Supporting Information

Polymorphism in Benzene-1,3,5-tricarboxamide Supramolecular Assemblies in Water: a Subtle Trade-off between Structure and Dynamics

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1. Materials

All chemicals were purchased from commercial sources and used as received unless otherwise specified. Dichloromethane (DCM) and tetrahydrofuran (THF) were dried on a MBraun MB-SPS-800 solvent purification system. Dimethylformamide (DMF) was dried over molecular sieves (4Å). Water was purified on an EMD Millipore Milli-Q Integral Water Purification System. 3,5-Bis((1-phenyl-2,5,8,11,14-pentaoxahexacosan-26-yl)carbamoyl)benzoic acid and N^1-(1-amino-3,6,9,12-tetraoxatetracosan-24-yl)-N^3,N^5-bis(1-hydroxy-3,6,9,12-tetraoxatetracosan-24-yl)benzene-1,3,5-tricarboxamide were prepared according to previously published literature procedures.[1]

2. Analytical Techniques

Nuclear magnetic resonance spectroscopy (NMR) was performed on Bruker Avance 400 MHz spectrometer, Varian Mercury Vx 400 MHz spectrometer, or a Varian Mercury Plus 200 MHz spectrometer. Chemical shifts (δ) are expressed in ppm and are referred to the residual peak of the solvent. Peak multiplicity is abbreviated as s: singlet; d: doublet; t: triplet; dt: doublet of triplets; ddt: doublet of doublets of triplets; td: triplet of doublets; tt: triplet of triplets; q: quartet; qd: quartet of doublets; m: multiplet.

Infrared spectroscopy was performed on a Perkin Elmer Spectrum One 1600 FT-IR spectrometer or a Perkin Elmer Spectrum Two FT-IR Spectrometer equipped with a Perkin Elmer Universal ATR Sampler Accessory. Solution FT-IR experiments were performed using a CaF_2 liquid cell with a 0.05 mm pathlength purchased from New Era Enterprises, Inc.

Matrix assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry was performed on a Bruker autoflex speed spectrometer or a PerSeptive Biosystems Voyager DE-PRO spectrometer using α-cyano-4-hydroxycinnamic acid (CHCA) and 2-[(2*E*)-3-(4-tert-butylphenyl)-2-methylprop-2-enylidene]malononitrile (DCTB) as matrices.

Liquid chromatography-mass spectrometry (LCMS) was performed on a system consisting of the following components: Shimadzu SCL-10A VP system controller with Shimadzu LC-10AD VP liquid chromatography pumps (with an Alltima C18 3 u (50 × 2.1 mm) reversed-phase column and gradients of water–acetonitrile supplemented with 0.1% formic acid, a Shimadzu DGU 20A3 prominence degasser, a Thermo Finnigan surveyor auto sampler, a Thermo Finnigan surveyor PDA detector and a Thermo Scientific LCQ Fleet. Gradients were run from 5% MeCN to 100% MeCN over a 15-minute period.

Ultraviolet-visible (UV-vis) absorbance spectroscopy was performed on a Jasco V-650 UV-vis spectrometer with a Jasco ETCT-762 temperature controller.

Fluorescence spectroscopy was performed using a Tecan Safire 2 Microplate Reader. Reactions were monitored by thin-layer chromatography (TLC) using precoated 0.25 mm, 60-F254 silica gel plates from Merck. Flash chromatography was performed on a Biotage Isolera One using Biotage SNAP or SNAP Ultra columns.

Total internal reflection fluorescence (TIRF) microscopy was performed on a Nikon N-STORM system. Nile Red was excited using a 561 nm laser. Fluorescence was collected by means of a Nikon 100x, 1.4NA oil immersion objective and passed through a quad-band pass dichroic filter (97335 Nikon). All material was recorded with an EMCCD camera (ixon3, Andor, pixel size 0.17µm). Samples were flown in a chamber between glass microscope coverslips (No. 1.5, 24x24x0.17 mm) and glass slides which were separated by double-sided tape.

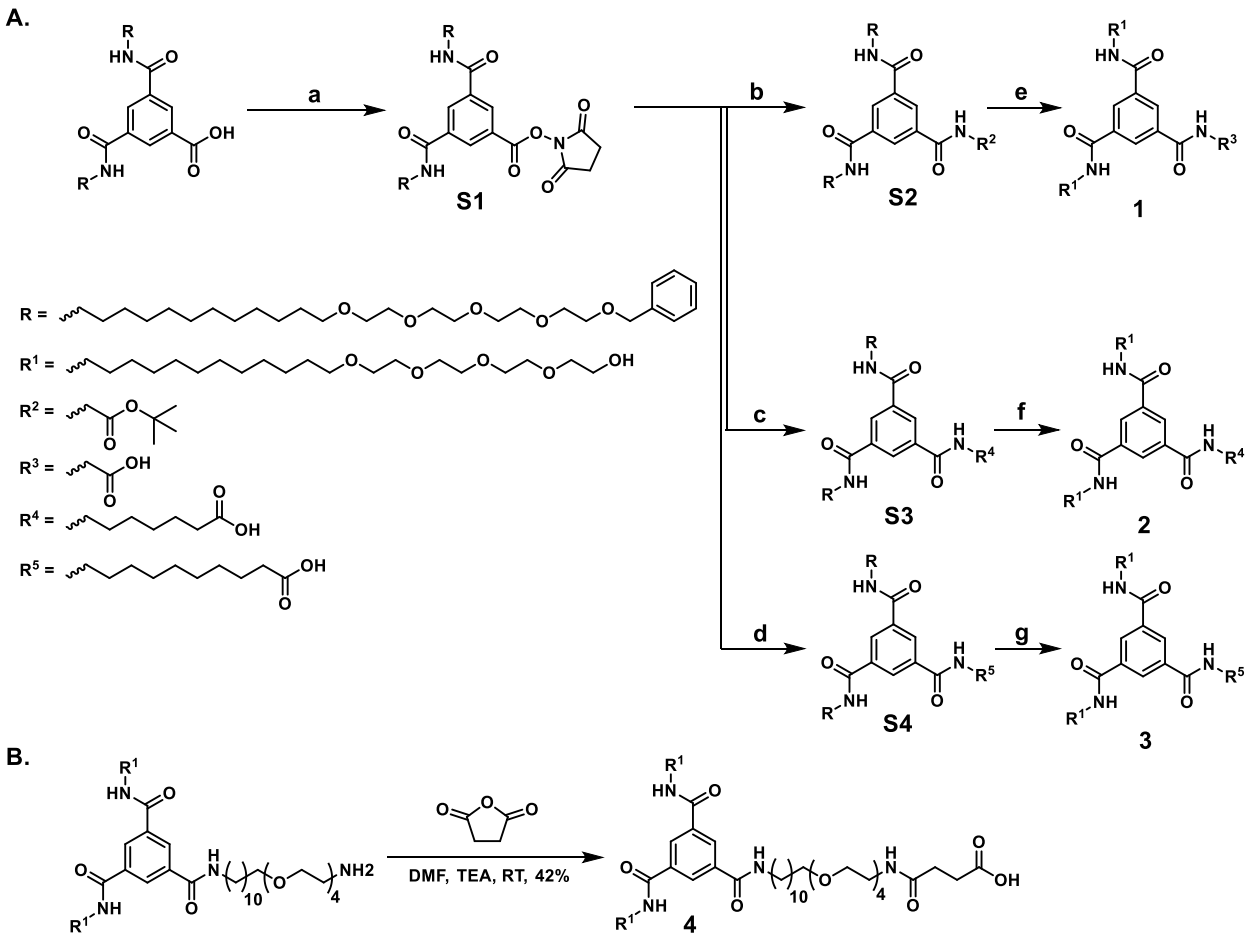
Cryogenic transmission electron microscopy (cryo-TEM) was performed using a Tecnai Sphera microscope operating at 200 kV, a detailed description for these experiments is included in section 6 of this Supporting Information.

Hydrogen-deuterium exchange mass spectrometry (HDX-MS) was performed using a Waters XevoTM G2 QTof mass spectrometer with a capillary voltage of 2.7 kV and a cone voltage of 20 V. The source temperature was set at 100 ℃, the desolvation temperature at 400 ℃ and the gas flow was set to 500 L/h. A Harvard 11 Plus syringe pump was used to introduce BTA solutions to the mass spectrometer at a flow rate of 50 µL/min. Details on the calculation of the BTA3D percentages as a fuction of time are described elsewhere.[2,3]

Small-Angle X-Ray Scattering (SAXS) measurements were performed at the BM29 BioSAXS beamline at the ESRF (Grenoble, France). A sample-to-detector distance of 2.864 m was used together with an X-ray photon wavelength of 0.992 Å. SAXS images were recorded using a 2D Pilatus 1M detector from Dectris with single photon counting capability and dynamic range of 20 bits. The detector's active area is 16.9 cm x 17.9 cm with pixel size of 172 microns, 981 by 1043 array. The 2D images were radially averaged to obtain the intensity I(q) vs q profiles. The beam centre and the q range calibrations were achieved by using the position of the diffraction peaks of a standard wet rat tail collagen fiber. The liquid samples were probed in quartz capillary as a part of automated sample changer allowing temperature variations (from 4 to 60°C). Standard data reduction procedures, i.e. subtraction of the empty capillary contribution, correction for the sample absorption, were applied. Water was used as secondary standard calibrants to perform intensity calibration on an absolute scale in cm^{-1}.

SAXS measurements were also performed on SAXSLAB GANESHA 300 XL SAXS system equipped with a GeniX 3D Cu Ultra Low Divergence micro focus sealed tube source producing X-rays with a wavelength λ = 1.54 Å at a flux of 1x108 ph/s and a Pilatus 300K silicon pixel detector with 487 x 619 pixels of 172x172 µm in size placed at two sample-to-detector distances of 713 and 1513 mm respectively to access a q-range of $0.07 \leq q \leq 4.45$ nm^{-1} with $q = 4\pi/\lambda(\sin\theta/2)$. Silver behenate was used for calibration of the beam centre and the q range. Samples were contained in 2 mm quartz capillaries (Hilgenberg Gmbh, Germany). The two-dimensional SAXS patterns were brought to an absolute intensity scale using the calibrated detector response function, known sample-to-detector distance, measured incident and transmitted beam intensities, and azimuthally averaged to obtain one-dimensional SAXS profiles. The scattering curves of the supramolecular polymers were obtained by subtraction of the scattering contribution of the solvent and quartz cell.

3. Synthetic Procedures



Scheme S1. A. Synthesis of BTAs **1-3** from a common NHS ester precursor. Reagents and conditions: a. NHS, EDC, DMAP, DCM, 66%; b. glycine *t*-butyl ester hydrochloride, TEA, DCM, 67% ; c. 7-amino heptanoic acid, DIPEA, DMF, 41%; d. 11-amino undecanoic acid, DMF, DIPEA, 98%; e. 1. H_2, Pd/C 2. TFA, DCM, quant.; f. H_2, Pd/C, AcOH, EtOH, 81%; g. H_2, $Pd(OH)_2$/C, AcOH, MeOH, quant.) **B.** Synthesis of BTA **4** by ring-opening of succinic anhydride with an amine functionalized water-soluble BTA.

2,5-Dioxopyrrolidin-1-yl 3,5-bis((1-phenyl-2,5,8,11,14-pentaoxahexacosan-26-yl)carbamoyl)benzoate **(S1)**. A round bottom flask equipped with a magnetic stirring bar was charged with 3,5-bis((1-phenyl-2,5,8,11,14-pentaoxahexacosan-26-yl)carbamoyl)benzoic acid (1.26 mmol, 1.40 g), dry DCM (100 mL) and *N*-hydroxysuccinimide (1.89 mmol, 0.22 g). The reaction mixture was then cooled to 0 °C and following reagents were added to the stirred mixture in the following order: 4-dimethylaminopyridine (0.25 mmol, 30.5 mg) and *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC) (2.52 mmol, 0.39 g). The reaction vessel was then sealed under an Ar atmosphere and warmed to room temperature to stir overnight. The solvent was removed *in vacuo* and the product was purified by column chromatography (silica gel, gradient from 0% to 10% vol% MeOH in $CHCl_3$) to yield **S1** as a waxy solid (0.99 g, 65.7%). ^{1}H

NMR (400 MHz, $CDCl_3$) δ = 8.60 (s, 2H), 8.51 (s, 21H), 7.33 (m, 10H), 6.62 (m, 2H), 4.54 (s, 4H), 3.66 (m, 32H), 3.42 (m, 8H), 2.93 (m, 4H), 1.66 (m, 8H), 1.55 (m, 8H), 1.26 (m, 28H). ^{13}C NMR (400 MHz, $CDCl_3$) δ = 168.91, 164.96, 138.14, 136.03, 128.34, 127.75, 127.59, 77.32, 77.00, 76.68, 73.24, 71.50, 70,61, 70.58. 70.02, 69.32, 40.37, 29.43, 29.37, 29.23, 26.95, 26.02. MALDI-TOF: Exact mass of **S1**: 1207.79 g/mol; observed: 1230.80 g/mol $[M+Na]^+$ and 1246.77 g/mol $[M+K]^+$.

*Tert-butyl (3,5-bis((1-phenyl-2,5,8,11,14-pentaoxahexacosan-26-yl)carbamoyl)benzoyl)glycinate (**S2**).* A screw cap vial equipped with a magnetic stirring bar was charged with **S1** (25.0 mg, 21.2 µmol) and dry DCM (1 mL). A solution of glycine *tert*-butyl ester hydrochloride (8.4 mg, 64.0 µmol) and triethylamine (50 µL, 0.36 mmol) in dry DCM (1 mL) was prepared in a separate vessel and added to the vial containing the **S1** solution. The reaction mixture was sealed under argon and stirred for 14 h at room temperature. The reaction mixture was concentrated under reduced pressure and the crude product was purified via column chromatography (silica gel, gradient from 0 to 5% vol% MeOH in $CHCl_3$) to yield **S2** as a colorless oil (17 mg, 67%). ^1H NMR (400 MHz, $CDCl_3$) δ = 8.35 (s, 3H), 7.33 (m, 10H), 7.12 (m, 1H), 6.56 (m, 2H), 4.55 (s, 4H), 4.13 (m, 2H), 3.73-3.53 (m, 32H), 3.50-3.37 (m, 8H), 1.71-1.44 (m, 21H), 1.41-1.15 (m, 32H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 168.83, 165.65, 138.23, 135.48, 134.47, 73.26, 71.54, 70.61, 70.06, 69.44, 42.62, 40.38, 29.60, 29.51, 29.46, 29.51, 29.46, 29.41, 29.24, 28.09, 26.95, 26.05. MALDI-TOF: Exact mass of **S2**: 1221.80 g/mol; observed: 1244.79 g/mol $[M+Na]^+$ and 1260.76 g/mol $[M+K]^+$.

7-(3,5-Bis((1-phenyl-2,5,8,11,14-pentaoxahexacosan-26-yl)carbamoyl)benzamido)heptanoic acid **(S3)**. A screw top vial was charged with **S1** (0.165 mmol, 200.0 mg) and dry DMF (10 mL). 7-Aminoheptanoic acid (0.497 mmol, 58.2 mg) and diisopropylethylamine (0.33 mmol, 42.8 mg) were then added. The vial was then sealed under argon and stirred overnight at 50 °C. DMF was then removed under high vacuum and the resulting crude product mixture was purified by column chromatography (silica gel, gradient from 0% to 10% vol% MeOH in $CHCl_3$) to yield **S3** (80.9 mg, 40.6%). ^1H NMR (400 MHz, $CDCl_3$) δ = 8.62 (s, 2H), 8.37 (s, 1H), 7.32 (m, 10H), 6.73 (bs, 2H), 4.55 (s, 4H), 3.72-3.53 (m, 32H), 3.51-3.37 (m, 10H), 2.42 (t, *J* = 6.00, 2H), 1.68-1.48 (m, 12H), 1.41-1.18 (m, 36H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 166.43, 165.93, 138.20, 134.91, 128.77, 128.36, 127.77, 127.62, 77.35, 77.23, 77.03, 76.71, 73.25, 71.55, 70.62, 70.59, 70.03, 69.42, 40.49, 29.53, 29.48, 29.37, 26.98, 26.06. FT-IR ν (cm^{-1}): 3342, 2065, 2925, 2854, 1713, 1663, 1648, 1595, 1535, 1454, 1350, 1289, 1102, 946, 879, 739, 699, 616. MALDI-TOF: Exact mass of **S3**: 1207.79 g/mol; observed: 1230.80 g/mol $[M+Na]^+$ and 1246.77 g/mol $[M+K]^+$.

11-(3,5-Bis((1-phenyl-2,5,8,11,14-pentaoxahexacosan-26-yl)carbamoyl)benzamido)undecanoic acid (**S4**). A 12 mL screw-top vial equipped with a magnetic stirring bar was charged with **S1** (0.251 mmol, 304.0 mg) and dry DMF (3 mL) and allowed to stir until homogeneous. In a separate vial, 11-aminoundecanoic acid (0.50 mmol, 101 mg) was dissolved in dry DMF (2 mL) and diisopropylethylamine (22 µL). The solution containing 11-aminoundecanoic acid was then transferred *via* syringe to the vial containing **S1**. The vial was then sealed under argon atmosphere and allowed to stir at room temperature. After 24 h, DMF was removed *in vacuo* and the crude reaction mixture was immediately purified by column chromatography (silica gel, 0 to 10% vol% MeOH in $CHCl_3$) yielding **S4** as a colorless waxy solid (320 mg, 98%). ^1H NMR (400

MHz, $CDCl_3$) δ = 8.58 (s, 2H), 8.41 (s, 1H), 7.46 (m, 1H), 7.37-7.28 (m, 10 H), 6.85 (m, 2H), 4.55 (s, 4H), 3.70-3.59 (m, 28H), 3.59-3.53 (m, 4H), 3.50-3.38 (m, 10H), 2.36 (t, *J* = 6.87 Hz, 2H), 1.71-1.50 (m, 12H), 1.44-1.20 (m, 44H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 166.18, 138.21, 134.76, 128.36, 127.77, 127.61, 73.25, 71.55, 70.60, 70.04, 69.42, 40.09, 33.62, 29.60, 29.54, 29.51, 29.47, 29.44, 29.27, 28.53, 27.69, 26.97, 26.06, 25.87, 24.23. MALDI-TOF: Exact mass of **S4**: 1291.88 g/mol; observed mass: 1314.87 g/mol $[M+Na]^+$, 1330.85 g/mol $[M+K]^+$.

*Tert-butyl (3,5-bis((1-phenyl-2,5,8,11,14-pentaoxahexacosan-26-yl)carbamoyl)benzoyl)glycinate (**1**).* A round bottom flask equipped with a magnetic stirring bar was charged with **S2** (0.01 mmol, 15.0 mg) and EtOAc (5 mL). The reaction flask was then evacuated and refilled with nitrogen three times to degas the solution. Pd/C (1.3 mg, 10 wt% loading) was then added and the flask was fitted with a H_2-gas filled balloon and left to stir at room temperature. After 16 h, the reaction mixture was filtered over celite and concentrated *in vacuo* to afford the benzyl-deprotected product which was then used without further purification. The benzyl-deprotected **S2** was dissolved in DCM (1.4 mL) and transferred to a screw top vial equipped with a magnetic stirring bar. TFA (0.6 mL) was then added and the reaction mixture was allowed to stir at room temperature. After 5 hours, the DCM and TFA were removed under high vacuum to afford **1** as a white waxy solid, in quantitative yield without need for further purification. ^1H NMR (400 MHz, $CDCl_3$) δ = 8.53-8.25 (m, 3H), 8.13-7.94 (m, 1H), 4.36-4.10 (m, 4H), 4.1-3.53 (m, 32 H), 3.52-3.11 (m, 8H), 1.74-1.43 (m, 8H), 1.40-1.10 (m, 32 H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ = 166.70, 164.20, 70.50, 70.16, 69.89, 61.54, 40.53, 29.45, 29.37, 26.89, 26.98.

*7-(3,5-Bis((1-hydroxy-3,6,9,12-tetraoxatetracosan-24-yl)carbamoyl)benzamido)heptanoic acid **(2)**.* A round bottom flask was charged with **S3** (0.109 mmol, 135 mg), 10 mL ethanol and 5 mL glacial acetic acid. The mixture was sparged with N_2-gas for 10 minutes, then Pd/C (20.0 mg, 10 wt% loading) was added. The mixture was sparged with N_2-gas for an additional 5 minutes. A balloon filled with H_2-gas was connected to the flask and the reaction was left to stir vigorously at room temperature overnight. The reaction mixture was then filtered over celite and concentrated *in vacuo*. Additional column chromatography was necessary (silica gel, 0 to 10% vol% MeOH in $CHCl_3$), yielding **2** as a colorless oil, which solidified upon standing (93.1 mg, 81%). ^1H NMR (400 MHz, $CDCl_3$) δ = 8.56 (s, 2H), 8.44 (s, 1H), 7.64 (bs, 2H), 7.47 (bs, 2H), 3.79-3.51 (m, 32H), 3.49-3.29 (m, 10H), 2.30 (t, *J* = 6.78, 2H), 1.70-1.46 (m, 12H), 1.39-1.19 (m, 36H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 177.81, 166.49, 166.18, 134.98, 134.87, 128.74, 77.36, 77.24, 77.04, 76.72, 72.52, 70.44, 69.90, 61.55, 40.46, 40.25, 34.38, 29.51, 29.48, 29.43, 29.27, 28.64, 28.11, 27.00, 26.00, 25.87, 23.53. FT-IR ν (cm^{-1}): 3342, 3077, 2925, 2855, 1726, 1649, 1542, 1463, 1289, 1107, 708. MALDI-TOF: Exact mass of **2**: 1055.72 g/mol; observed mass: 1078.75 g/mol $[M+Na]^+$, 1094.74 g/mol $[M+K]^+$.

*11-(3,5-Bis((1-hydroxy-3,6,9,12-tetraoxatetracosan-24-yl)carbamoyl)benzamido)undecanoic acid (**3**).* A two neck round bottom flask equipped with magnetic stirring bar was charged with **S4** (0.32 mg, 0.265 mmol), EtOAc (30 mL). The solution of **S4** was sparged with N_2 gas for 20 minutes, a catalytic amount of Pd/C (20 mg, 10 wt% loading) was then added, and the reaction mixture was sparged with N_2 gas for an additional 5 minutes. The reaction flask was then fitted with a balloon filled with H_2-gas. The reaction flask was then evacuated and refilled with H_2 gas three times and left to stir at room temperature overnight under a H_2 atmosphere. The reaction mixture was then filtered over celite and concentrated under

reduced pressure. The crude product was purified via column chromatography (silica gel, gradient from 0 to 10% vol% MeOH in $CHCl_3$) to yield **3** as a colorless waxy solid in quantitative yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.56 (s, 2H), 8.42 (s, 2H), 7.53 (s, 1H), 7.04 (s, 1H), 3.75-3.69 (m, 4H), 3.67-3.54 (m, 28H), 3.50-3.38 (m, 10H), 2.33 (t, *J* = 6.7 Hz *,* 2H), 1.67-1.50 (m, 12H), 1.43-1.20 (m, 44H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 166.22, 134.75, 181.70, 72.54, 71.54, 70.53, 70.48, 70.23, 69.95, 61.62, 40.45, 29.47, 29.43, 29.39, 29.23, 26.94, 26.00. FT-IR ν (cm^{-1}): 3316, 3073, 2922, 2853, 1712, 1646, 1537, 1463, 1349, 1288, 1104, 941, 885, 840, 707, 626. MALDI-TOF: Exact mass of **3**: 1111.79 g/mol; observed mass: 1134.78 g/mol $[M+Na]^+$, 1150.74 g/mol $[M+K]^+$.

*1-(3,5-Bis((1-hydroxy-3,6,9,12-tetraoxatetracosan-24-yl)carbamoyl)phenyl)-1-oxo-15,18,21,24-tetraoxa-2,27-diazatriacontan-30-oic acid (**4**)*. A screw cap vial equipped with a magnetic stirring bar was charged with N^1-(1-amino-3,6,9,12-tetraoxatetracosan-24-yl)-N^3,N^5-bis(1-hydroxy-3,6,9,12-tetraoxatetracosan-24-yl)benzene-1,3,5-tricarboxamide (50.0 mg, 38.8 µmol), succinic anhydride (4.0 mg, 39.7 µmol), and dry DMF (1 mL) and sealed under an argon atmosphere. The reaction mixture was then stirred overnight at room temperature. DMF was then removed under reduced pressure and the crude product was purified via column chromatography (silica gel, gradient from 0 to 10% vol% MeOH in $CHCl_3$) to yield **4** as a colorless waxy solid (22.4 mg, 42%). ^{1}H NMR (200 MHz, $CDCl_3$) δ = 8.44 (s, 2H), 7.10 (m, 1H), 6.96 (m, 3H), 3.79-3.31 (m, 60H), 2.69-2.46 (m, 4H), 1.71-1.47 (m, 12H), 1.44-1.30 (m, 48H). MALDI-TOF: Exact mass of **4**: 1386.96 g/mol; observed mass: 1409.96 g/mol $[M+Na]^+$.

4. Preparation of BTA solutions

Typical preparation of an assembled BTA solution. BTA **2** (6 mg) was weighed into a 2 mL screw-cap vial equipped with a magnetic stirring bar. Ultra-pure water (1 mL) was then added and the vial was sealed. The sealed vial was then stirred in a water bath at 80℃ for 10 minutes, then vortexed for 15 seconds, then sonicated for 15 seconds. The heat/stir-vortex-sonication process was repeated an additional 4 times then the sample was placed on a benchtop to cool to room temperature for a minimum 2 hours to produce a solution containing BTA assemblies. The same heat/stir-vortex-sonication protocol was applied to all of the reported BTAs. It is important to note that all BTA stock solutions and dilutions of the stock solutions were prepared with ultra-pure water (pH = 7.0). No pH adjustments were made to the BTA stock solutions and diluted stock solutions.

*Typical preparation of a concentrated BTA **2** solution in MeOD for FT-IR in solution*. A 2 mL vial, equipped with a magnetic stirring bar, was charged with BTA **2** (25 mg) and MeOD (0.5 mL, Cambridge Isotope Laboratories). The vial was sealed under argon atmosphere and stirred at room temperature until homogeneous. The same procedure was used to prepare solutions of BTA **3** in MeOD for solution FT-IR experiments.

*Typical preparation of a concentrated BTA **2** solution in D_2O for FTIR in solution.* A 2 mL vial, equipped with a magnetic stirring bar, was charged with BTA **2** (10 mg) and D_2O (0.5 mL, Cambridge Isotope

Laboratories). The vial was sealed under argon atmosphere and stirred at 80℃ until homogeneous. After heating, the viscous solution was allowed to cool to room temperature for a minimum of 2 h. The same procedure was used to prepare solutions of BTA **3** in D_2O for solution FT-IR experiments.

5. Co-assembly of BTAs 2 and 3 with Nile Red

*Typical co-assembly of Nile Red with BTA **2**.* A stock solution of Nile Red in MeCN was prepared at a concentration of 0.8 mg/mL (2.51 mM). Then, 1 µL of the Nile Red stock solution was added to a screw top vial containing a 100 µM solution of BTA **2** (diluted from previously described heat/stir-vortex-sonication assembly protocol). The Nile Red/BTA **2** solution was then heated to 80℃ for 20 minutes in a heating block and allowed to cool to room temperature overnight in a dark environment. The final concentrations of Nile Red and BTA **2** were 2.5 µM and 100 µM, respectively. This heat/cool protocol was used to prepare Nile Red co-assemblies with BTA **3** and a control sample consisting of a 2.5 µM solution of Nile Red in H_2O.

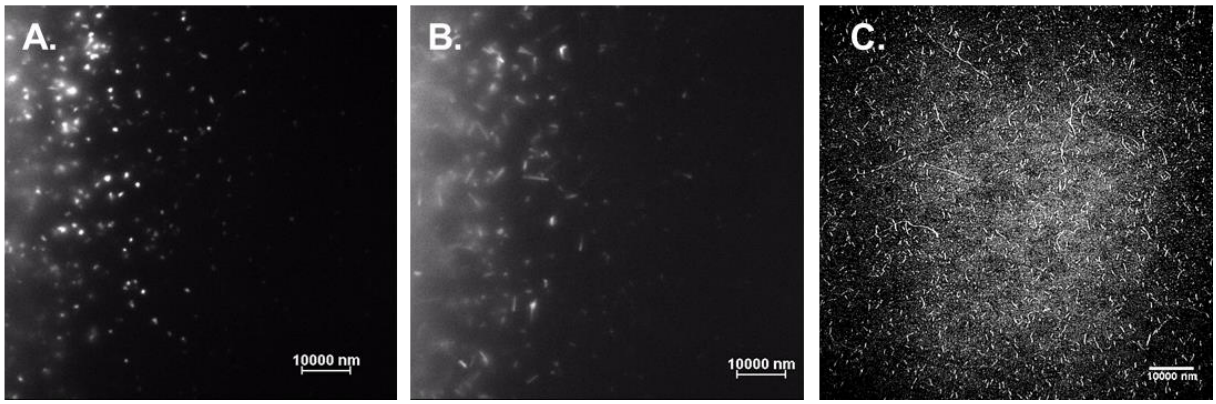


Figure S1. Fluorescence micrographs of Nile Red stained BTA **2** (A), BTA **3** (B), and BTA **4** (C). Total internal reflection fluorescence (TIRF) images and videos were acquired with a Nikon N-STORM system. Nile red was excited using a 561 nm laser. Fluorescence was collected by means of a Nikon 100x, 1.4NA oil immersion objective and passed through a quad-band pass dichroic filter (97335 Nikon). All material was recorded with an EMCCD camera (ixon3, Andor, pixel size 0.17µm). Samples were flown in a chamber between glass microscope coverslips (No. 1.5, 24x24x0.17 mm) and glass slides which were separated by double-sided tape.

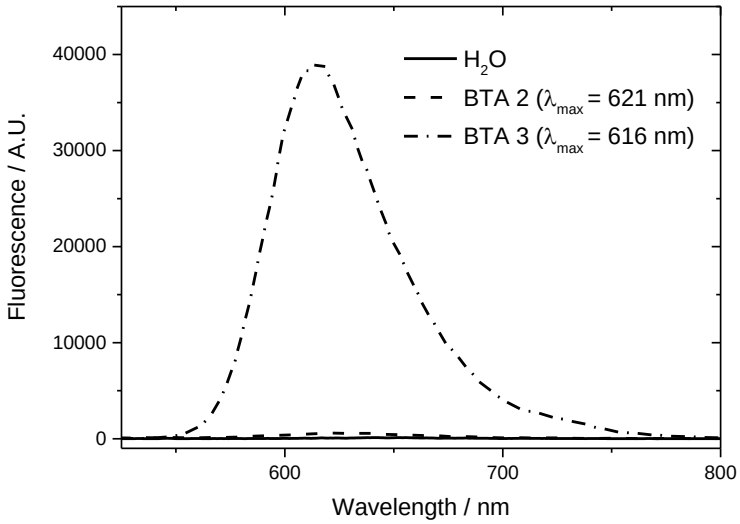


Figure S2. Fluorescence emission of Nile Red in water, co-assembled with BTA **2**, and co-assembled with BTA **3.** Nile Red fluorescence emission spectra were recorded from 520 nm to 800 nm upon excitation at 500 nm.

6. Imaging BTA assemblies by cryo-TEM

*Cryogenic transmission electron microscopy of BTA **2, 3, and 4**.* Cryogenic transmission electron microscopy was performed on assembled BTA samples at a concentration of 1 mg/mL. Vitrified films were prepared in a 'Vitrobot' instrument (PC controlled vitrification robot, patent applied, Frederik et al 2002, patent licensed to FEI) at a relative humidity of 100%. The BTA **2** and BTA **4** samples were vitrified from 22°C, and the BTA **3** sample was vitrified from both 22°C and 60°C. All Quantifoil grids (R 2/2, Quantifoil Micro Tools GmbH) were surface plasma treated just prior to use (Cressington 208 carbon coater operating at 5 mA for 40 s). For the films prepared from 22°C, 3µL of the sample was applied on a Quantifoil grid in the preparation chamber of the 'Vitrobot'. Excess sample was removed by blotting using filter paper for 3 s at –3 mm, and the thin film thus formed was plunged (acceleration about 3 g) into liquid ethane just above its freezing point. To prepare films from 60°C, the 'Vitrobot' was set to a temperature of 65°C and the tweezer and pipet tips used were equilibrated at the same temperature in the preparation chamber. Meanwhile, a BTA **3** sample was equilibrated at 60°C using an oil bath. At the elevated temperature of the preparation chamber, an increased amount of moisture was required in order to saturate the air with water vapor. To reach 100% relative humidity, after installing a freshly surface plasma treated grid into the preparation chamber, both the temperature of the chamber was set to 60°C and the humidifier was continuously operated. Subsequently, 3µL of the sample was applied on the grid and excess sample was removed by blotting using filter paper for 2 s at -3 mm. The thin film thus formed was plunged (acceleration about 3 g) into liquid ethane just above its freezing point. The vitrified films were transferred to a cryoholder (Gatan 626) and observed at temperatures below -170 °C in a Tecnai Sphera

microscope operating at 200 kV. Micrographs were taken at low dose conditions, using a defocus setting of 15 µm at 14500 magnification and a defocus settings of 2 and 5 µm at 25000 magnification.

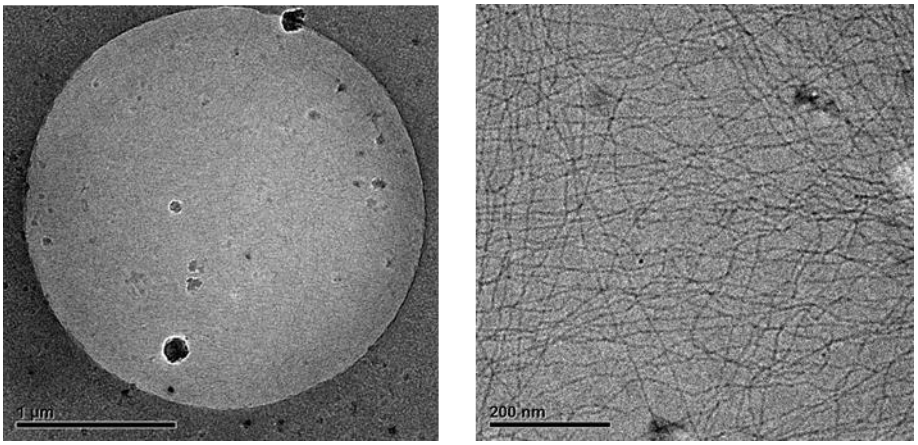


Figure S3. Cryo-TEM of BTA **4** 1-dimensional supramolecular polymers in water.

7. HDX-MS analysis of BTA 2 and 3

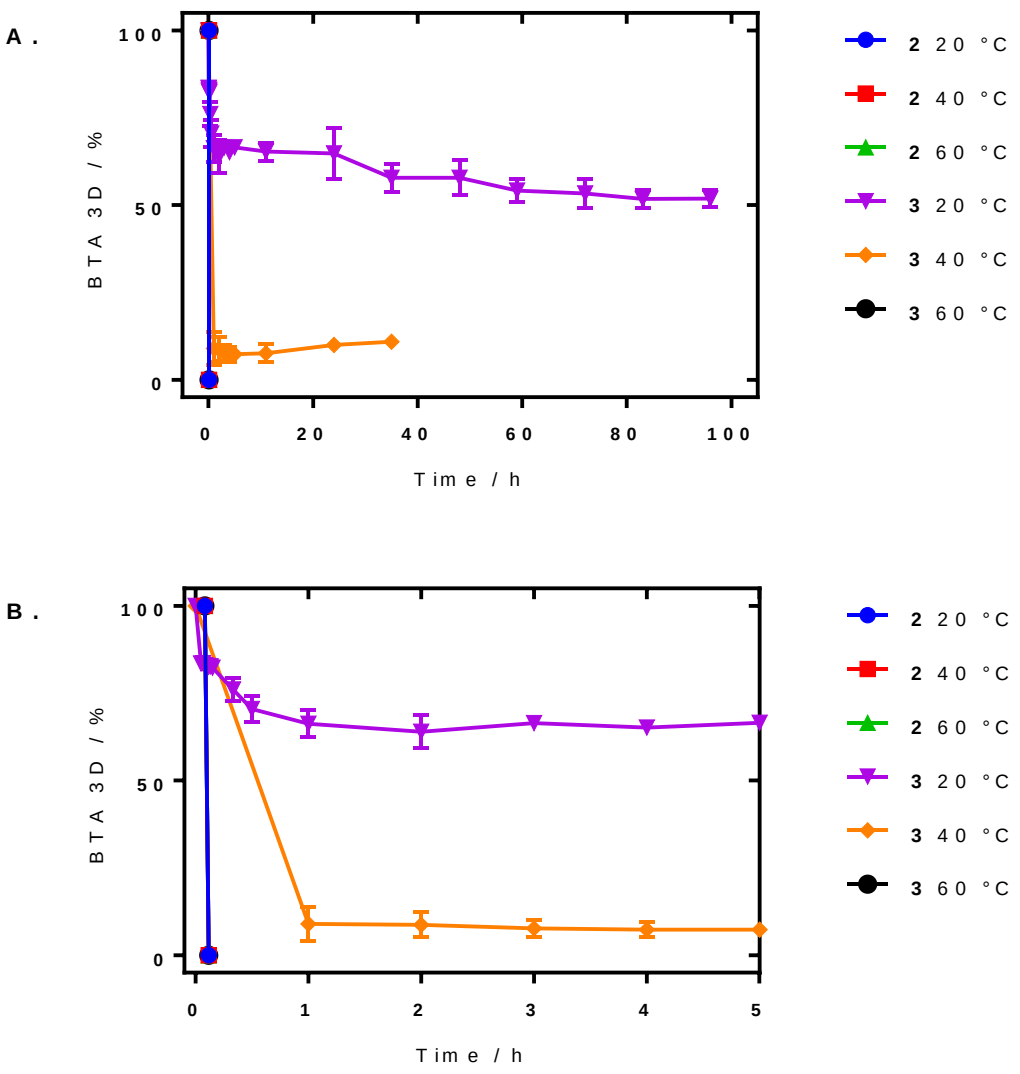


Figure S4. A. HDX-MS analysis of BTA **2** and **3** at 20, 40, and 60℃ over the course of 100 h. Upon 100-fold dilution in D_2O at all temperatures, all labile BTA **2** protons (O-H, N-H) are exchanged to deuteriums (O-D, N-D). At 60℃, BTA **3** is immediately exchanged to the fully deuterated species. B. Expanded view of HDX-MS analysis of BTA **2** and **3** at 20, 40, and 60℃ from 0 to 5 h.

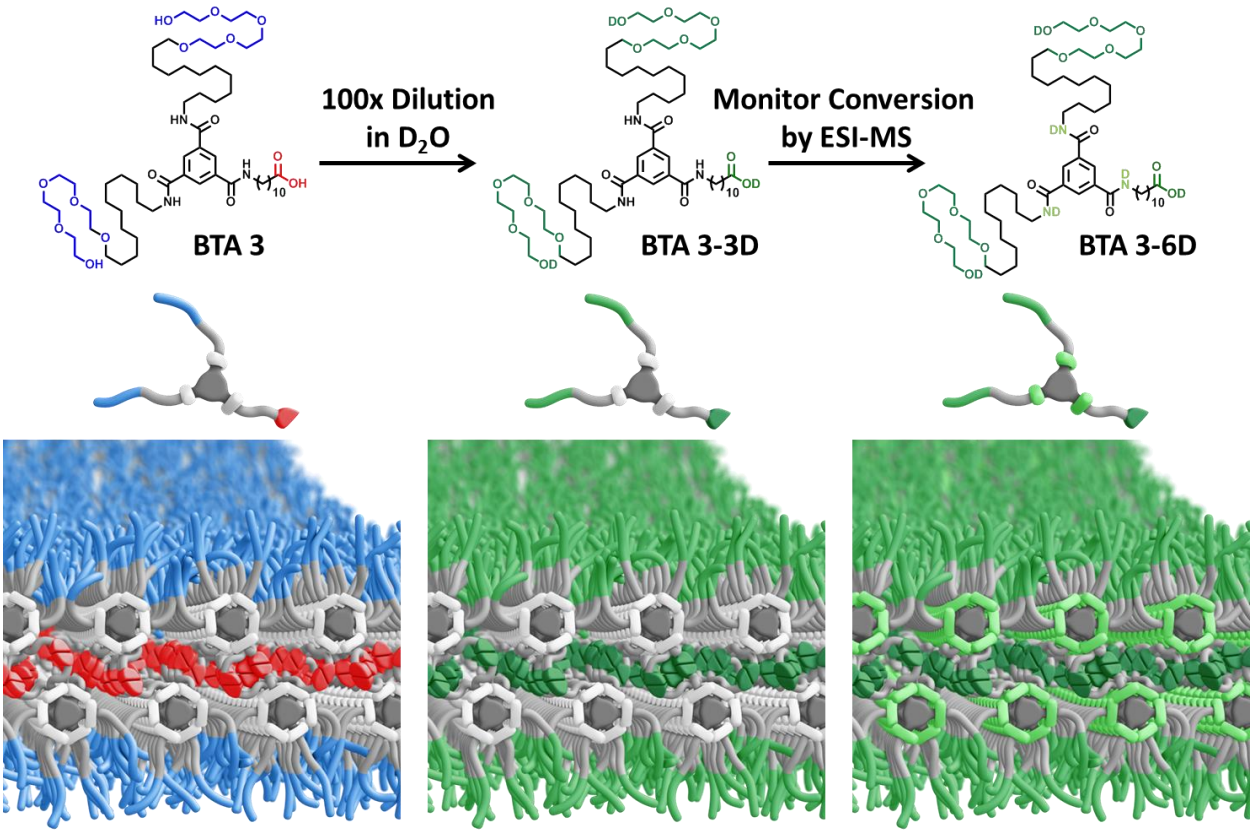


Figure S5. Illustrations representing the Hydrogen-Deuterium exchange process.

8. Analysis of SAXS data

SAXS data were fitted by SASview 4.1.0 which is originally developed under NSF Award DMR-0520547. SasView also contains code developed with funding from the EU Horizon 2020 programme under the SINE2020 project Grant No 654000. In this study, the scattering intensity, I(q), is expressed as $P_1(q)$ + $P_2(q)$ + BKG, where $P_1(q)$ and $P_2(q)$ represent the form factor of hollow cylinder and rectangular parallelepiped, respectively. BKG represents for the incoherent scattering background. The form factor of the hollow cylinder is expressed as the following equation

$$\mathrm{P_1(q)} = M \times V_{shell} \Delta\rho_1^2 \int_0^1 F^2\left[q, R_o(1-x^2)^{1/2}, R_c(1-x^2)^{1/2}\right] \left[\frac{\sin(qHx)}{qHx}\right]^2 dx$$

$$F[q,y,z] = \frac{1}{1-\gamma^2}\left[\Lambda(qy) - \gamma^2\Lambda(qz)\right]$$

$$\Lambda(\alpha) = \frac{2J_1(a)}{a}$$

$$\gamma = {}^{R_c}/_{R_o}$$

$$V_{shell} = \pi(R_o^2 - R_c^2)L$$

where *M* is the scale factor, V_{shell} is the volume of the shell of the hollow cylinder, $\Delta\rho_1$ is the scattering length density difference between the shell and the core and solvent. Here, solvent and core share the same scattering length density (*i.e.* water). R_o and R_c are the outer and core radius of the cylinder. The shell thickness is equal to $R_o - R_c$. $\mathrm{H} = {}^{L}/_{2}$ where *L* is the length of the cylinder, J_1 is the first order Bessel function and the integral over *x* is the orientational average. The core radius and the shell thickness of the hollow cylinder as well as the scale factor are the fitting parameters that we focus on. A Gaussian distribution in radius was added into the fitting parameters while the polydispersity index (PDI) is defined as the ${}^{SD}/_{\overline{R_c}}$ where SD is the standard deviation of the Gaussian distribution and $\overline{R_c}$ is the mean core radius.

The form factor of the rectangular parallelepiped could be expressed as the following equation

$$\mathrm{P}_2(\mathrm{q}) = N \times V_P \Delta\rho_2^2 \int_0^1 \phi_Q(\mu\sqrt{1-\sigma^2}, a)[S\mu c\sigma/2]^2 d\sigma$$

$$\phi_Q(\mu, a) = \int_0^1 \left\{ S\left[\frac{\mu}{2}\cos(\frac{\pi}{2}u)\right] S\left[\frac{\mu a}{2}\sin(\frac{\pi}{2}u)\right]\right\}^2 du$$

$$S(x) = \frac{\sin x}{x}$$

$$\mu = qB$$

where *N* is the scale factor, V_p is the volume of the rectangular parallelepiped and is equal to ABC where A, B and C represent the thickness, width and length of the rectangular parallelepiped. The order of A, B and C was assigned in such way that a = A/B < 1, b = B/B = 1 and c = C/B > 1. $\Delta\rho_2$ is the scattering length density difference between the rectangular parallelepiped and the solvent (water). This model allows us to get the volume fraction and the geometry of the rectangular parallelepiped.

By obtaining the scaling factor, *M* and *N*, from $P_1(q)$ and $P_2(q)$ enable us to calculate the relative volume fraction of the hollow cylinder and rectangular parallelepiped. For the different concentrations of BTA3 at the room temperature, we used simultaneous fit to constrain the geometric parameters of the hollow cylinder and rectangular parallelepiped. In the fitting process of temperature-dependent scattering profiles, we constrained the wall thickness of the hollow cylinder ($R_o - R_c$) and the thickness of the rectangular parallelepiped (A) to be the same at a given temperature.

Table S1. Shared parameters used in the global fit of SAXS profiles in Figure 3

Hollow Cylinder		**Parrellelpiped**	
Core Radius (Å)	132.1 ± 0.2	Length_a (Å)	50.3 ± 0.2
Shell Thickness (Å)	47.1 ± 0.3	Length_b (Å)	250 (Fixed)
Length (Å)	1126 ± 6	Length_c (Å)	628 ± 22

Table S2. Concentration-specific parameters used in the global fit of SAXS profiles in Figure 3

Hollow Cylinder		**Parallelpiped**	
6 mg/mL			
Scale	$4.3 \times 10^{-3} \pm 8 \times 10^{-6}$	Scale	$3.0 \times 10^{-3} \pm 1 \times 10^{-5}$
Volume Ratio	58.9 %	Volume Ratio	41.1 %
Polydispersity of Radius	$7.4 \times 10^{-2} \pm 1 \times 10^{-3}$		
3 mg/mL			
Scale	$1.1 \times 10^{-3} \pm 3 \times 10^{-6}$	Scale	$4.9 \times 10^{-4} \pm 4 \times 10^{-6}$
Volume Ratio	69.2 %	Volume Ratio	30.8 %
Polydispersity of Radius	$1.6 \times 10^{-1} \pm 1 \times 10^{-3}$		
1.5 mg/mL			
Scale	$1.5 \times 10^{-4} \pm 2 \times 10^{-6}$	Scale	$5.3 \times 10^{-5} \pm 3 \times 10^{-6}$
Volume Ratio	73.9 %	Volume Ratio	26.1 %
Polydispersity of Radius	$2.2 \times 10^{-1} \pm 9 \times 10^{-3}$		
0.75 mg/mL			
Scale	$1.0 \times 10^{-4} \pm 3 \times 10^{-7}$	Scale	$7.7 \times 10^{-6} \pm 5 \times 10^{-7}$
Volume Ratio	92.9 %	Volume Ratio	7.1 %
Polydispersity of Radius	$2.4 \times 10^{-1} \pm 2 \times 10^{-3}$		

Table S2. Temperature-specific parameters used in the fit of SAXS profiles in Figure 7

	20°C	30°C	40°C	50°C	60°C	70°C
Hollow Cylinder						
Scale	1.1e-3 ± 1e-4	9.2e-4 ± 1.6e-4	6.8e-4 ± 1.5e-4	9.5e-4 ± 2.2e-4	8.2e-4 ± 1.7e-4	6.5e-4 ± 1.4e-4
Volume Ratio	62.9%	58.2%	53.5%	96.1%	94.7%	90.0%
Core Radius (Å)	124 ± 3	135 ± 4	144 ± 5	117 ± 6	119.5 ± 5.5	117 ± 6
Shell Thickness (Å)	52 ± 2	47 ± 2	51 ± 2	39 ± 3	40.2 ± 1.8	46 ± 4
Length (Å)	460 ± 55	432 ± 94	544 ± 118	540 ± 84	527.1 ± 83.3	533 ± 93
PD of Core Radius	1.2e-1 ± 3e-2	1.2e-1 ± 3e-2	1.3e-1 ± 3e-2	2.5e-1 ± 7e-2	2.2e-1 ± 5.9e-2	1.2e-1 ± 3e-2
Parallelepiped						
Scale	6.5e-4 ± 1.5e-4	6.6e-4 ± 1.5e-4	5.9e-4 ± 1.4e-4	3.9e-5 ± 2.1e-5	4.6e-5 ± 1.6e-5	7.2e-5 ± 1.3e-5
Volume Ratio	37.1%	41.8%	46.5%	3.9%	5.3%	10.0%
Length_a (Å)	52 ± 2	47 ± 1	51 ± 2	39 ± 3	53 ± 14	46 ± 4
Length_b (Å)	250 (fixed)	250 (fixed)	250 (fixed)	250 (fixed)	250 (fixed)	250 (fixed)
Length_c (Å)	413 ± 123	350 ± 104	438 ± 45.8	455 ± 3036	687 ± 4305	723 ± 2742
BKG	1.6e-4 ± 1e-4	6.9e-5 ± 4e-5	2.1e-4 ± 1.1e-4	2.6e-4 ± 1.1e-4	7.2e-4 ± 3.4e-4	8.9e-4 ±1.5e-4

9. Selected Spectra

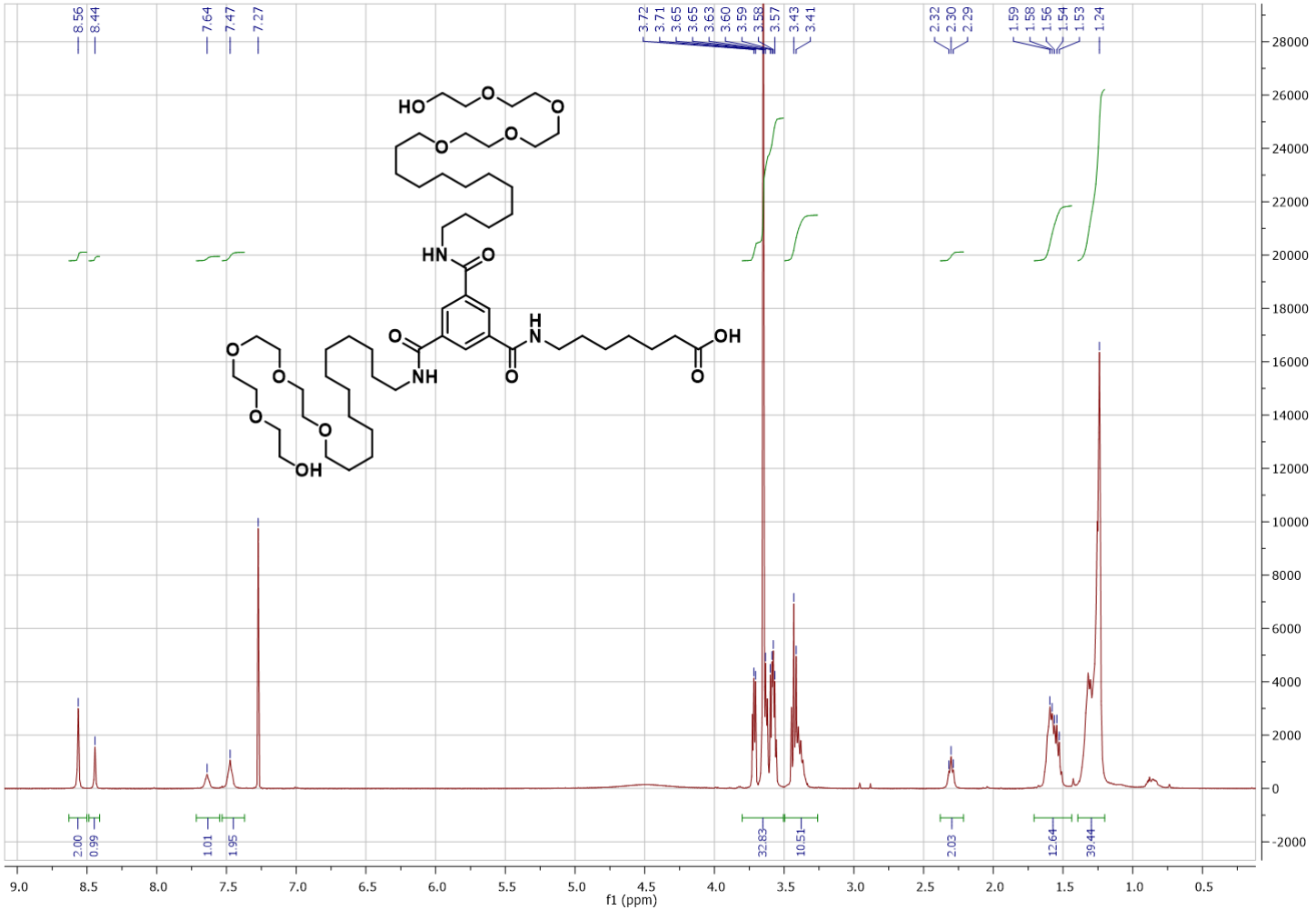


Figure S6. ^{1}H NMR spectrum of **2** ($CDCl_3$, 100 MHz)

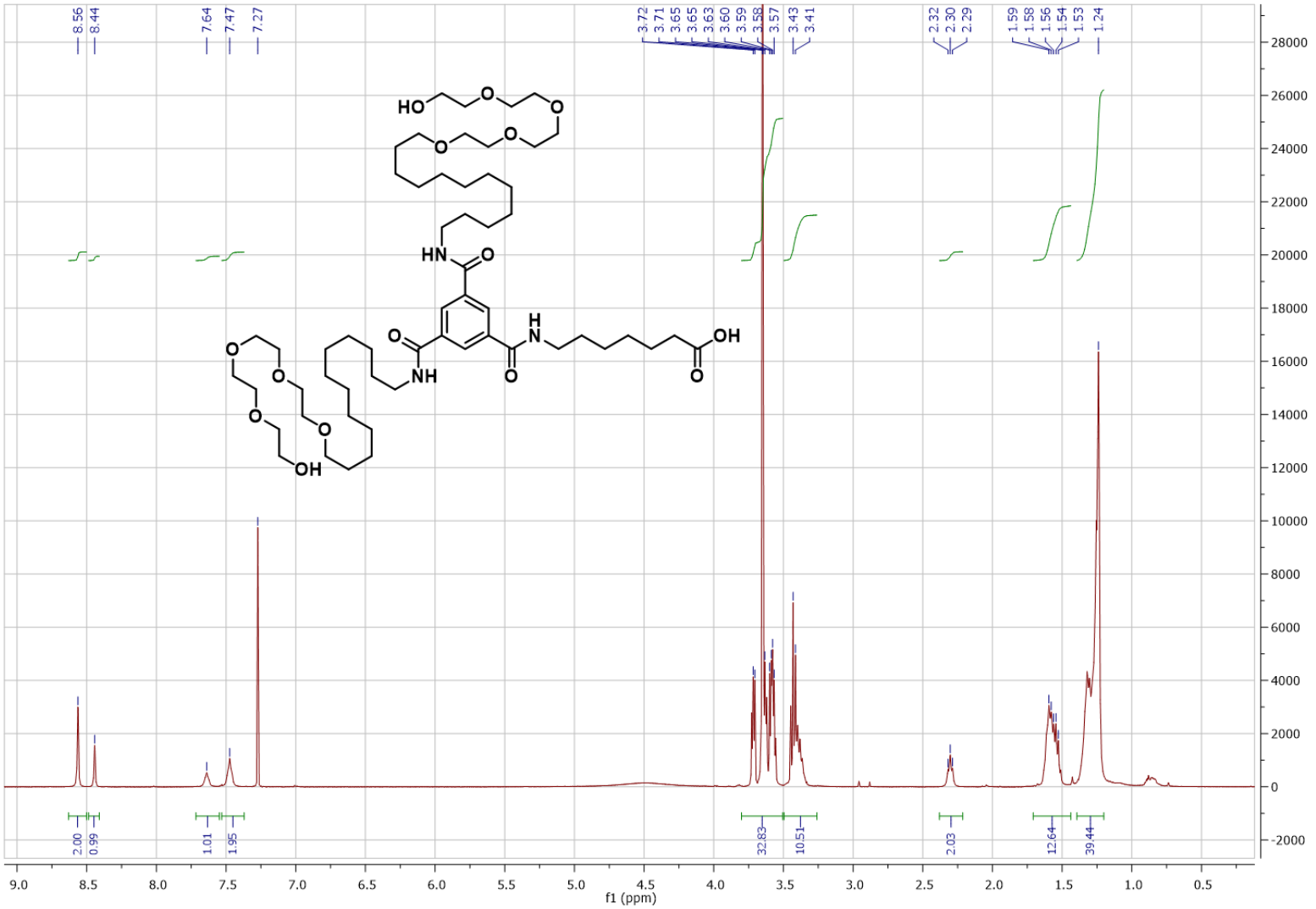


Figure S7. ^{13}C NMR spectrum of **1** ($CDCl_3$, 100 MHz)

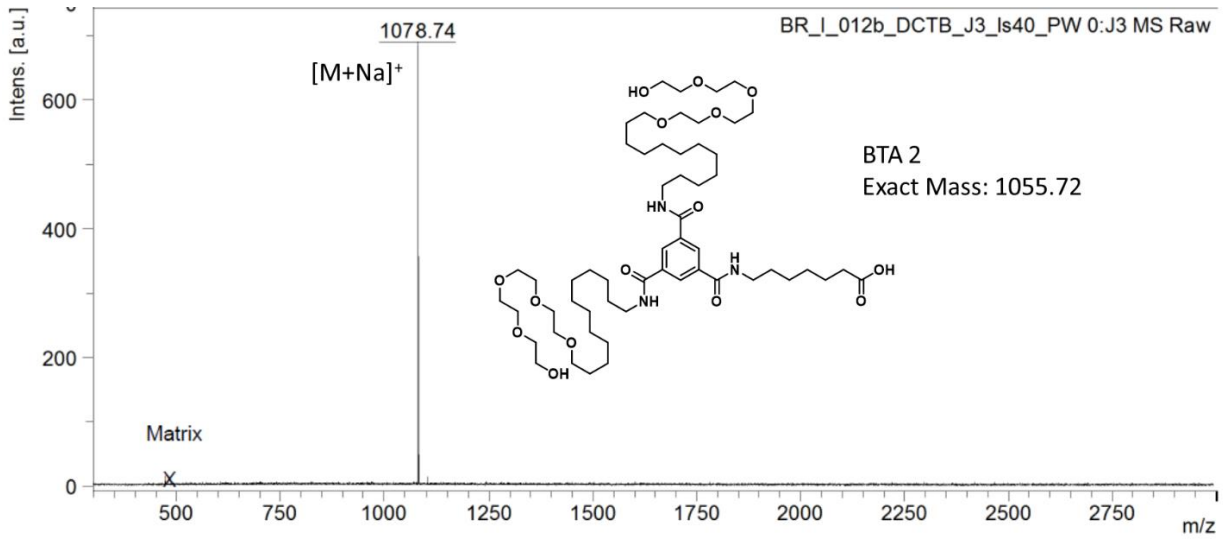


Figure S8. MALDI-TOF analysis of BTA **2**

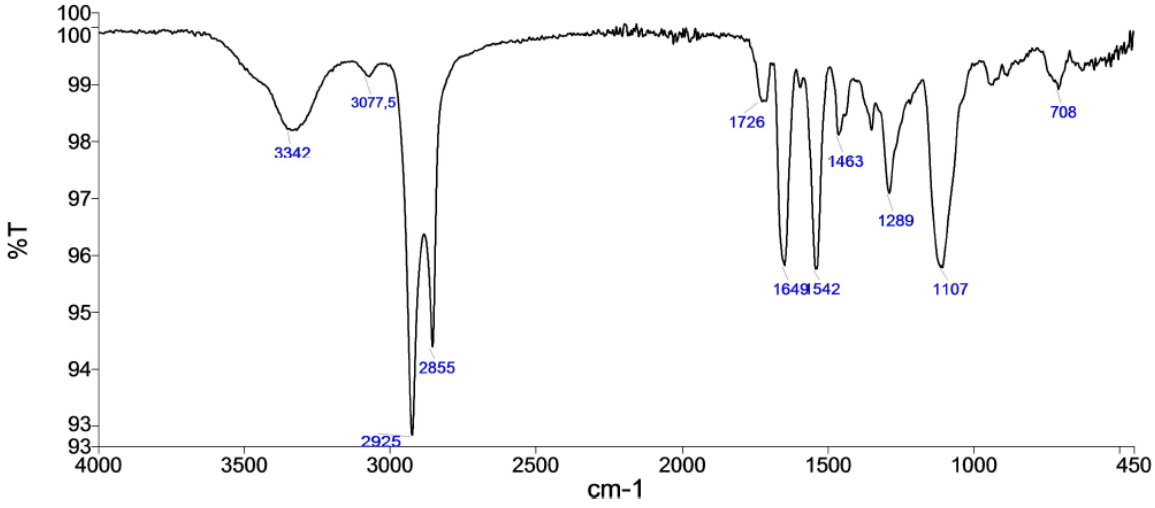


Figure S9. FT-IR spectrum of BTA **2** (thin film)

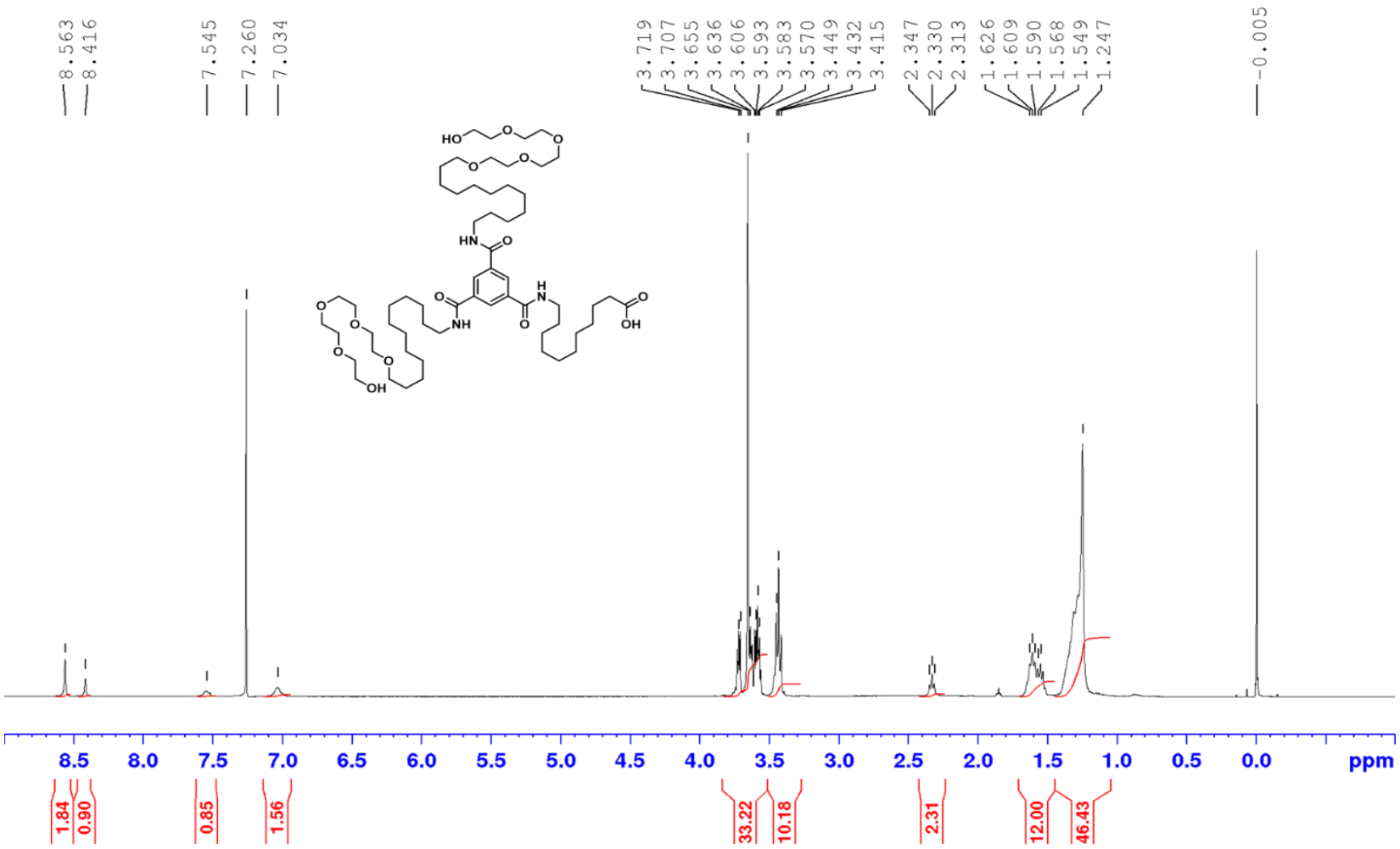


Figure S10. ^{1}H NMR spectrum of **3** ($CDCl_3$, 400 MHz)

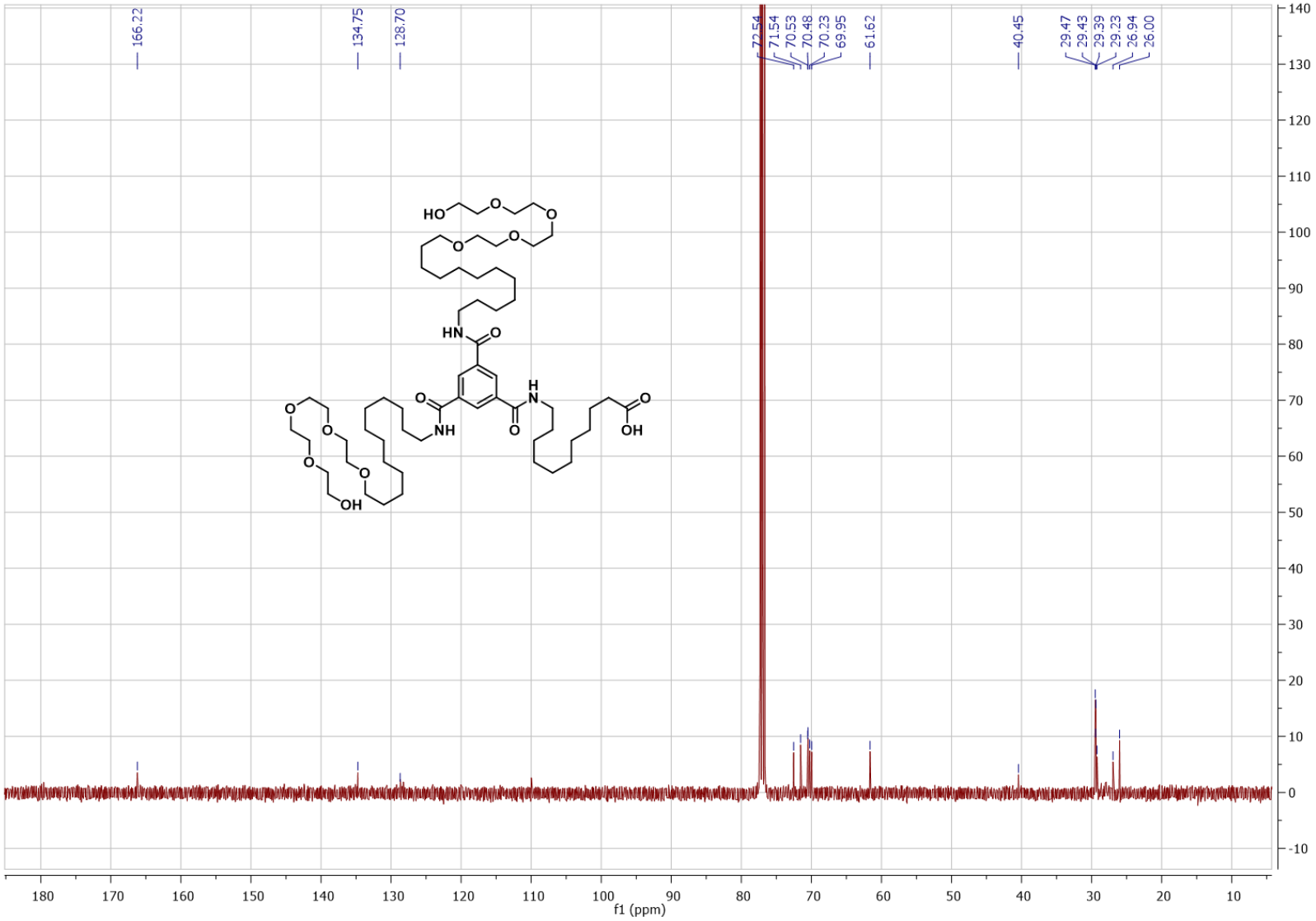


Figure S11. ^{13}C NMR spectrum of **3** ($CDCl_3$, 100 MHz)

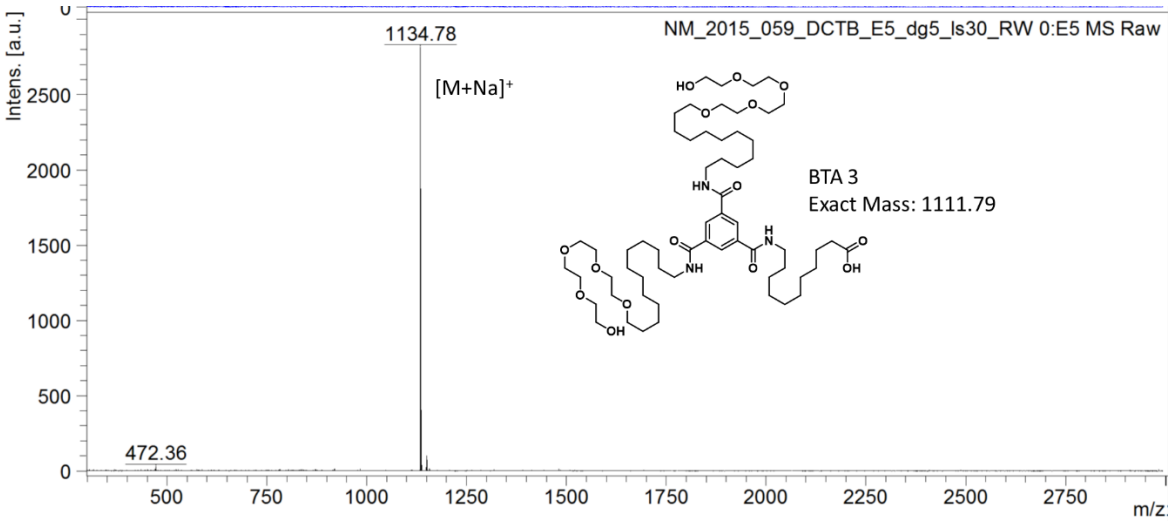


Figure S12. MALDI-TOF analysis of BTA **3**

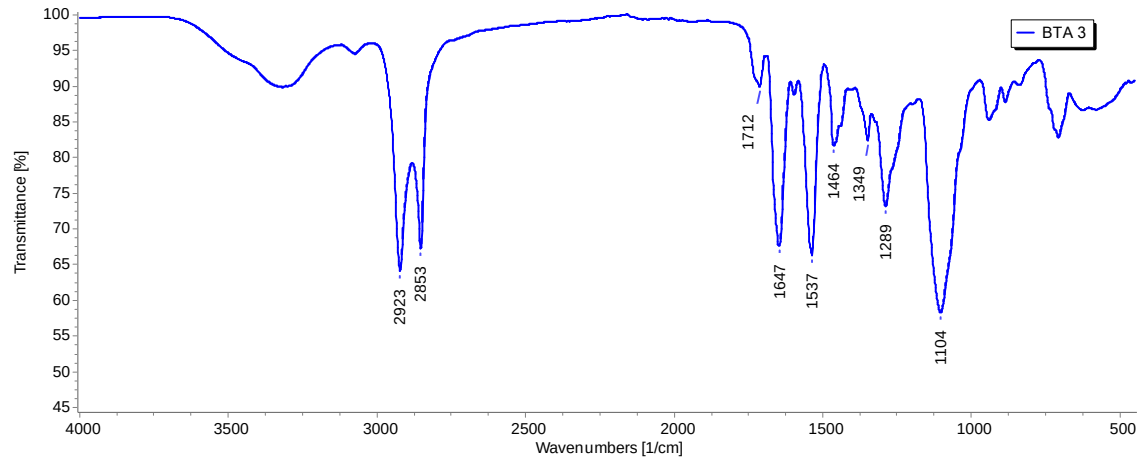


Figure S13. FT-IR spectrum of BTA **3** (thin film)

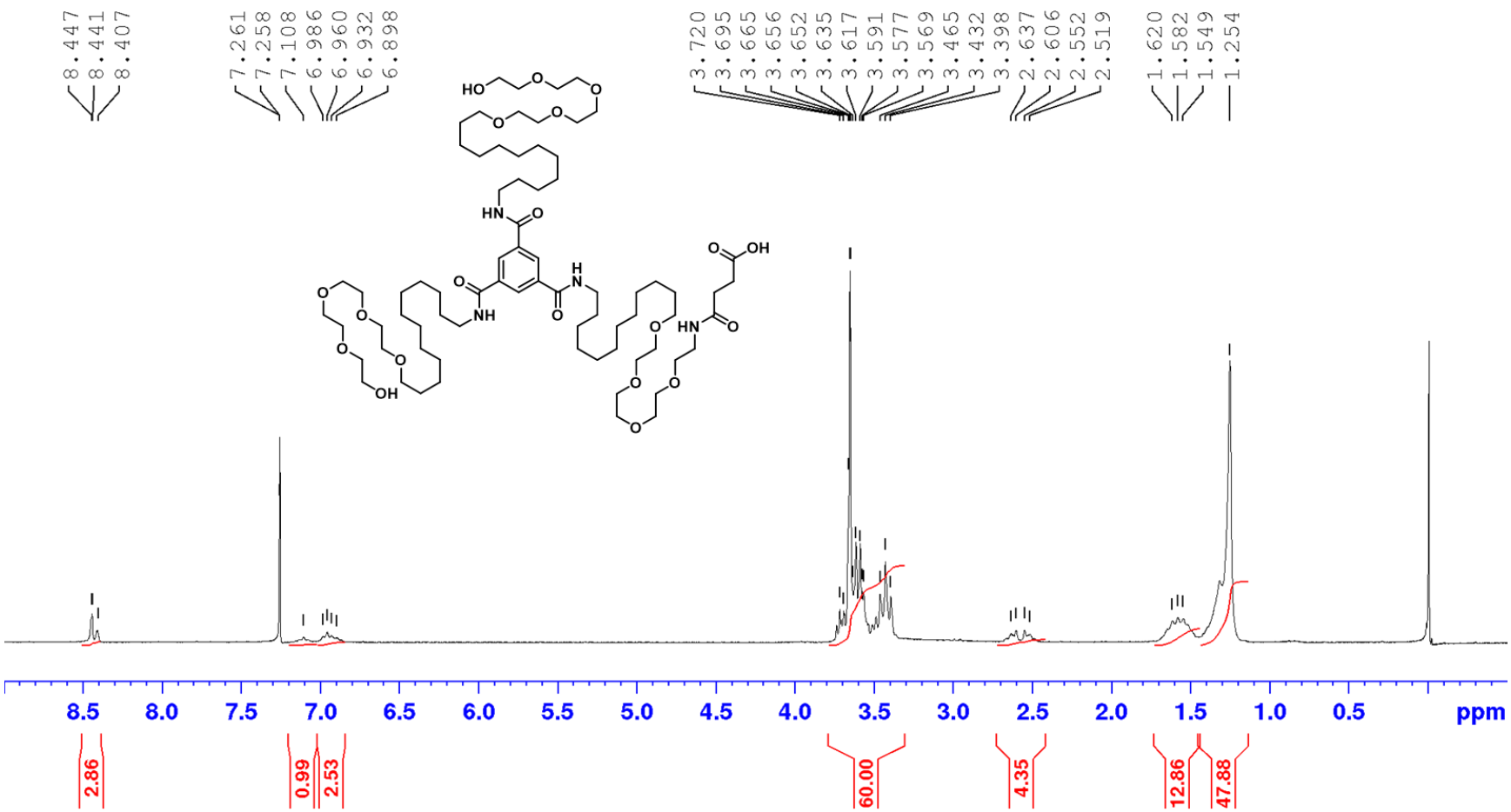


Figure S14. ^{1}H NMR spectrum of BTA **4** (CDCl**3**, 400 MHz)

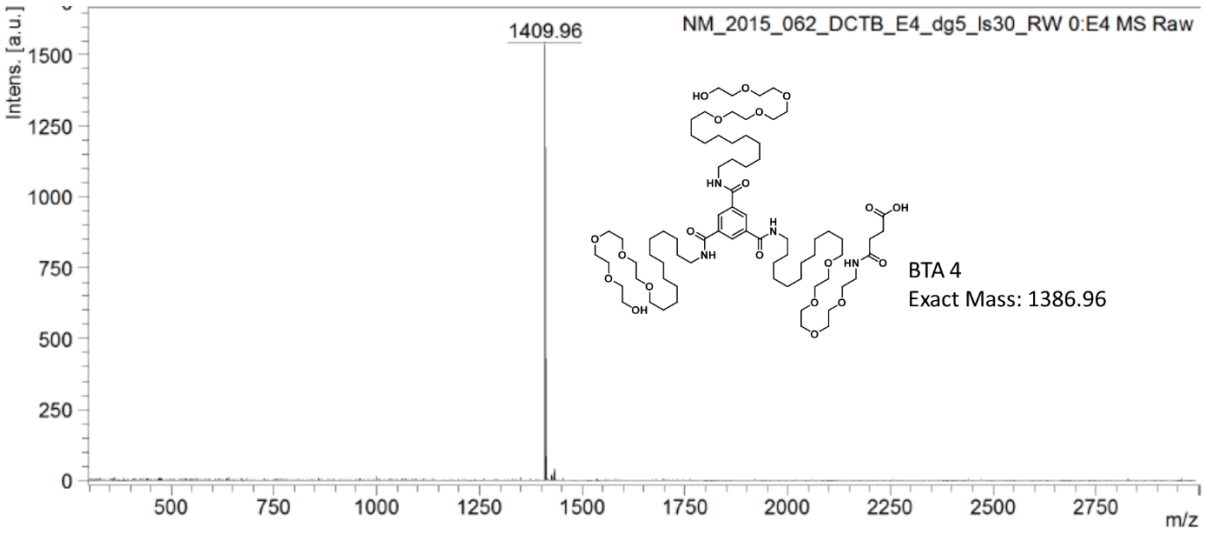


Figure S15. MALDI-TOF analysis of BTA **4.**

10. References

1. Albertazzi, L.; van der Zwaag, D.; Leenders, C. M. A.; Fitzner, R.; van der Hofstad, R. W.; Meijer, E. W. *Science* **2014**, *344*, 491-495.

2. Lou, X.; Lafleur, R. P. M.; van Dongen J. L. J.; Leenders, C. M. A.; Baker M. B.; Palmans, A. R. A.; Meijer E. W. *Nat. Commun.* **2017**, *8*, 15420.

3. Thota, B.; Lou, X.; Bochicchio, D.; Paffen, T. F. E.; Lafleur, R. P. M.; van Dongen, J. L. J., Haag R.; Pavan, G. M.; Palmans, A. R. A.; Meijer; E. W. *Angew. Chem. Int. Ed*. **2018**, *57*, 6843-6847.