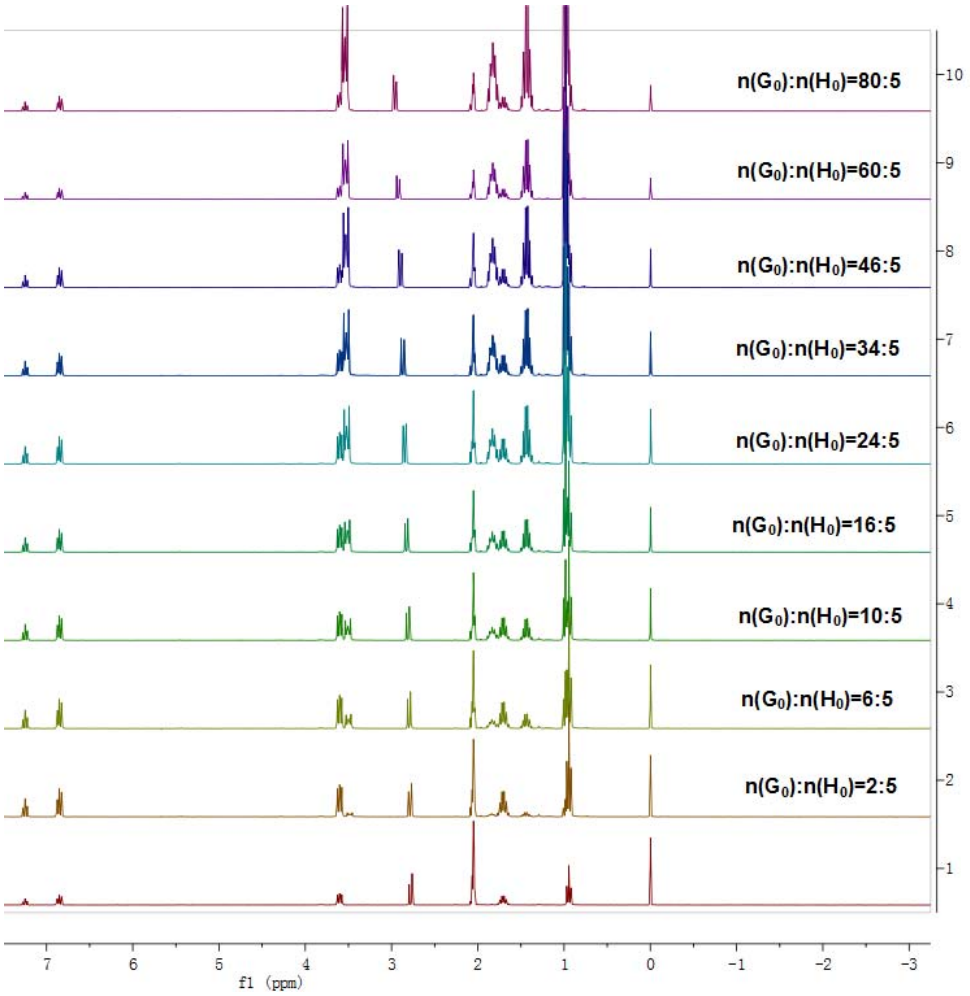


Fig. S25 ^{1}H NMR titrations of **5** in presence of n-Bu_4NCl in acetone-d_6.

References:

1. J. P. Hagemann and P. T. Kaye, *Synthetic Commun.*, 1997, **27**, 2539-2546.
2. M. X. Wang and H. B. Yang, *J. Am. Chem. Soc.*, 2004, **126**, 15412-15422.
3. S. Quici, A. Manfredi, G. Pozzi, M. Cavazzini and A. Rozzoni, *Tetrahedron*, 1999, **55**, 10487-10496.
4. M. Iqbal, J. Huskens, M. Sypula, G. Modolo and W. Verboom, *New. J. Chem.*, 2011, **35**, 2591-2600.
5. H. -B. Yang, D. -X. Wang, Q. -Q. Wang and M. -X. Wang, *J. Org. Chem.*, 2007, **72**, 3757-3763.

13 ^{1}H NMR spectra and ^{13}C NMR spectra of the compounds

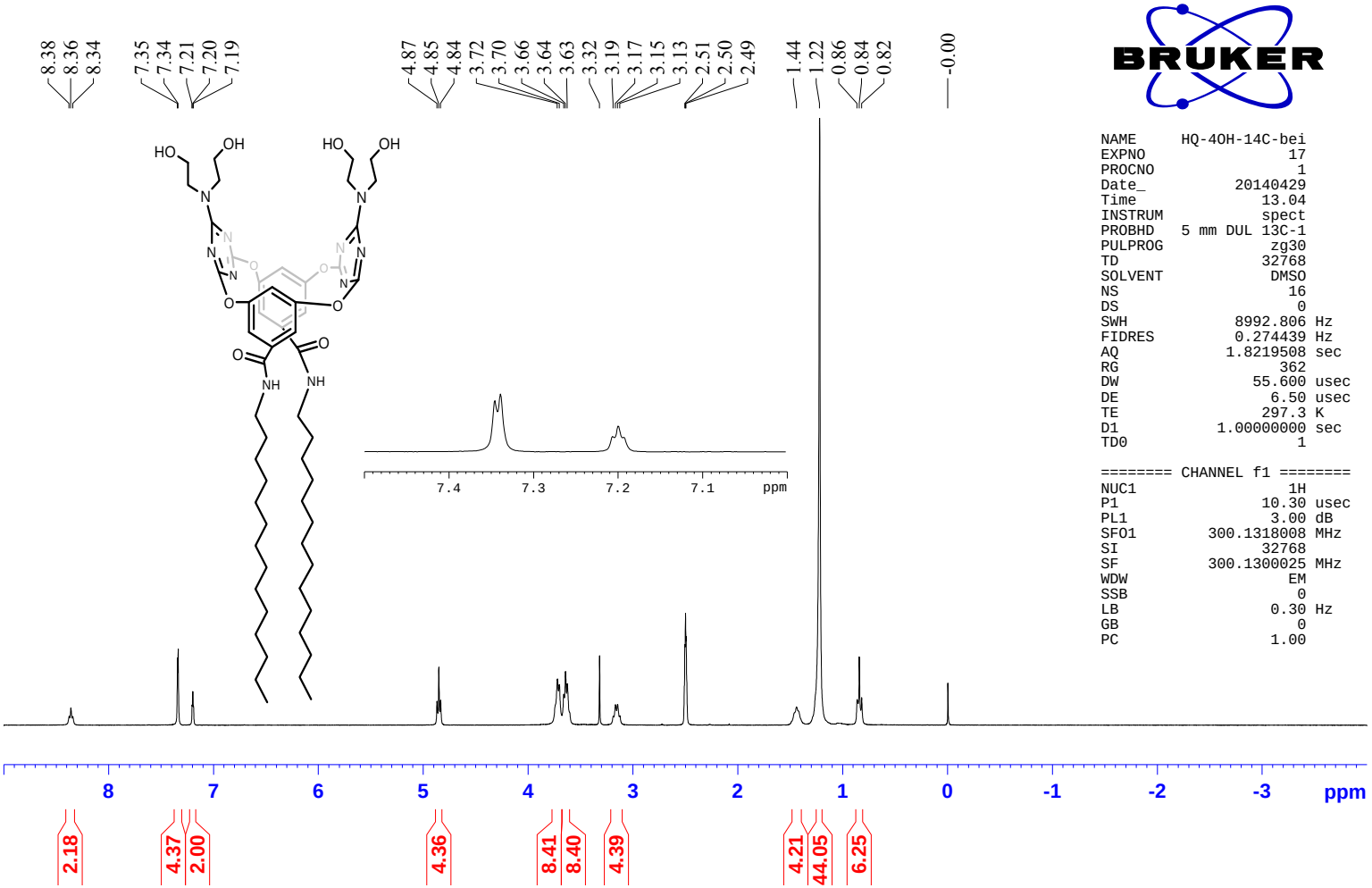
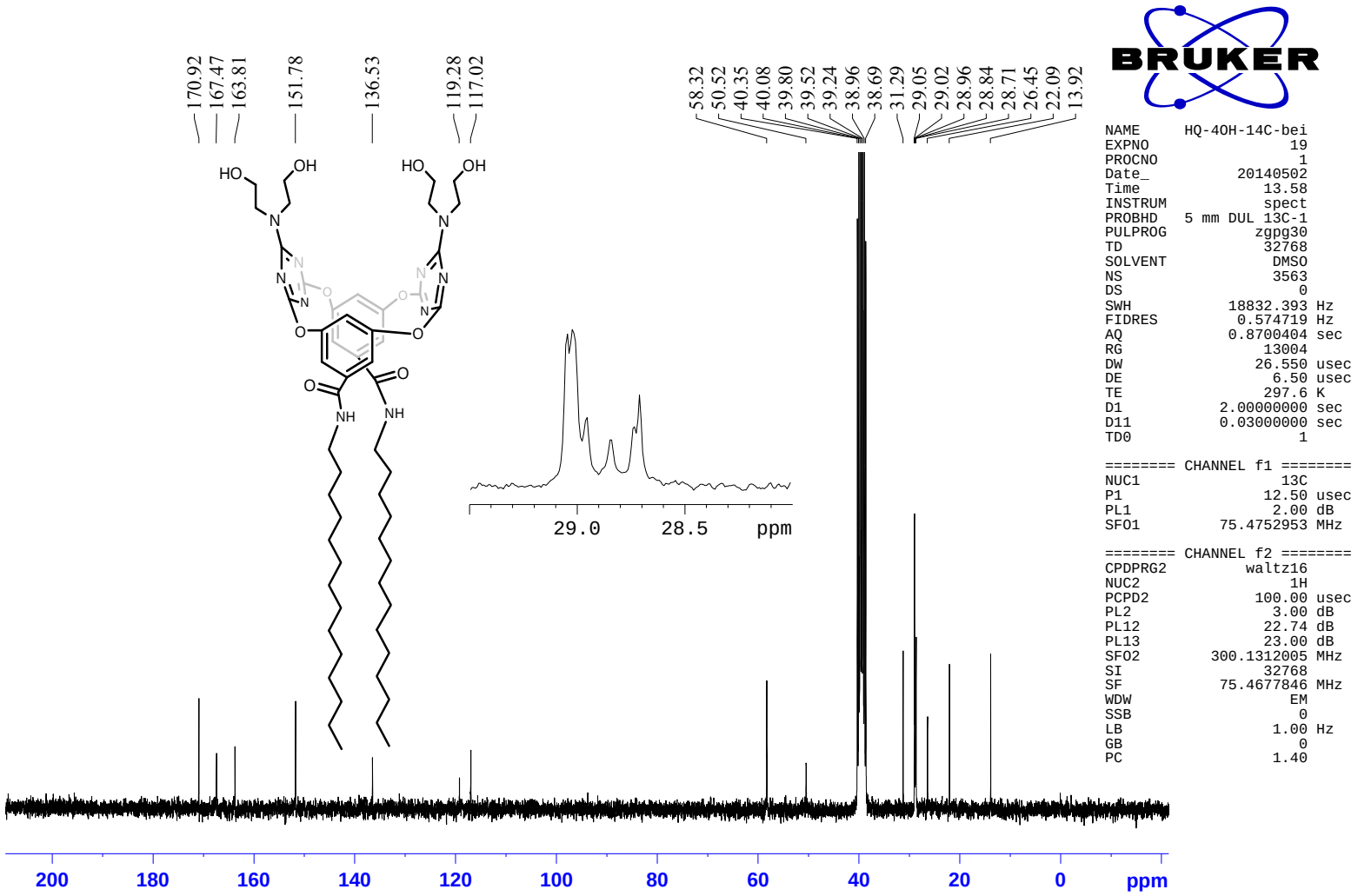


Figure S26 ^{1}H NMR spectrum of **3a.**




```
NAME        HQ-4OH-14C-bei
EXPNO                   19
PROCNO                   1
Date_             20140502
Time                 13.58
INSTRUM              spect
PROBHD    5 mm DUL 13C-1
PULPROG           zgpg30
TD                  32768
SOLVENT               DMSO
NS                    3563
DS                       0
SWH              18832.393 Hz
FIDRES           0.574719 Hz
AQ               0.8700404 sec
RG                   13004
DW                  26.550 usec
DE                   6.50 usec
TE                  297.6 K
D1              2.00000000 sec
D11             0.03000000 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                   13C
P1                   12.50 usec
PL1                   2.00 dB
SFO1            75.4752953 MHz

======== CHANNEL f2 ========
CPDPRG2            waltz16
NUC2                   1H
PCPD2               100.00 usec
PL2                   3.00 dB
PL12                 22.74 dB
PL13                 23.00 dB
SFO2           300.1312005 MHz
SI                    32768
SF              75.4677846 MHz
WDW                      EM
SSB                      0
LB                    1.00 Hz
GB                       0
PC                    1.40
```

Figure S27 ^{13}C NMR spectrum of **3a.**

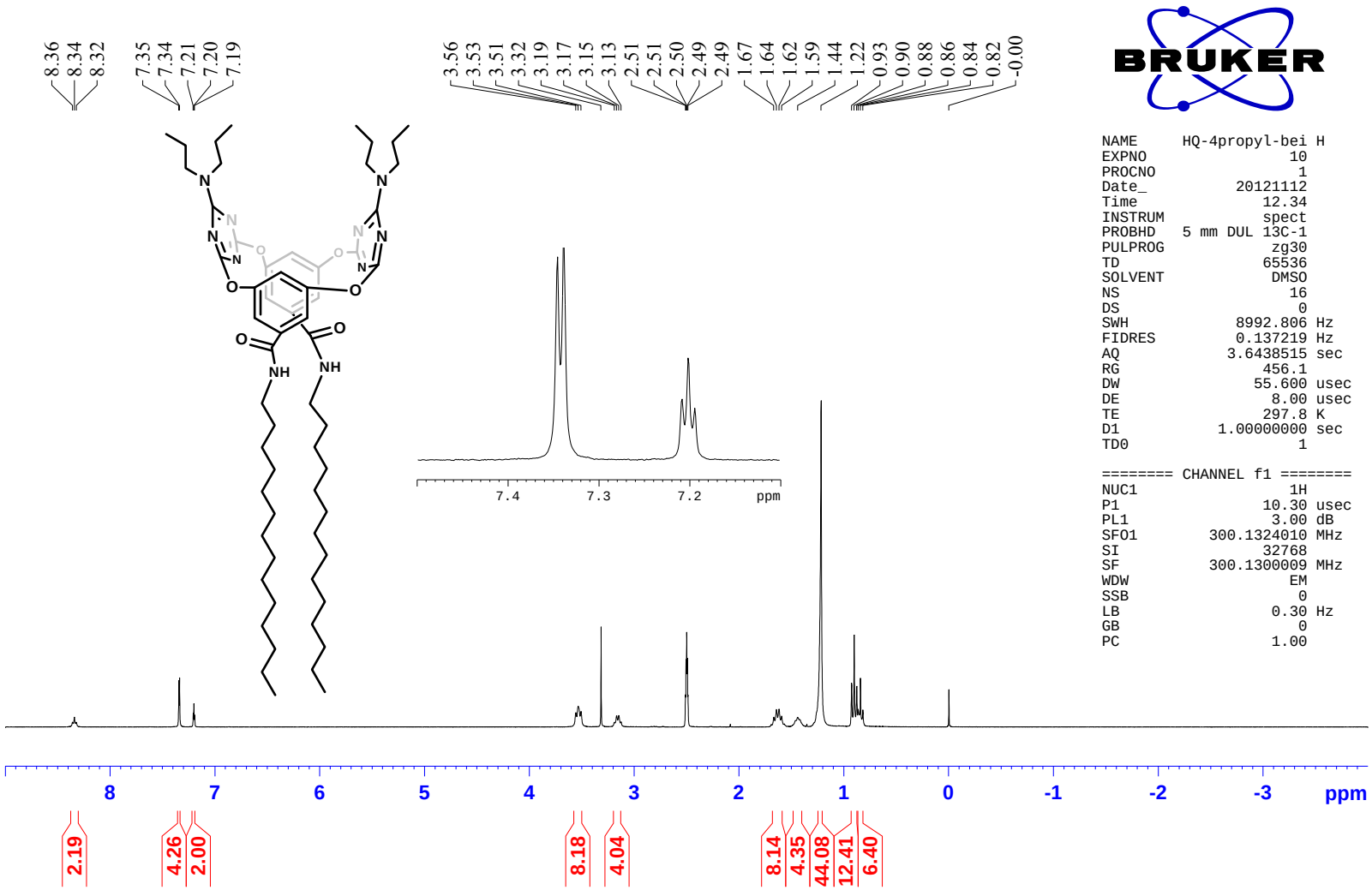


Figure S28 ^{1}H NMR spectrum of **3b.**

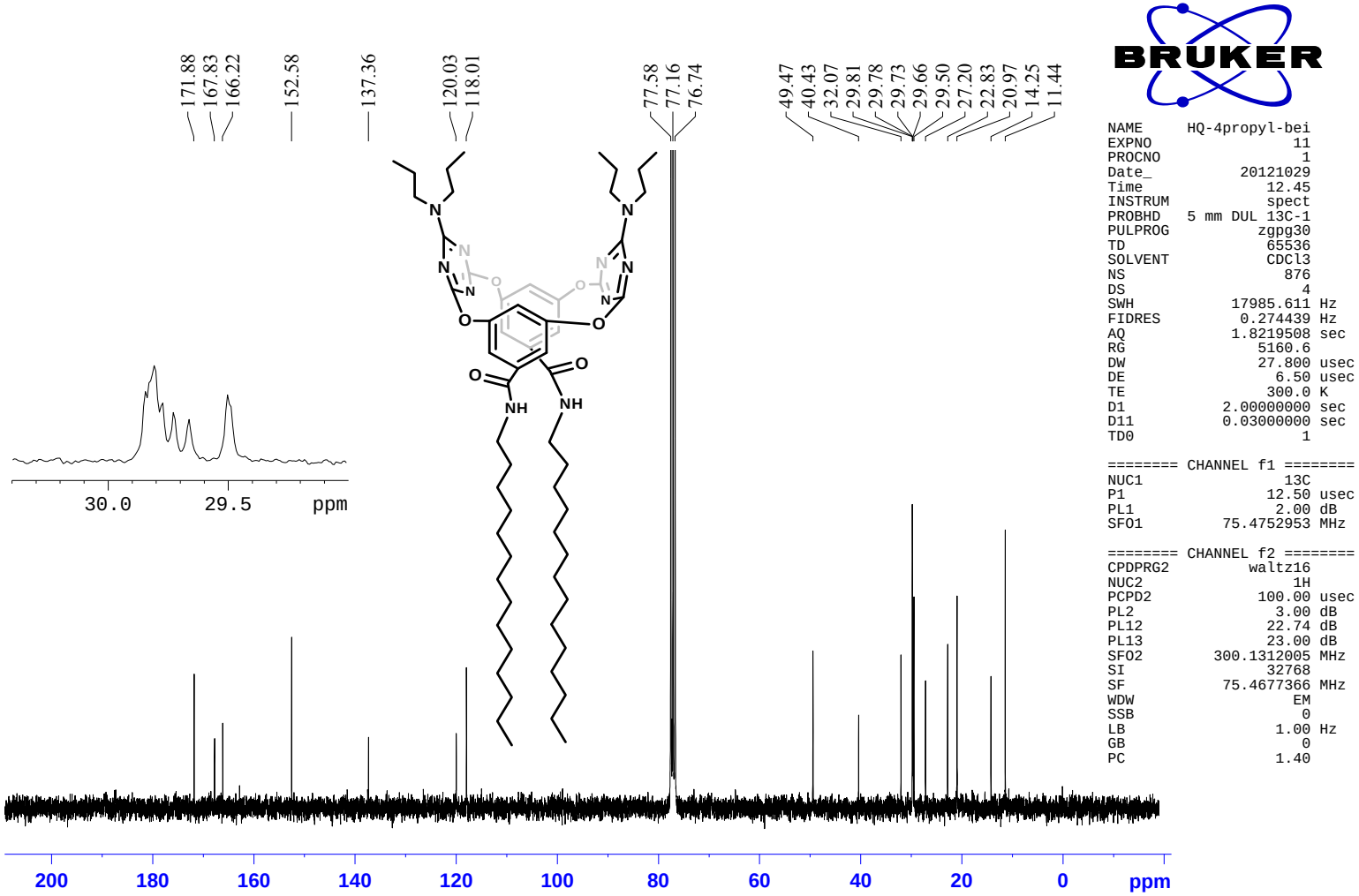


Figure S29 ^{13}C NMR spectrum of **3b.**

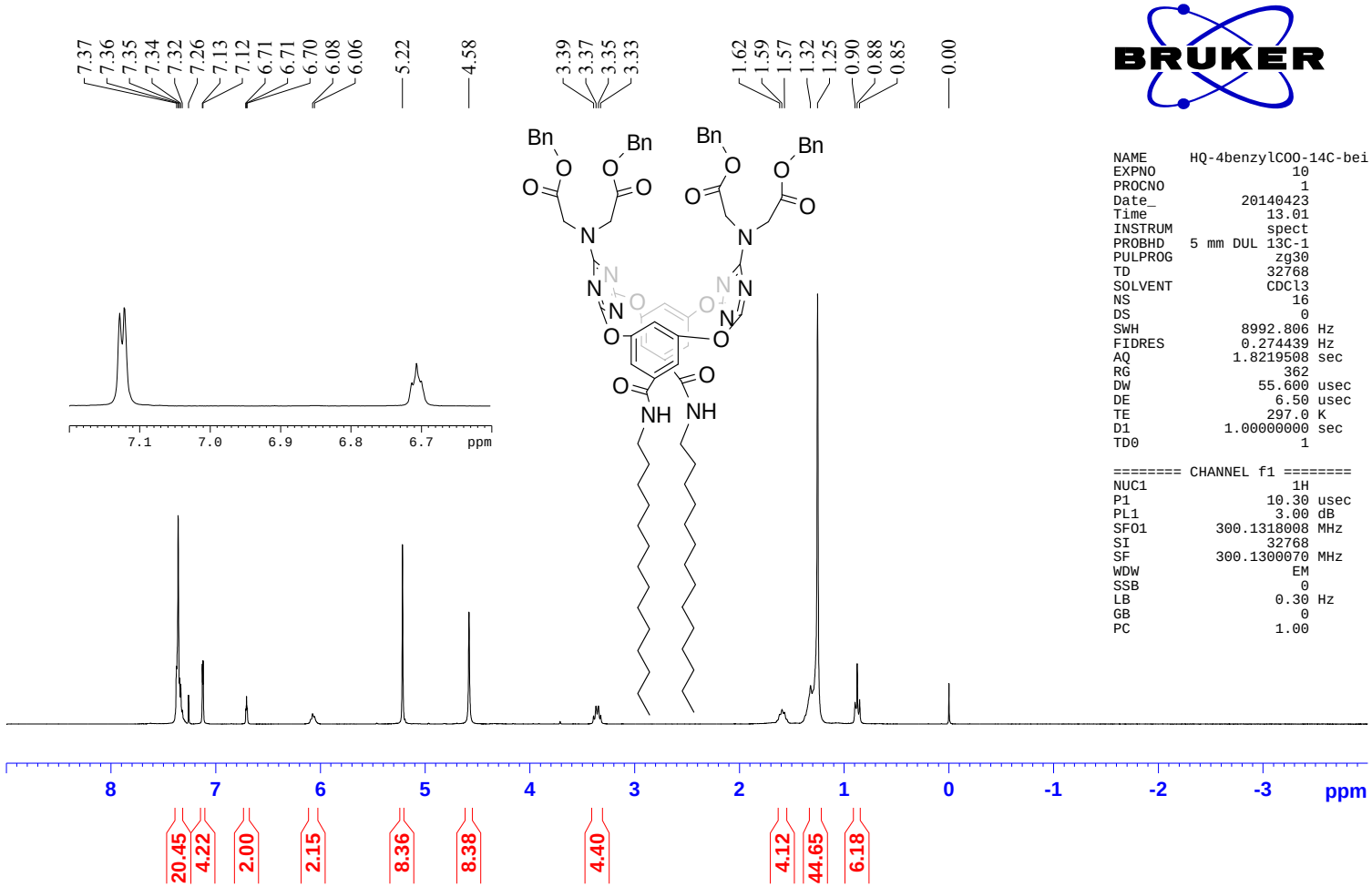
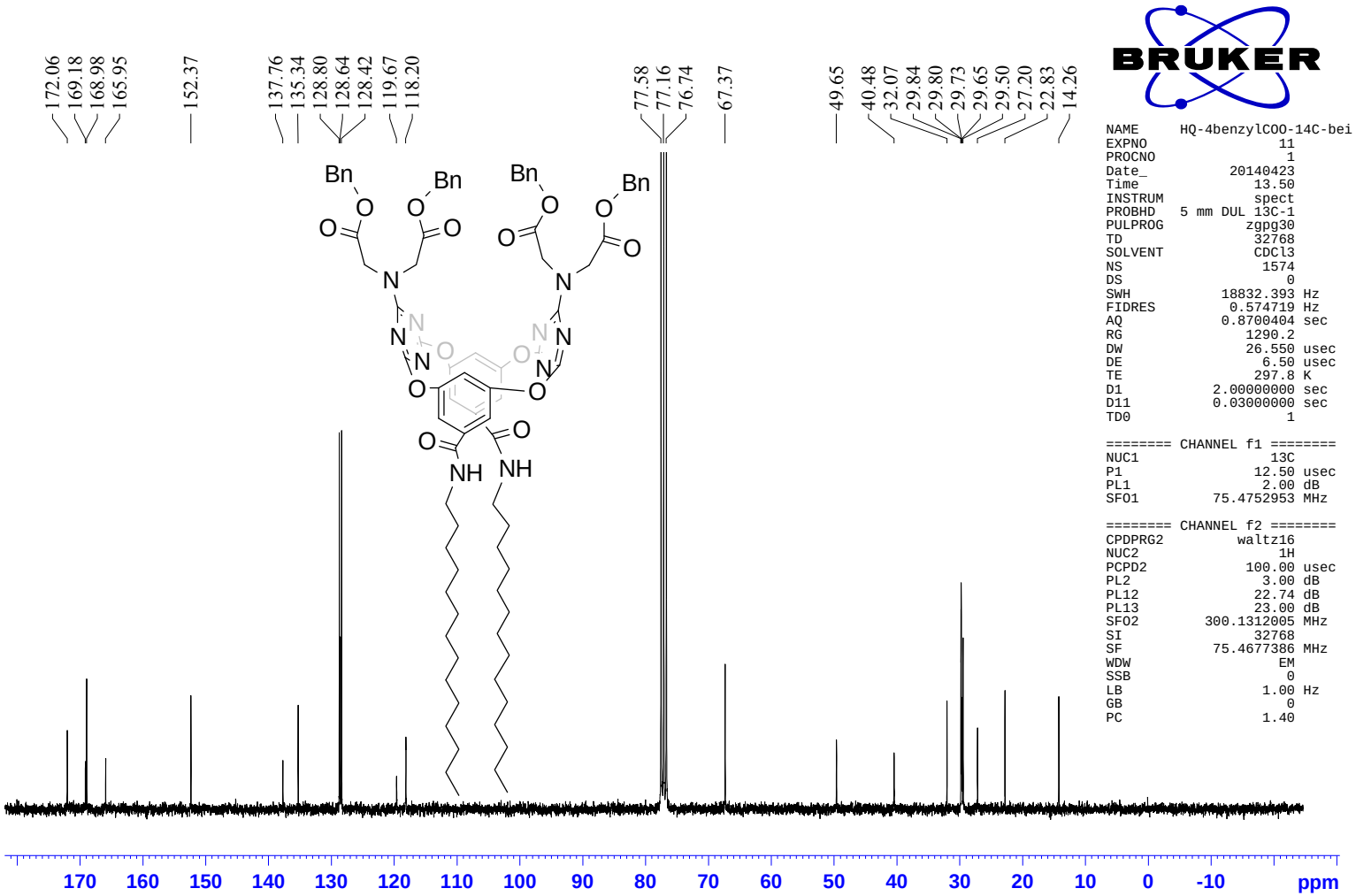


Figure S30 ^{1}H NMR spectrum of **3c`.**




```
NAME      HQ-4benzylCOO-14C-bei
EXPNO                    11
PROCNO                    1
Date_              20140423
Time                  13.50
INSTRUM               spect
PROBHD   5 mm DUL 13C-1
PULPROG              zgpg30
TD                    32768
SOLVENT               CDCl3
NS                     1574
DS                        0
SWH               18832.393 Hz
FIDRES             0.574719 Hz
AQ                 0.8700404 sec
RG                    1290.2
DW                    26.550 usec
DE                     6.50 usec
TE                    297.8 K
D1                2.00000000 sec
D11               0.03000000 sec
TD0                       1

======== CHANNEL f1 ========
NUC1                    13C
P1                    12.50 usec
PL1                    2.00 dB
SFO1             75.4752953 MHz

======== CHANNEL f2 ========
CPDPRG2              waltz16
NUC2                    1H
PCPD2                100.00 usec
PL2                    3.00 dB
PL12                  22.74 dB
PL13                  23.00 dB
SFO2            300.1312005 MHz
SI                    32768
SF               75.4677386 MHz
WDW                      EM
SSB                       0
LB                     1.00 Hz
GB                        0
PC                     1.40
```

Figure S31 ^{13}C NMR spectrum of **3c`.**

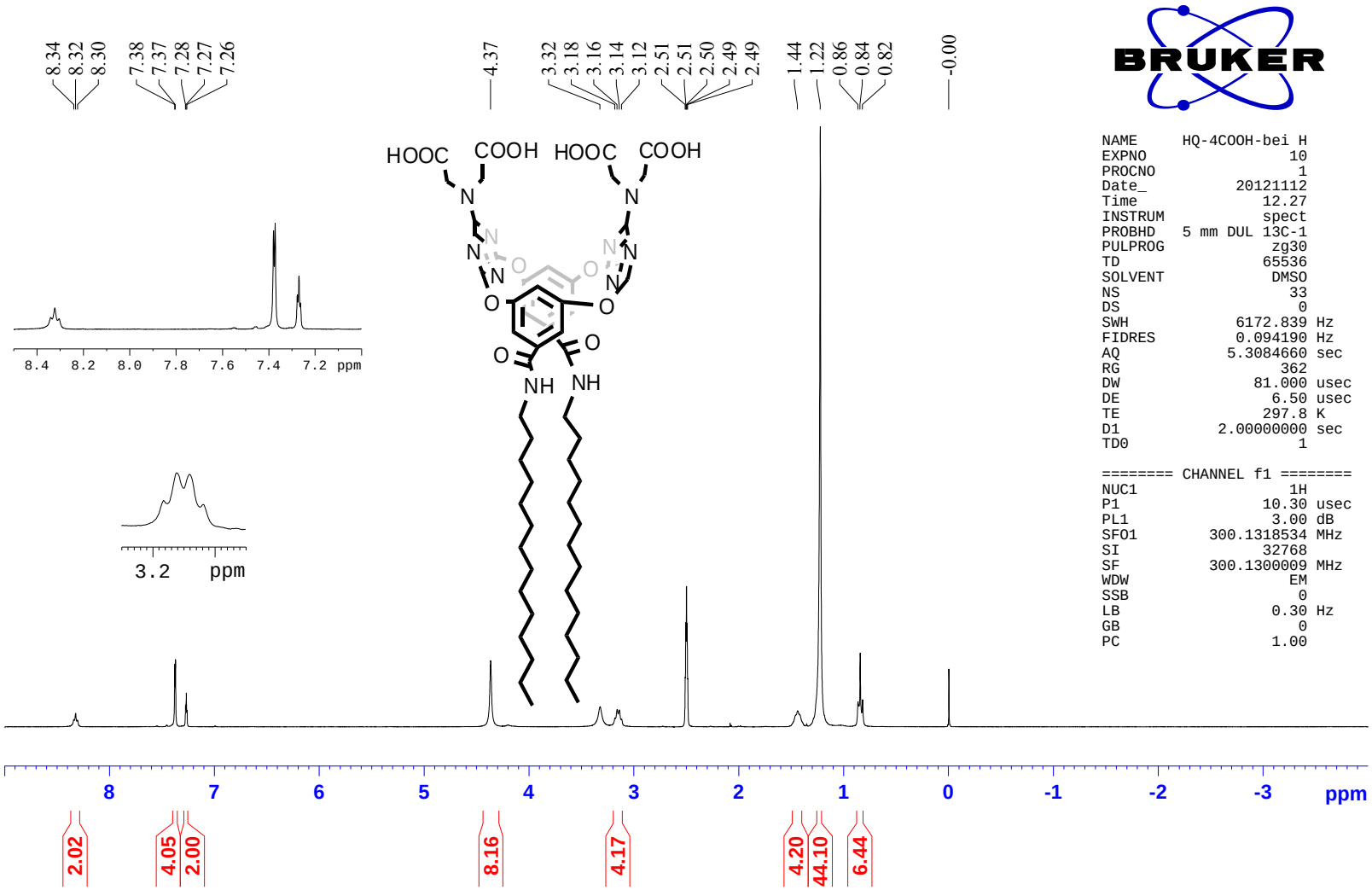


Figure S32 ^{1}H NMR spectrum of **3c.**

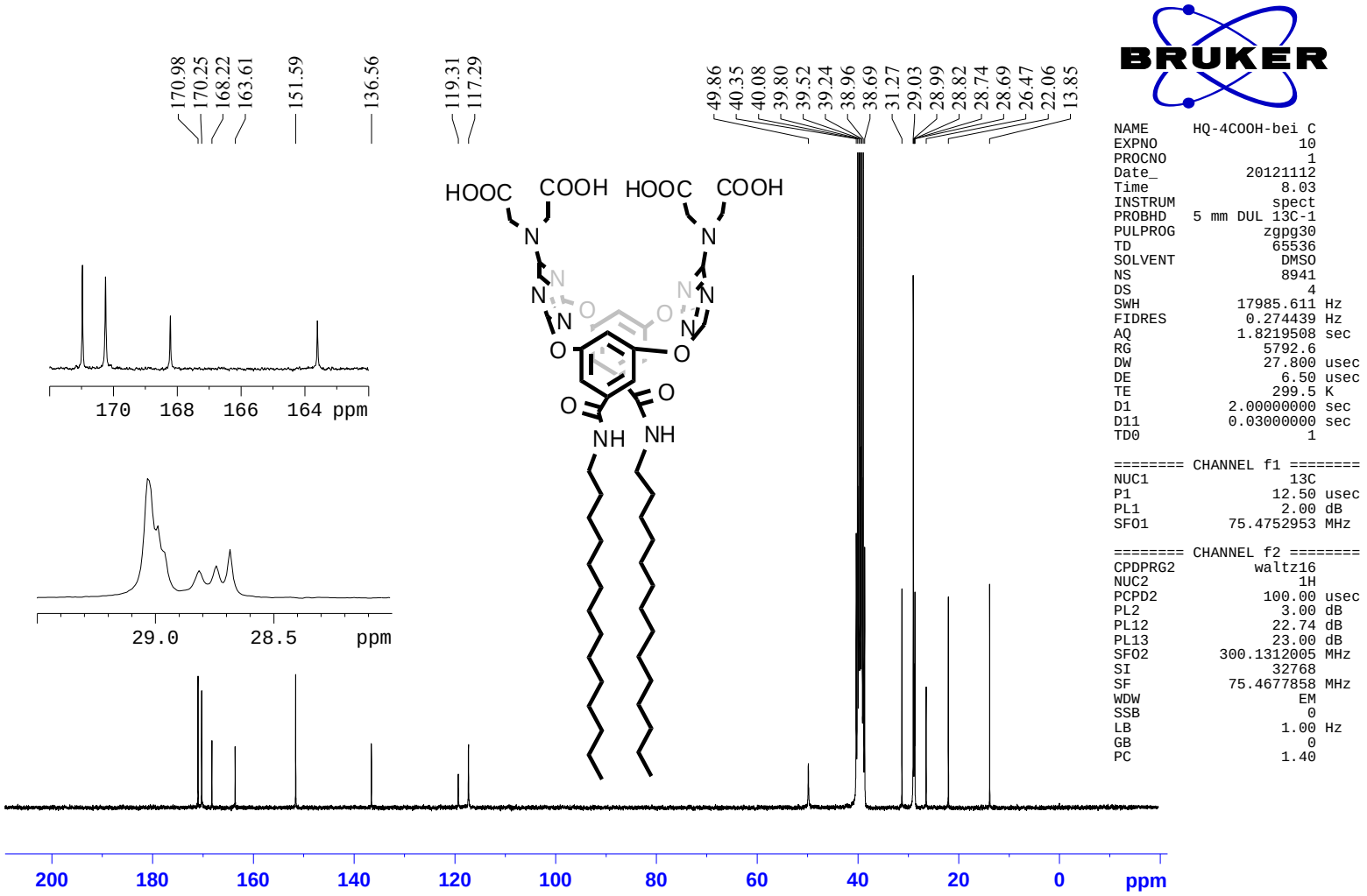


Figure S33 ^{13}C NMR spectrum of **3c.**

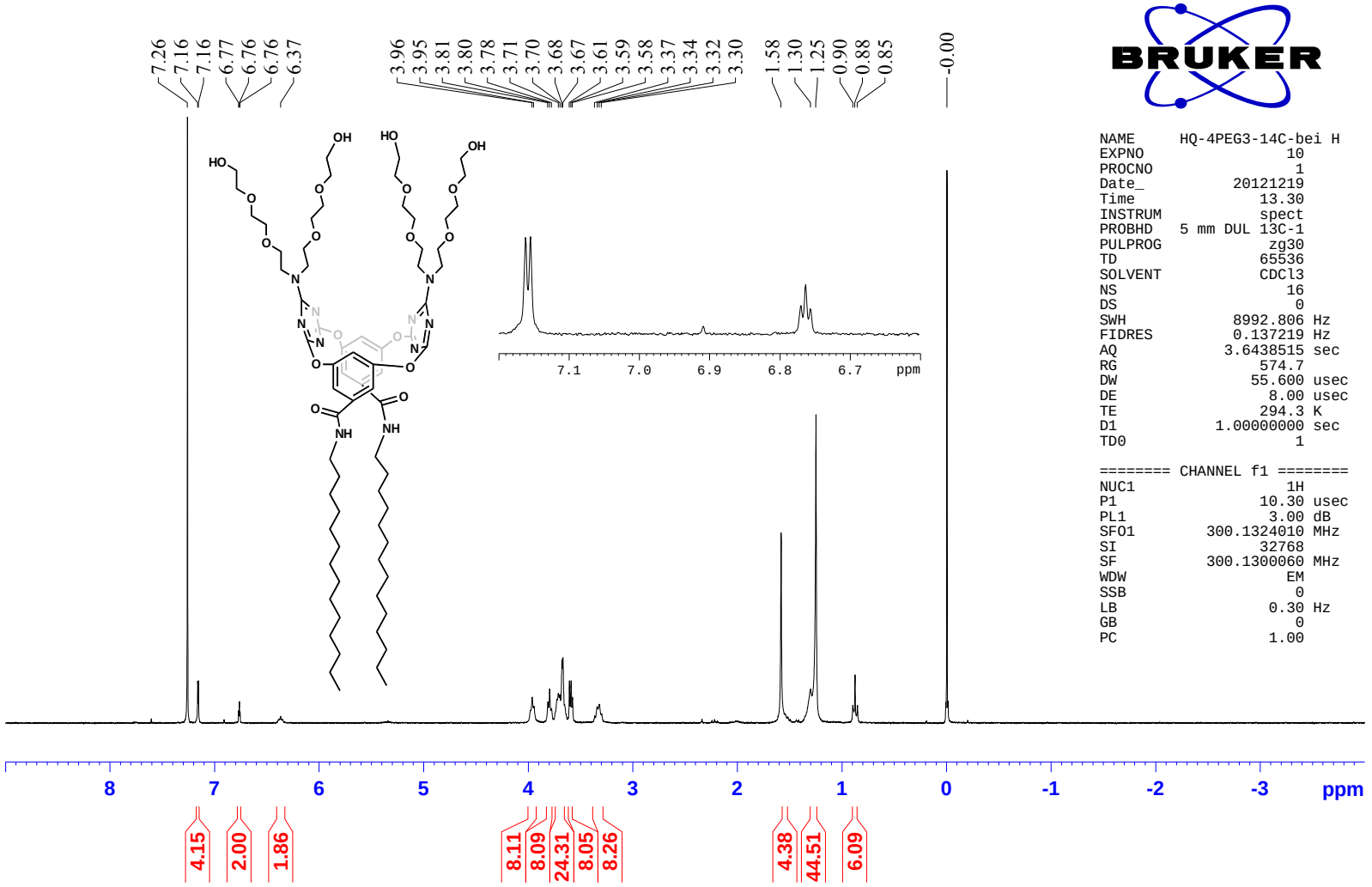


Figure S34 ^{1}H NMR spectrum of **3d.**

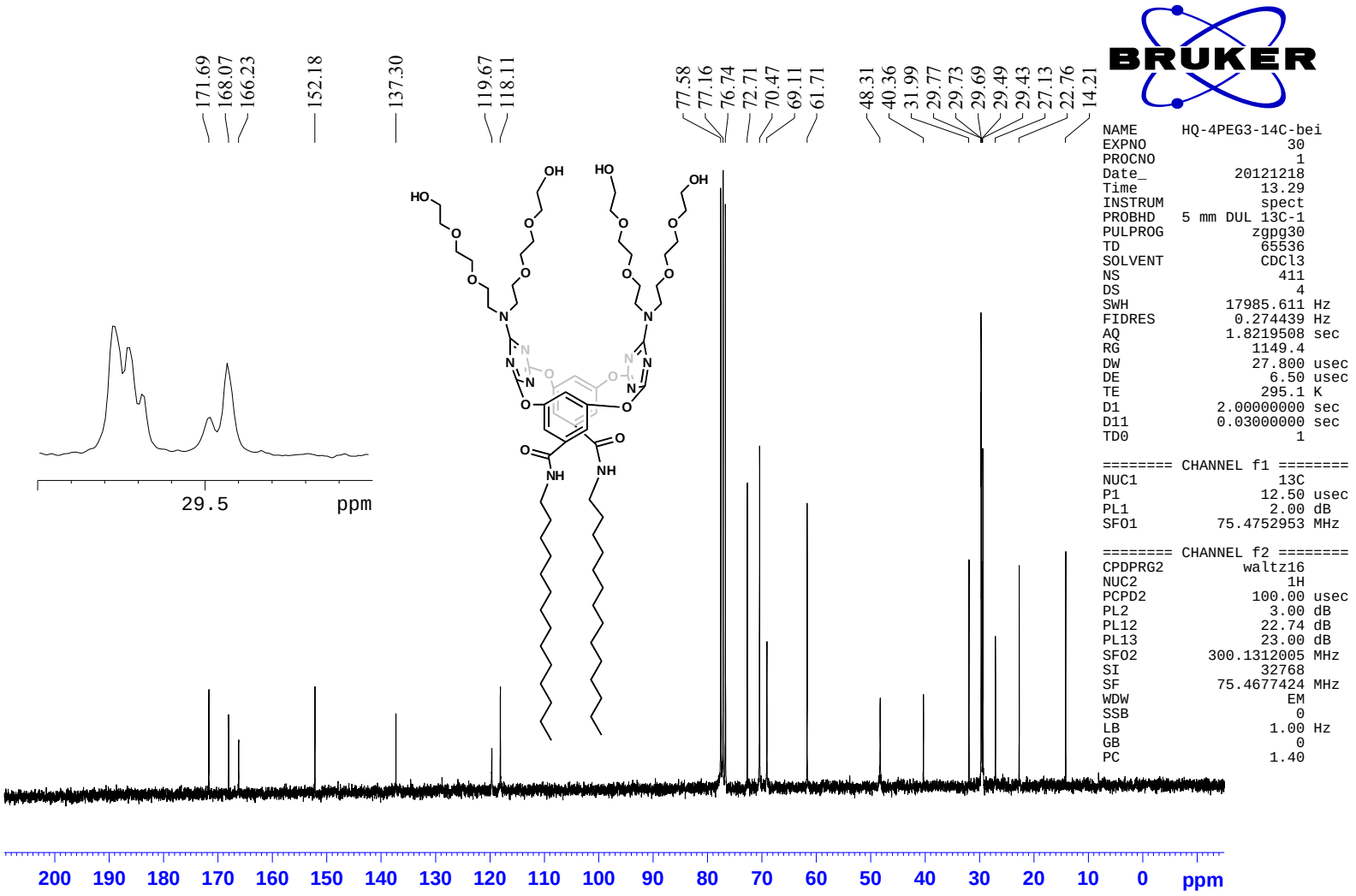


Figure S35 ^{1}C NMR spectrum of **3d.**

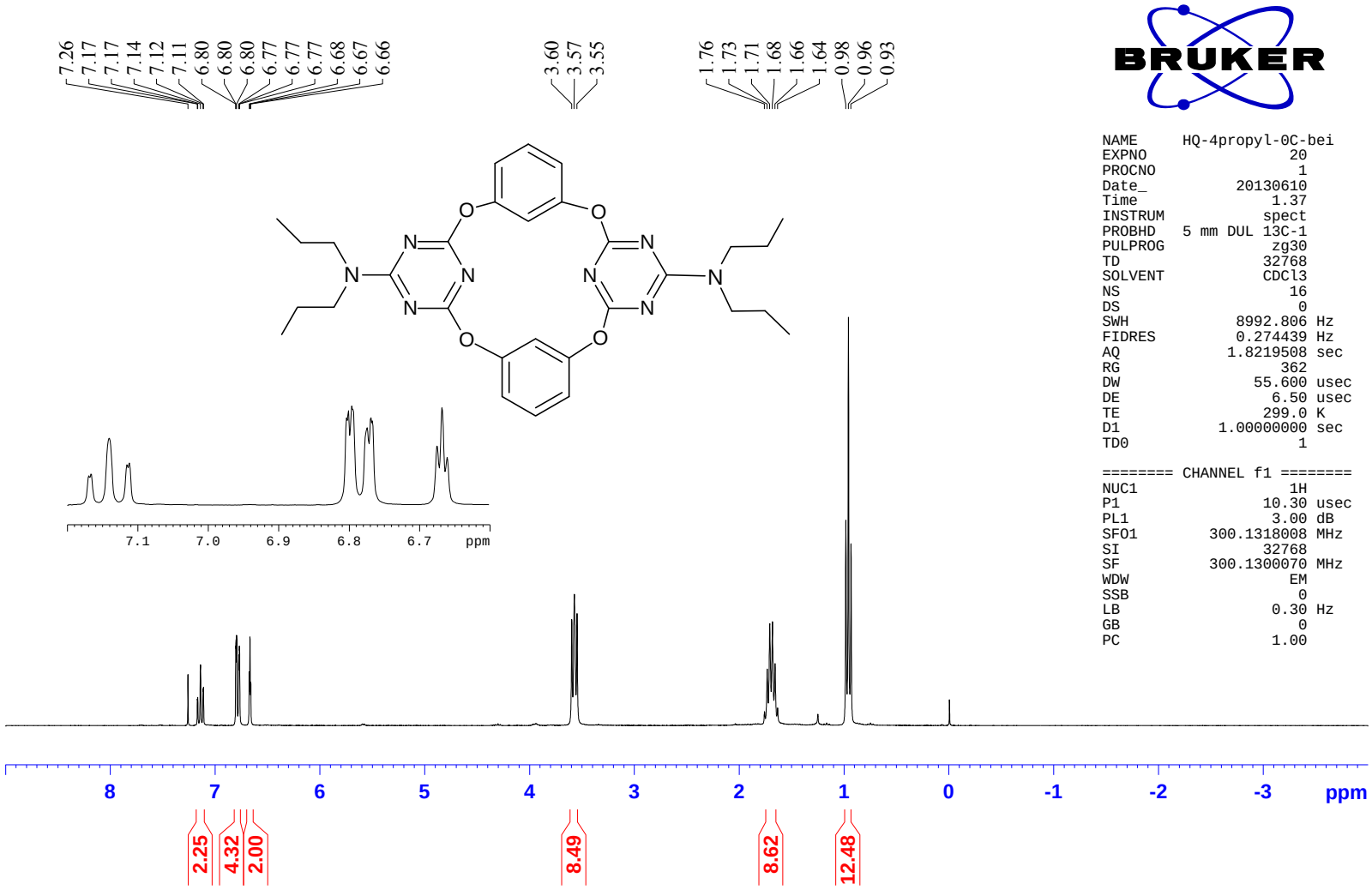


Figure S36 ^{1}H NMR spectrum of **5.**

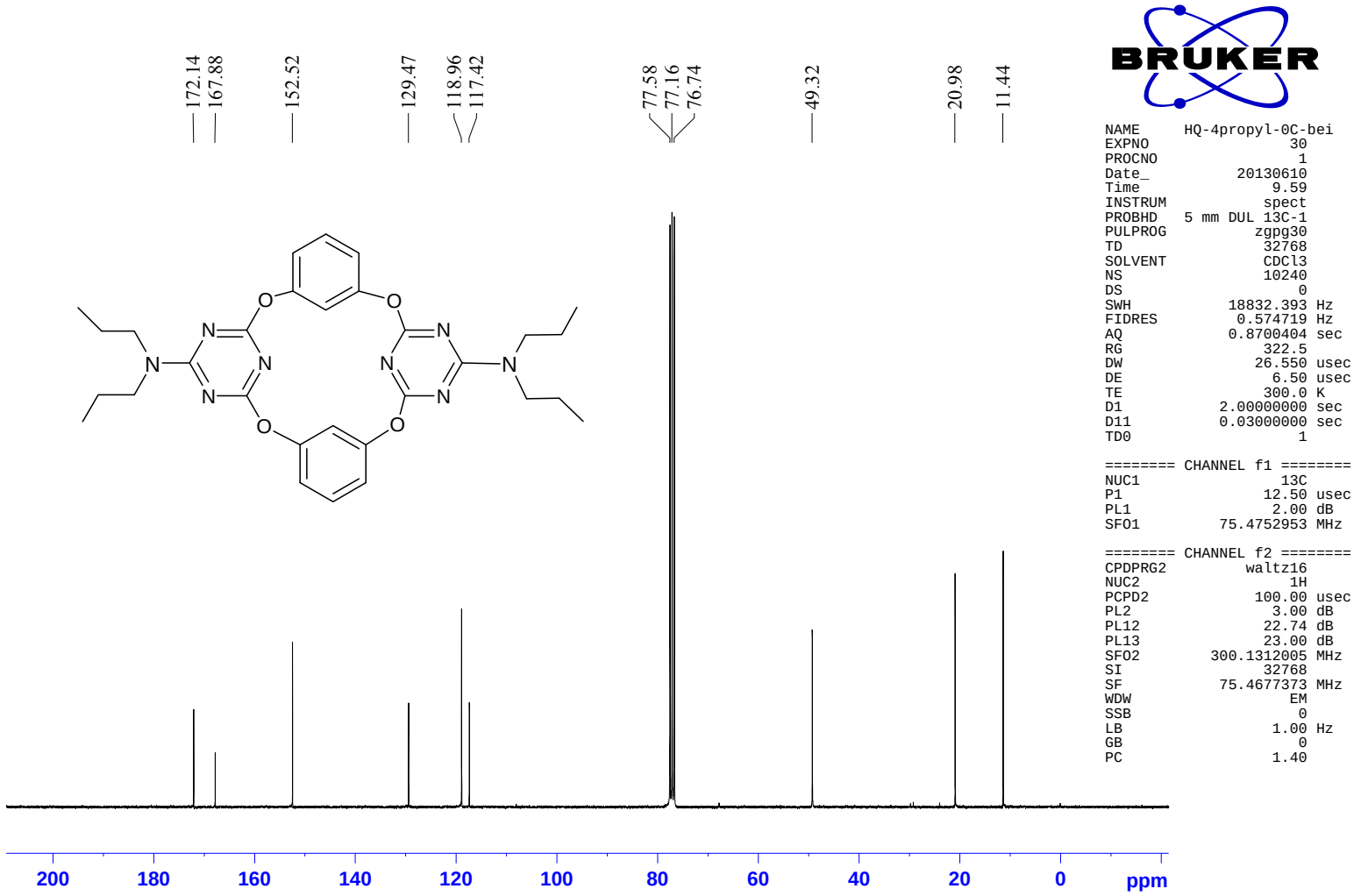


Figure S37 ^{13}C NMR spectrum of **5.**