Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting information

Shape modulation of squaramide-based supramolecular polymer nanoparticles

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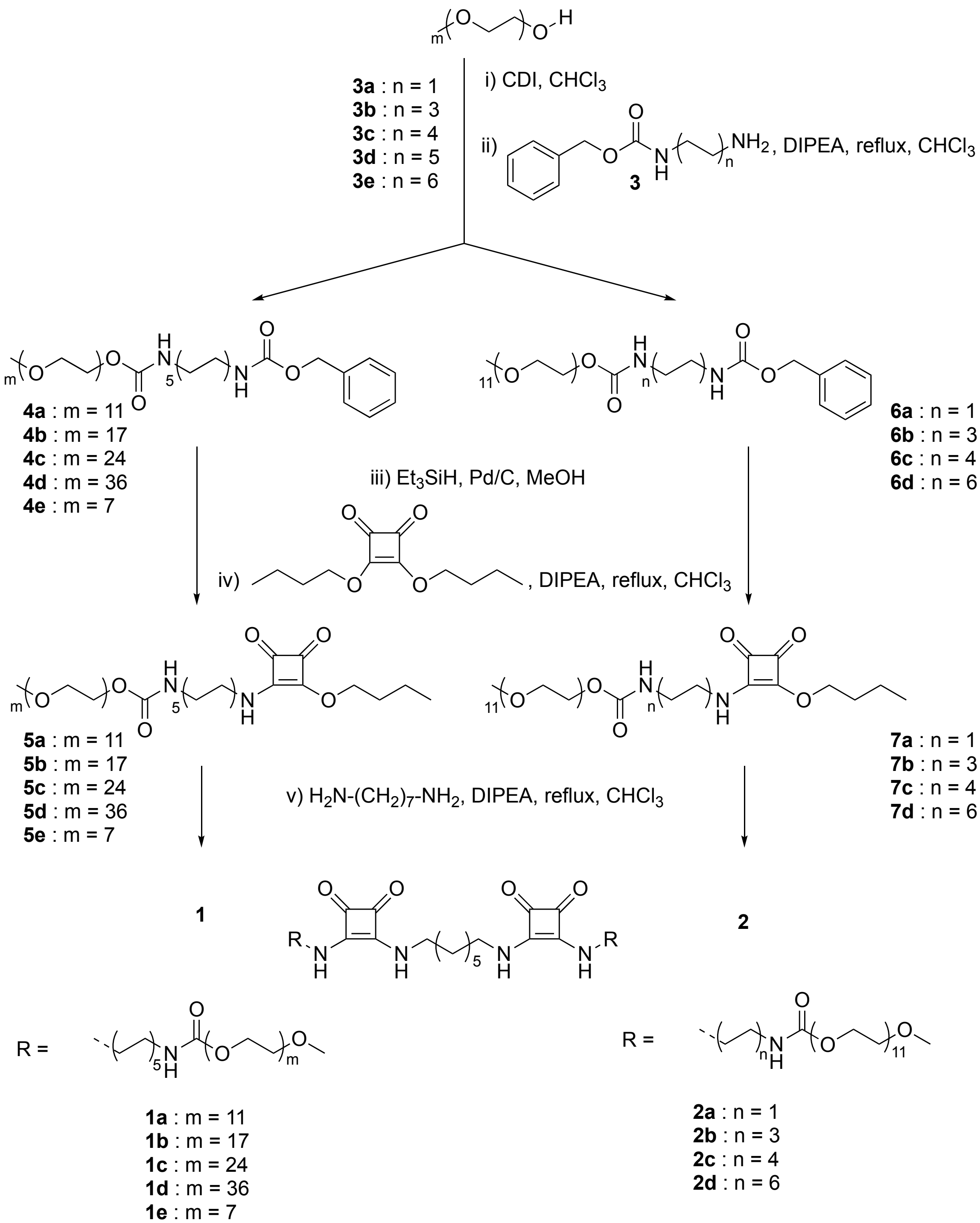
Contents

1. Characterization methods

The squaramide-based bolaamphiphiles were purified using a Grace Reveleris X1 flash chromatography system equipped with a C18 column. ^{1}H NMR and ^{13}C NMR spectra were acquired on a Bruker Ascend 850, Bruker DMX-400, Bruker AV-III-600 MHz and Bruker DPX-300 MHz at 298K. LC-MS analysis was performed on a Finnigan Surveyor HPLC system equipped with a Gemini C18 50×4.60 mm column (UV detection at 200-600 nm), coupled to a Finnigan LCQ Advantage Max mass spectrometer with ESI, or with a TSQ Quantum Access MAX system equipped with a Gemini 3 μm C18 110 Å 50×4.60 mm column (UV detection at 214 nm and 254 nm). The mobile phase consisted of a gradient of 10-90% of H_2O-CH_3CN with 0.1% trifluoroacetic acid over 13.5 minutes. MALDI-TOF-MS (Matrix-assisted laser desorption ionization–time-of-flight) spectra were recorded on a Bruker microflex LRF mass spectrometer in reflection positive-ion mode using α-cyano-4-hydroxycinnamicacid as a matrix on a ground steel target plate.

2. Synthetic routes
Synthesis of squaramide-based bolaamphiphiles



Scheme 1. Synthetic route of **1a-e** and **2a-d**.

The synthesis of **3d**, **4a**, **5a** and **1a** were reported in an earlier publication.[1] A similar synthetic approach was followed for the synthesis of the rest of the monomers, which is reported below.

Synthesis of 3a

1,n-alkyldiamine (**a:** n=2, 1.8 g, 30 mmol) was dissolved in 50 mL CH_2Cl_2 and cooled to 0°C. Benzyl chloroformate (2.6 g, 15 mmol) was dissolved in 75 mL CH_2Cl_2 and added dropwise over 1 hour to a solution while stirring. The reaction was allowed to reach room temperature and allowed to stir overnight. After completion, the solution was washed 3x with brine, dried with $MgSO_4$, and the CH_2Cl_2 was evaporated under reduced pressure. The crude product was purified by normal phase chromatography by using $CH_2Cl_2/CH_3OH/Et_3N$ (99/0/1 - 75/24/1 v/v/v).

3a: Yield: 2.47 g, 85.0% ^{1}H-NMR (δ_H[ppm], DMSO-d_6, 300 MHz): 7.38-7.27 (m, 5H), 6.53 (br s), 5.00 (s, 2H), 3.08-3.02 (m, 2H), 2.66-2.61 (t, 2H). ^{13}C-NMR (δ_C[ppm], DMSO-d_6, 75 MHz) 156.71, 137.56, 128.78, 128.20, 128.16, 65.72, 42.79, 41.11.

Synthesis of 3b-3e

A similar synthetic approach to **3d** was carried out for the synthesis of **3b**, **3c** and **3e**. N-(Benzyloxycarbonyloxy)succinimide (**b:** 2.2 g, 8.8 mmol, **c:** 0.9 g, 3.6 mmol, **e:** 1.8 g, 7.2 mmol) was dissolved in 150 mL chloroform and added dropwise over 1 hour to a cooled (0°C), stirring solution of 1,n-alkyldiamine (**b:** n = 6; 5.0 g, 43.0 mmol, **c:** n = 8; 2.5 g, 17.3 mmol, **e:** n = 12; 8.6 g, 43.0 mmol) dissolved in 150 mL chloroform. Afterwards, the reaction was allowed to reach room temperature and stirred for an additional 18 hours. At the end of the reaction, the solution of **3b** was evaporated to dryness, dissolved in ethyl acetate and washed 3x with water. Subsequently, the aqueous layers were combined and adjusted to pH 12 with NaOH and saturated with NaCl. This solution was extracted 3x with ethyl acetate and the combined organic layers were washed 3x with brine, dried over $MgSO_4$ and evaporated to yield compound **3b** as a white crystalline solid.

For **3c** and **3e**, the respective solutions were evaporated to dryness and 200 mL of ethyl acetate was added. Subsequently, 200 mL of a 1M HCl solution were added to these solutions resulting in a precipitate in the organic layer. The precipitates were collected by filtration and washed with ethyl acetate to obtain the final compounds as white crystalline solids.

3b: Yield: 1.69 g, 76.5% ^{1}H-NMR (δ_H[ppm], DMSO-d_6, 400 MHz): 7.41-7.26 (m, 5H), 5.05 (s, 2H), 3.55-3.48 (m, 2H), 3.02-2.96 (q, 2H), 1.49-1.22 (m, 8H). ^{13}C-NMR (δ_C[ppm], DMSO-d_6, 100 MHz): 156.61, 137.86, 128.88, 128.27, 65.60, 51.08, 41.70, 32.93, 30.97, 30.18, 29.96, 27.30, 26.67, 26.56.

3c: Yield: 0.68 g, 67.6% ^{1}H-NMR (δ_H[ppm], DMSO-d_6, 400 MHz): 8.12 (s, 3H), 7.38-7.23 (m, 5H), 5.00 (s, 2H), 3.00-2.95 (q, 2H), 2.74-2.70 (t, 2H), 1.59-1.54 (m, 2H), 1.41-1.36 (m, 2H), 1.30-1.22(m, 8H) ^{13}C-NMR (δ_C[ppm], DMSO-d_6, 100 MHz): 156.55, 137.79, 129.55, 128.15, 127.47, 65.51, 40.70, 40.62, 40.41, 29.82, 28.95, 28.93, 27.34, 26.57, 26.28.

3e: Yield: 1.73 g, 71.7% ^{1}H-NMR (δ_H[ppm], DMSO-d$_6$, 400 MHz): 8.07 (s, 3H), 7.35-7.23 (m, 5H), 4.98 (s, 2H), 2.96-2.93 (q, 2H), 2.71-2.69 (t, 2H), 1.55-1.50 (m, 2H), 1.37-1.35 (m, 2H), 1.26-1.21 (m, 16H) ^{13}C-NMR (δ_C[ppm], DMSO-d$_6$, 100 MHz): 156.66, 137.91, 129.02, 128.81, 128.18, 65.62, 40.88, 39.09, 29.99, 29.54, 29.46, 29.33, 29.16, 27.51, 26.83, 26.47.

Synthesis of 4b-e

Oligo(ethylene glycol) methyl ether with various repetition units (**b:** n = 17, 0.5 g, 0.64 mmol; **c:** n = 24, 0.3 g, 0.27 mmol; **d:** n = 36, 0.3 g, 0.19 mmol; and **e:** n = 7, 0.5 g, 1.47 mmol) were activated with 1,1'-carbonyldiimidazole (CDI) (**b:** n = 17, 0.18 g, 1.10 mmol; **c:** n=24, 0.07 g, 0.40 mmol; **d:** n = 36.45 mg, 0.30 mmol; and **e:** n = 7, 0.36 g, 2.21 mmol) in a minimal amount of chloroform (~1 mL). The solution was stirred until complete conversion was observed by LC-MS. To the resulting solution, **3d** (**b:** n = 17, 0.29 g, 1 mmol; **c:** n = 24, 0.13 g, 0.43 mmol; **d:** n = 36, 0.12 g, 0.39 mmol; and **e:** n = 7, 0.9 g, 2.94 mmol) a few drops of DIPEA, and chloroform (up to 10 mL) were added and refluxed overnight. The product was purified by flash column chromatography using an CH_3CN/H_2O gradient from 10-90% over 30-45 minutes on a C18 silica column. The product was concentrated by evaporation and lyophilized to obtain a white solid.

4b: Yield: 0.35 g, 49.1% ^{1}H-NMR (δ_H[ppm], $CDCl_3$, 600 MHz): 7.35-7.29 (m, 5H), 5.08 (s, 2H), 4.88 (br s, NH), 4.78 (br s, NH), 4.20-4.19 (t, 2H), 3.75-3.62 (m, 64H), 3.55-3.53 (t, 2H), 3.37 (s, 3H), 3.18-3.12 (m, 4H), 2.09 (br s, 2H), 1.47-1.46 (m, 4H), 1.27-1.25 (m, 12H). ^{13}C-NMR (δ_C[ppm], $CDCl_3$, 150 MHz): 156.53, 136.80, 128.63, 128.24, 128.20, 72.05, 70.72, 70.68, 70.64, 69.82, 66.67, 63.91, 59.17, 41.23, 41.15, 30.06, 29.53, 29.33, 26.83. LC-MS: 7.53 min, m/z: 1113.20 $[M+H]^+$. MALDI-TOF-MS: *m/z* calc: 1112.68; found: 1136.19 $[M+Na]^+$.

4c: Yield: 0.30 g, 76.6% ^{1}H-NMR (δ_H[ppm], $CDCl_3$, 600 MHz): 7.37-7.31 (m, 5H), 5.10 (s, 2H), 4.23-4.21 (t, 2H), 3.79-3.65 (m, 92H), 3.57-3.55 (t, 2H), 3.39 (s, 3H), 3.21-3.16 (m, 4H), 1.98 (br s, NH), 1.50-1.47 (m, 4H), 1.28 (m, 12H). ^{13}C-NMR (δ_C[ppm], $CDCl_3$, 150 MHz): 156.43, 136.77, 128.57, 128.13, 72.02, 70.69, 70.65, 70.60, 69.77, 66.63, 63.88, 59.10, 41.11, 30.01, 29.47, 29.27, 26.77. LC-MS: 6.79 min, m/z: 1421.93 $[M+H]^+$. MALDI-TOF-MS: *m/z* calc: 1420.87; found: 1444.08 $[M+Na]^+$.

4d: Yield: 0.26 g, 71.9% ^{1}H-NMR (δ_H[ppm], $CDCl_3$, 600 MHz): 7.29-7.23 (m, 5H), 5.02 (s, 2H), 4.89 (s, 1H), 4.14-4.12 (t, 2H), 3.59-3.46 (m, 142H), 3.31 (s, 3H), 3.12-3.06 (m, 4H), 1.41-1.40 (m, 4H), 1.20-1.19 (m, 12H). ^{13}C-NMR (δ_C[ppm], $CDCl_3$, 150 MHz): 156.33, 136.65, 128.40, 128.00, 127.65, 71.85, 70.52, 70.48, 70.42, 69.59, 66.39, 63.68, 58.95, 41.00, 40.93, 29.84, 29.31, 29.11, 26.61. LC-MS: 6.61 min, m/z: 1950.47 $[M+H]^+$. MALDI-TOF-MS: *m/z* calc: 1949.18; found: 1972.32 $[M+Na]^+$.

4e: Yield: 0.62 g, 62.7% ^{1}H-NMR (δ_H[ppm], $CDCl_3$, 400 MHz): 7.29-7.23 (m, 5H), 5.03 (s, 2H), 4.15-4.12 (t, 2H), 3.67-3.54 (m, 24H), 3.50-3.47 (t, 2H), 3.31 (s, 3H), 3.13-3.05

(m, 4H), 1.44-1.39 (m, 4H), 1,26-1.21 (m, 12H). ^{13}C-NMR (δ_C [ppm], $CDCl_3$, 100 MHz): 156.43, 136.69, 128.44, 128.02, 127.98, 71.86, 70.58, 70.56, 70.53, 70.50, 70.47, 70.43, 69.63, 66.44, 63.71, 60.51, 58.95, 41.05, 40.98, 40.90, 29.95, 29.89, 29.36, 29.17, 26.66. LC-MS: 7.81 min, m/z: 673.20 $[M+H]^+$. MALDI-TOF-MS: *m/z* calc: 672.42; found: 694.93 $[M+Na]^+$, 710.94 $[M+K]^+$.

Synthesis of 5b-e

Compound **4** (**b:** 0.24 g, 0.22 mmol; **c:** 0.30 g, 0.21 mmol; **d:** 0.26 g, 0.13 mmol; **e:** 0.20 g, 0.30 mmol) was dissolved in 1-3 mL methanol, and a catalytic amount of Pd/C was added. Subsequently, triethylsilane (**b:** 0.41 mL, 2.6 mmol; **c:** 0.43 mL, 2.7 mmol; **d:** 0.18 mL, 1.1 mmol; **e:** 0.41 mL, 2.6 mmol) was added dropwise to the reaction mixture. The solution became effervescent due to the *in situ* formation of H_2 (g). Complete deprotection was confirmed by TLC-MS (additional Et_3SiH was added in case the deprotection was incomplete) and the solution was filtered over Celite in order to remove Pd/C. The filtrate was concentrated by rotary evaporation and a gentle stream of air was used to dry the product. The white solid was redissolved in chloroform (~10 mL), and 3,4-dibutoxy-3-cyclobutene-1,2-dione was added (**b:** 50 µL, 0.23 mmol; **c:** 55 µL, 0.25 mmol; **d:** 28 µL, 0.13 mmol; **e:** 85 µL, 0.39 mmol) with a few drops of DIPEA. The reaction mixture was stirred and refluxed overnight. The crude product was purified by flash column chromatography using a gradient of 10-90% CH_3CN/H_2O over 30-45 minutes on a C18 silica column. The product was concentrated by evaporation and lyophilized overnight to obtain compound **5b-e** as a white solid.

5b: Yield: 143 mg, 58.6% ^{1}H-NMR (δ_H[ppm], $CDCl_3$, 600 MHz): 4.90 (br s, 1H), 4.74-4.69 (t, 2H), 4.21-4.20 (t, 2H), 3.75-3.72 (t, 2H), 3.67-3.63 (m, 64H), 3.55-3.54 (t, 2H), 3.42-3.40 (m, 2H), 3.37 (s, 3H), 3.16-3.13 (t, 2H), 1.89-1.77 (m, 2H), 1.60-1.58 (m, 2H), 1.48-1.43 (m, 4H), 1.28-1.24 (m, 12H), 0.98-0.95 (t, 3H). ^{13}C-NMR (δ_C[ppm], $CDCl_3$, 150 MHz): 189.19, 183.01, 177.49, 172.56, 156.56, 73.53, 72.03, 70.70, 70.65, 70.62, 70.61, 69.80, 68.11, 63.93, 59.18, 45.00, 41.10, 32.14, 30.75, 30.00, 29.42, 29.25, 29.15, 26.76, 26.40, 25.73, 18.78, 13.81. LC-MS: 7.17 min, m/z: 1131.33$[M+H]^+$. MALDI-TOF-MS: *m/z* calc: 1130.69; found: 1153.99 $[M+Na]^+$.

5c: Yield: 164 mg, 54.0% ^{1}H-NMR (δ_H[ppm], $CDCl_3$, 600 MHz): 4.91 (br s, 1H), 4.76-4.74 (t, 2H), 4.24-4.23 (t, 2H), 3.76-3.66 (m, 90H), 3.58-3.57 (m, 4H), 3.45-3.44 (m, 2H), 3.40 (s, 3H), 3.19-3.16 (t, 2H), 1.82-1.81 (m, 2H), 1.63-1.61 (m, 2H), 1.51-1.43 (m, 4H), 1.36-1.26 (m, 12H), 1.01-0.96 (t, 3H). ^{13}C-NMR (δ_C[ppm], $CDCl_3$, 150 MHz): 189.02, 183.19, 177.34, 172.49, 156.44, 73.40, 71.94, 70.60, 70.56, 70.52, 69.69, 63.82, 59.04, 44.87, 40.99, 32.03, 30.64, 29.89, 29.28, 29.11, 29.02, 26.63, 26.28, 18.66, 13.68. LC-MS: 6.42 min, m/z: 1439.74 $[M+2H]^{+2}$. MALDI-TOF-MS: *m/z* calc: 1438.88; found: 1462.09 $[M+Na]^+$.

5d: Yield: 160 mg, 61.0% ^{1}H-NMR (δ_H[ppm], $CDCl_3$, 600 MHz): 6.59 (br s, 1H), 4.90 (br s, 1H), 4.72-4.70 (t, 2H), 4.18-4.16 (t, 2H), 3.73-3.48 (m, 142H), 3.39-3.35 (m, 5H), 3.13-3.10 (m, 2H), 1.77-1.75 (m, 2H), 1.60-1.55 (m, 2H), 1.46-1.41 (m, 4H), 1.28-1.25 (m, 12H), 0.95-0.93 (t, 3H). ^{13}C-NMR (δ_C[ppm], $CDCl_3$, 150 MHz): 177.34, 156.44, 73.40, 71.94, 70.60, 70.56, 70.52, 69.69, 63.82, 59.04, 44.87, 40.99, 32.03, 30.64, 29.89, 29.28, 29.11, 29.02, 26.63, 26.28, 18.66, 13.68. LC-MS: 7.28 min, m/z: 1968.20 $[M+H]^+$. MALDI-TOF-MS: *m/z* calc: 1967.19; found: 1990.30 $[M+Na]^+$.

5e: Yield: 179 mg, 87.1% ^{1}H-NMR (δ_H[ppm], $CDCl_3$, 400 MHz): 7.20 (br s, 1H), 5.03 (br s, 1H), 4.70-4.67 (t, 2H), 4.16-4.15 (t, 2H), 3.63-3.58 (m, 24H), 3.51-3.49 (m, 2H), 3.37-3.34 (m, 2H), 3.32 (s, 3H), 3.11-3.09 (t, 2H), 1.76-72 (m, 2H), 1.58-1.54 (m, 2H), 1.45-1.40 (m, 4H), 1.30-1.22 (m, 12H), 0.94-0.90 (t, 3H). ^{13}C-NMR (δ_C[ppm], $CDCl_3$, 100 MHz): 189.56, 182.73, 177.31, 172.48, 156.46, 73.27, 71.86, 70.49, 69.60, 68.51, 63.74, 58.93, 44.81, 40.96, 31.98, 30.99, 30.59, 29.86, 29.32, 29.13, 29.03, 26.63, 26.30, 18.61, 13.63. LC-MS: 7.33 min, m/z: 691.07$[M+H]^+$. MALDI-TOF-MS: *m/z* calc: 690.43; found: 694.93 $[M+Na]^+$, 710.94 $[M+K]^+$.

Synthesis of 6a-d

Undeca(ethylene glycol) methyl ether (**a:** 0.51 g, 1 mmol; **b:** 0.5 g, 1 mmol; **c:** 0.5 g, 1 mmol; **d:** 0.53 g, 1.02 mmol) was activated with 1,1'-carbonyldiimidazole (**a:** 0.19 g, 1.19 mmol; **b:** 0.25 g, 1.5 mmol; **c:** 0.25 g, 1.5 mmol; **d:** 0.20 g, 1.22 mmol) in chloroform (~1 mL) and was reacted until complete conversion was confirmed by LC-MS. To the resulting solution containing the activated undeca(ethylene glycol) methyl ether, **3a** (0.23 g, 1.18 mmol), **3b** (0.5 g, 2 mmol), **3c** (0.56 g, 2 mmol) and **3e** (0.41 g, 1.22 mmol) were added respectively, followed by the addition of few drops of DIPEA and left to reflux overnight. The product was purified by flash column chromatography using a gradient of 10-90% CH_3CN/H_2O over 30-45 minutes on a C18 silica column. The product was concentrated by evaporation and lyophilized overnight to obtain a white solid.

6a: Yield: 0.56 g, 77% ^{1}H-NMR (δ_H[ppm], $CDCl_3$, 400 MHz): 7.33-7.28 (m, 5H), 5.53 (s, 1H), 5.07 (s, 2H), 4.18-4.17 (m, 2H), 3.72-3.70 (m, 42H), 3.35 (s, 3H), 3.28 (m, 4H).^{13}C-NMR (δ_C[ppm], $CDCl_3$, 100 MHz): 156.95, 156.88, 136.60, 128.55, 128.18, 128.15, 71.97, 70.64, 70.60, 70.59, 70.55, 69.56, 66.73, 64.07, 59.09, 41.32, 41.40, 29.75.LC-MS: 5.20 min, m/z: 759.22 $[M+Na]^+$. MALDI-TOF-MS: *m/z* calc: 736.85; found: 759.65 $[M+Na]^+$.

6b: Yield: 0.43 g, 54.3% ^{13}C-NMR (δ_C[ppm], $CDCl_3$, 100 MHz): 156.46, 136.64, 128.51, 128.23, 128.08, 71.93, 70.60, 70.56, 70.51, 69.71, 66.61, 63.84, 60.71, 59.04, 40.85, 29.93, 29.89, 29.84, 26.25, 26.23. LC-MS: 6.51 min, m/z: 793.27$[M+H]^+$. MALDI-TOF-MS: *m/z* calc: 792.46; found: 815.20 $[M+Na]^+$, 831.20 $[M+K]^+$.

6c: Yield: 0.52 g, 63.3% ^{1}H-NMR (δ_H[ppm], $CDCl_3$, 400 MHz): 7.37-7.31 (m, 5H), 5.10 (s, 2H), 4.23-4.20 (m, 2H), 3.69-3.64 (m, 42H), 3.57-3.54 (m, 2H), 3.38 (s, 3H), 3.20-3.13 (m, 4H), 1.51-1.46 (m, 4H), 1.31-1.30 (m, 8H). ^{13}C-NMR (δ_C[ppm], $CDCl_3$, 100 MHz): 156.66, 136.68, 128.50, 128.15, 128.06, 71.93, 70.60, 70.56, 70.54, 70.51, 69.69, 66.56, 63.80, 59.03, 41.00, 29.93, 29.90, 29.11, 26.61. LC-MS: 7.06 min, m/z: 821.40 $[M+H]^+$. MALDI-TOF-MS: *m/z* calc: 820.49; found: 843.38 $[M+Na]^+$, 859.38 $[M+K]^+$.

6d: Yield: 0.45 g, 64.3% ^{1}H-NMR (δ_H[ppm], $CDCl_3$, 400 MHz): 7.38-7.31 (m, 5H), 5.11 (s, 2H), 4.24-4.21 (t, 2H), 3.71-3.55 (m, 44H), 3.40 (s, 3H), 3.23-3.17 (m, 4H), 1.84 (s, 2H), 1.50-1.49 (m, 4H), 1.20 (m, 16H). ^{13}C-NMR (δ_C[ppm], $CDCl_3$, 100 MHz): 156.25, 136.51, 128.50, 128.06, 71.94, 70.57, 70.52, 69.69, 66.56, 63.80, 59.03. LC-MS: 7.82 min, m/z: 876.76 $[M+H]^+$. MALDI-TOF-MS: *m/z* calc: 876.56; found: 899.60 $[M+Na]^+$.

Synthesis of 7a-d

Compound **6** (**a:** 0.55 g, 0.74 mmol; **b:** 0.30 g, 0.38 mmol; **c:** 0.075 g, 0.09 mmol; **d:** 0.44 g, 0.51 mmol) was dissolved in 1-3 mL methanol, and a catalytic amount of Pd/C was added, as described previously for compound **5**. The Cbz-deprotection of the amine moiety was achieved by the dropwise addition of Et_3SiH to provide an effervescent solution (**a:** 1.19 mL, 7.45 mmol; **b:** 0.6 mL, 3.8 mmol; **c:** 0.14 mL, 0.9 mmol; **d:** 0.81 mL, 5.1 mmol). Complete deprotection was confirmed by TLC-MS and the solution was filtered over Celite to remove the Pd/C. The filtrate was concentrated by rotary evaporation and a gentle stream of air to dry the product. The white solid was redissolved in chloroform (~ 10 mL) and 3,4-dibutoxy-3-cyclobutene-1,2-dione was added (**a:** 208 µL, 0.96 mmol; **b:** 106 µL, 0.49 mmol; **c:** 26 µL, 0.117 mmol; **d:** 142 µL, 0.66 mmol) with few drops of DIPEA. The reaction mixture was stirred and refluxed overnight. The crude product was purified by flash column chromatography using a gradient of 10-90% CH_3CN/H_2O over 30-45 minutes on a C18 silica column. The product was concentrated by evaporation and lyophilized overnight to obtain compound **7a-d** as a white solid.

7a: Yield: 240 mg, 42.6% ^{1}H-NMR (δ_H[ppm], $CDCl_3$, 400 MHz): 4.72-4.69 (m, 2H), 4.21-4.19 (t, 2H), 3.76-3.63 (m, 40H), 3.55-3.53 (m, 2H), 3.37 (s, 3H), 1.78-1.76 (m, 2H), 1.45-1.41 (m, 2H), 0.97-0.95 (t, 3H). ^{13}C-NMR (δ_C[ppm], $CDCl_3$, 100 MHz): 188.96, 183.45, 177.34, 172.98, 156.86, 73.41, 71.90, 71.85, 70.56, 70.52, 70.51, 70.48, 70.44, 70.40, 70.30, 70.28, 69.37, 64.04, 59.04, 44.79, 41.76, 41.70, 41.24, 32.03, 29.71, 18.66, 13.72. LC-MS: 4.82 min, m/z: 754.54 $[M+H]^+$. MALDI-TOF-MS: *m/z* calc: 754.44; found: 777.33 $[M+Na]^+$.

7b: Yield: 200 mg, 65.2% ^{1}H-NMR (δ_H[ppm], $CDCl_3$, 400 MHz): 6.70 (br s, 1H), 5.01 (br s, 1H), 4.77-4.72 (t, 2H), 4.22-4.20 (t, 2H), 3.75-3.63 (m, 42H), 3.58-3.54 (m, 2H), 3.38 (s, 3H), 3.21-3.16 (t, 2H), 1.81-1.77 (m, 2H), 1.66-1.60 (m, 2H), 1.59-1.36 (m, 8H), 0.99-0.95 (t, 3H). ^{13}C-NMR (δ_C[ppm], $CDCl_3$, 100 MHz): 177.39, 172.47, 156.54, 73.39, 71.92, 70.58, 70.54, 70.49, 69.64, 63.87, 59.00, 44.62, 40.67, 32.02, 30.48, 29.81, 26.06, 25.87, 18.63, 13.66. LC-MS: 6.09 min, m/z: 811.33 $[M+H]^+$. MALDI-TOF-MS: *m/z* calc: 810.47; found: 833.23 $[M+Na]^+$, 849.22 $[M+K]^+$.

7c: Yield: 48.33 mg, 63.1% ^{1}H-NMR (δ_H[ppm], $CDCl_3$, 400 MHz): 5.14 (br s, 1H), 4.69-4.66 (t, 2H), 4.15-4.13 (t, 2H), 3.63-3.57 (m, 42H), 3.50-3.48 (m, 2H), 3.32 (s, 3H), 3.18 (t, 2H), 2.80 (s, 2H), 1.75-1.71 (m, 2H), 1.55-1.53 (m, 2H), 1.44-1.37 (m, 4H), 1.25 (m, 8H), 0.93-0.90 (t, 3H). ^{13}C-NMR (δ_C[ppm], $CDCl_3$, 100 MHz): 177.22, 172.35, 156.38, 73.26, 71.82, 70.44, 70.37, 69.56, 63.69, 58.92, 44.73, 40.86, 31.95, 30.51, 29.79, 28.98, 26.50, 26.21, 18.59, 13.62. LC-MS: 6.67 min, m/z: 839.33 $[M+H]^+$. MALDI-TOF-MS: *m/z* calc: 838.50; found: 861.32 $[M+Na]^+$, 877.27 $[M+K]^+$.

7d: Yield: 310 mg, 69% ^{1}H-NMR (δ_H[ppm], $CDCl_3$, 400 MHz): 4.75-4.74 (m, 2H), 4.23-4.21 (t, 2H), 3.68-3.56 (m, 44H), 3.40 (s, 3H), 3.18-3.14 (t, 2H), 1.80-1.78 (m, 2H), 1.63-

1.60 (m, 2H), 1.48-1.46 (m, 4H), 1.28 (m, 16H), 1.02-0.97 (t, 3H). ^{13}C-NMR (δ_C[ppm], $CDCl_3$, 100 MHz): 189.53, 182.93, 177.52, 172.47, 156.51, 73.47, 71.98, 70.65, 70.61, 70.56, 69.75, 63.86, 59.11, 44.96, 41.10, 32.09, 31.16, 30.74, 30.00, 29.54, 29.29, 29.18, 26.79, 26.41, 18.73, 13.76. LC-MS: 7.39 min, m/z: 894.81 $[M+H]^+$. MALDI-TOF-MS: *m/z* calc: 894.57; found: 917.44 $[M+Na]^+$.

Synthesis of 1b-e

Compound **5** (**b:** 98 mg, 0.086 mmol; **c:** 80 mg, 0.056 mmol; **d:** 128 mg, 0.066 mmol; **e:** 180 mg, 0.26 mmol) was dissolved in 10 mL chloroform with a few drops of DIPEA. 1,7-heptanediamine (**b:** 6.5 µL, 0.043 mmol; **c:** 4.23 µL, 0.028 mmol; **d:** 5 µL, 0.033 mmol; **e:** 20 µL, 0.13 mmol) was added to the reaction mixture and refluxed overnight. If necessary, an additional amount of 1,7-heptanediamine (up to a maximum of 2 equivalents) was added until the starting material **5** disappeared. The completion of the reaction was verified by LC-MS, and purified by flash column chromatography using a gradient of 10-90% CH_3CN/H_2O over 30-45 minutes on a C18 silica column. The product was concentrated down by evaporation and lyophilized overnight to obtain a white solid.

1b: Yield: 53.2 mg, 54.7% ^{1}H-NMR (δ_H[ppm], $CDCl_3$, 400 MHz): 7.78 (br s, 2H), 7.54 (br s, 2H), 5.05 (br s, 2H), 4.25-4.20 (m, 4H), 3.74-3.65 (m, 136 H), 3.58-3.55 (t, 4H), 3.39 (s, 6H), 3.15-3.13 (m, 4H), 1.65-1.59 (m, 8H), 1.48-1.26 (m, 34H).^{13}C-NMR (δ_C[ppm], $CDCl_3$, 100 MHz): 182.62, 181.55, 168.97, 167.13, 156.53, 71.90, 70.56, 70.52, 70.50, 70.49, 70.46, 69.67, 63.77, 59.05, 44.75, 43.22, 41.06, 31.16, 29.95, 29.47, 29.27, 29.24, 26.75, 26.42, 24.80. LC-MS: 6.83 min, m/z: 1153.47 $[M+H+Na]^{2+}$. MALDI-TOF-MS: *m/z* calc: 2243.39; found: 2266.66 $[M+Na]^+$.

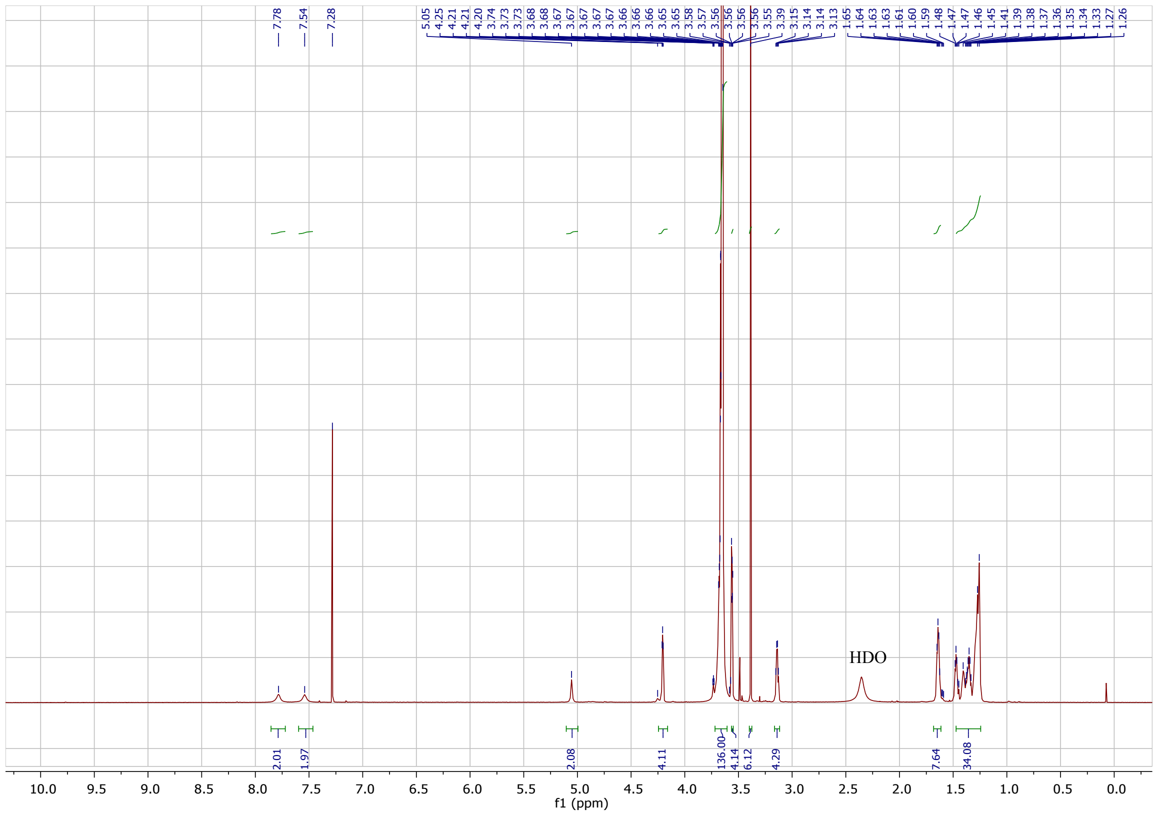


Figure S1. ^{1}H NMR of compound **1b** in $CDCl_3$.

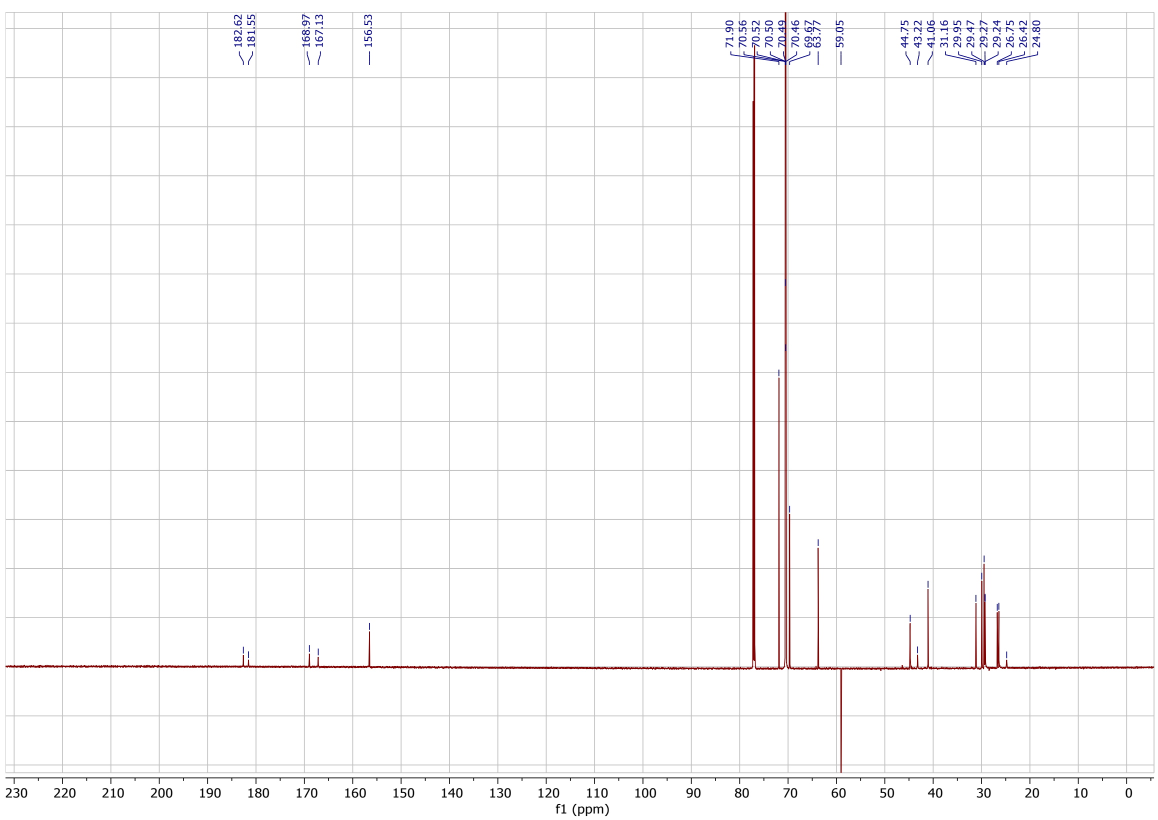


Figure S2. ^{13}C NMR of compound **1b** in $CDCl_3$.

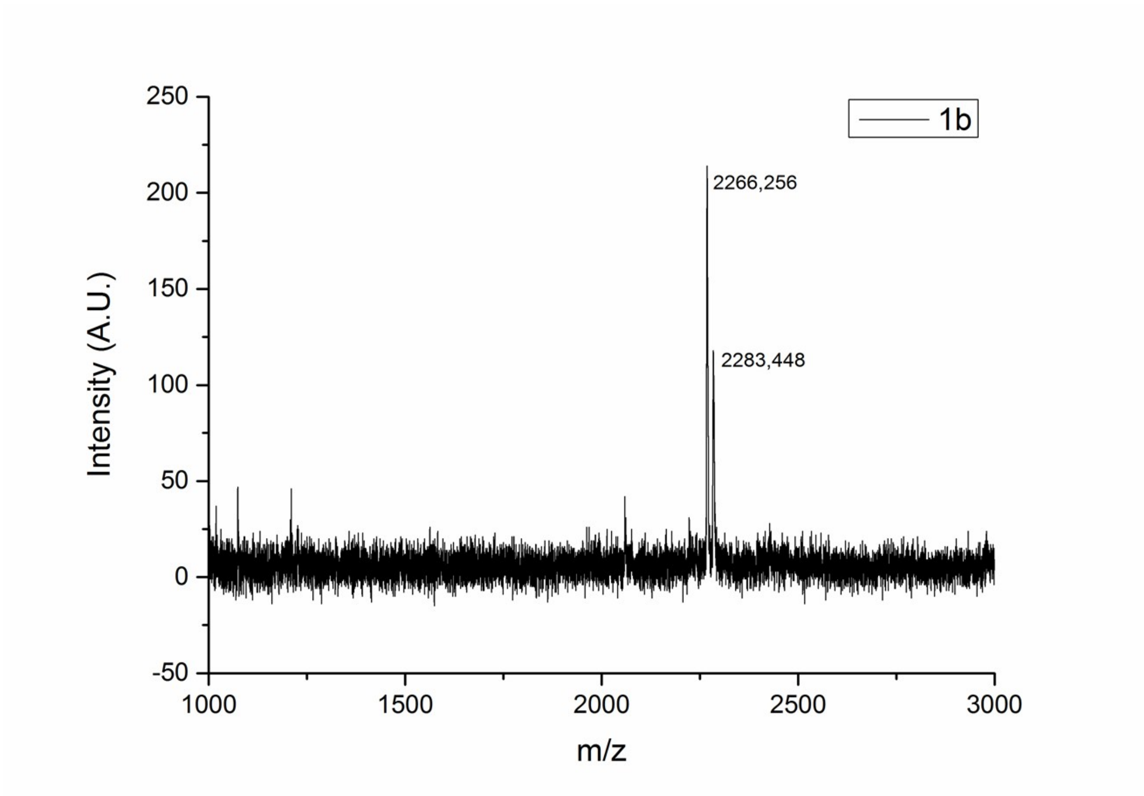


Figure S3. MALDI-TOF-MS spectrum of compound **1b**.

1c: Yield: 38.5 mg, 48.4% ^{1}H-NMR (δ_H[ppm], $CDCl_3$, 850 MHz): 4.22 (m, 4H), 3.75-3.56 (m, 196H), 3.40 (s, 6H), 3.16-3.15 (m, 4H), 1.65-1.64 (m, 8H), 1.50-1.27 (m, 34H).^{13}C-NMR (δ_C[ppm], $CDCl_3$, 212.5 MHz). 182.26, 182.13,168.80, 167.78, 156.53, 71.92, 71.89, 70.54, 70.52, 70.49, 70.44, 70.38, 70.34, 70.31, 69.81, 69.72, 63.66, 59.05, 44.60, 43.27, 41.08, 31.03, 29.86, 29.70, 29.42, 29.31, 29.18, 29.11,26.68, 26.36, 24.86. LC-MS: 6.60 min, m/z: 1431.60 $[M+2H]^{2+}$. MALDI-TOF-MS: *m/z* calc: 2859.75; found: 2883.15 $[M+Na]^{+}$.

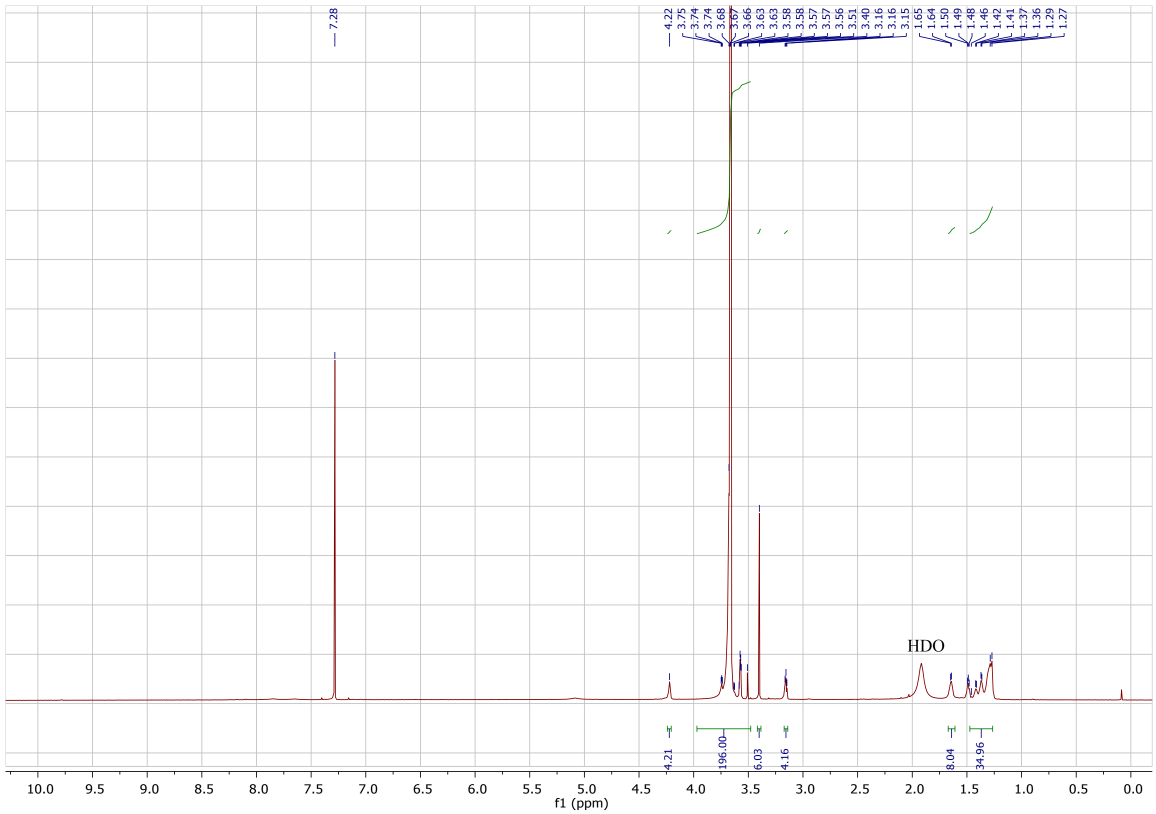


Figure S4. ^{1}H NMR of compound **1c** in $CDCl_3$.

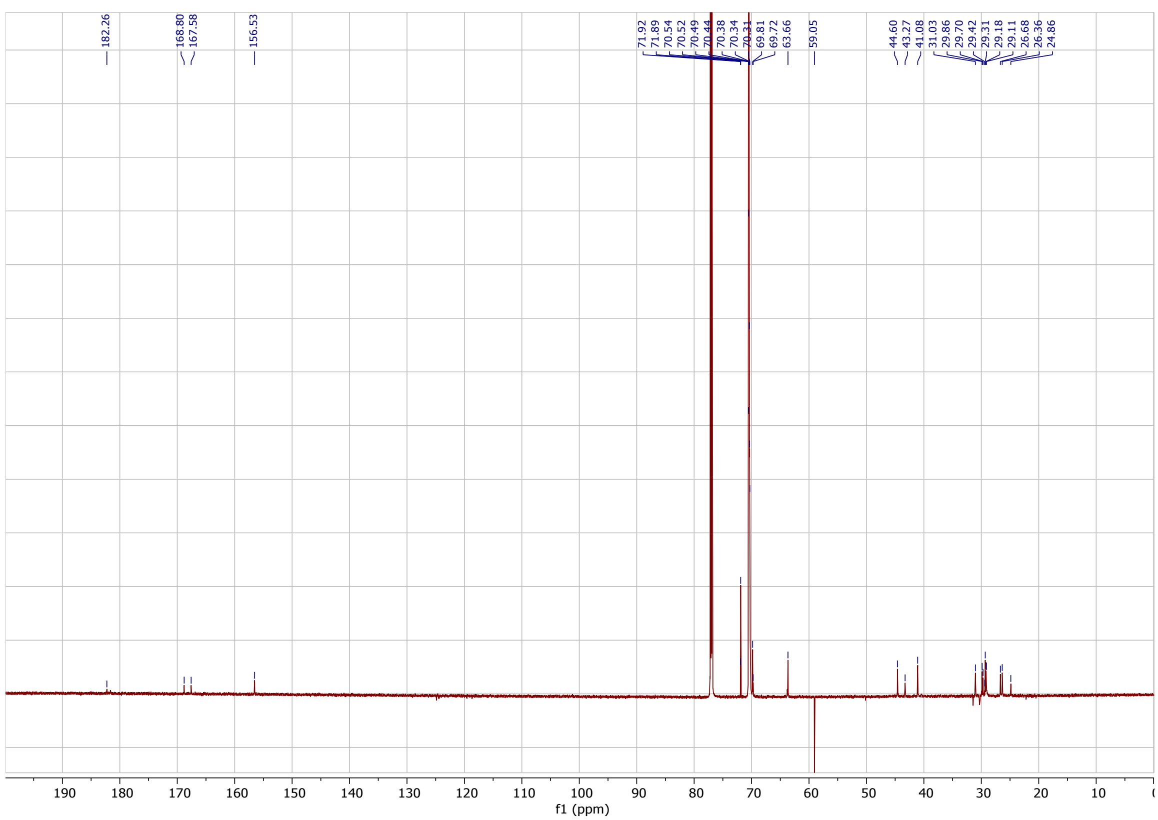


Figure S5. ^{13}C NMR of compound **1c** in $CDCl_3$.

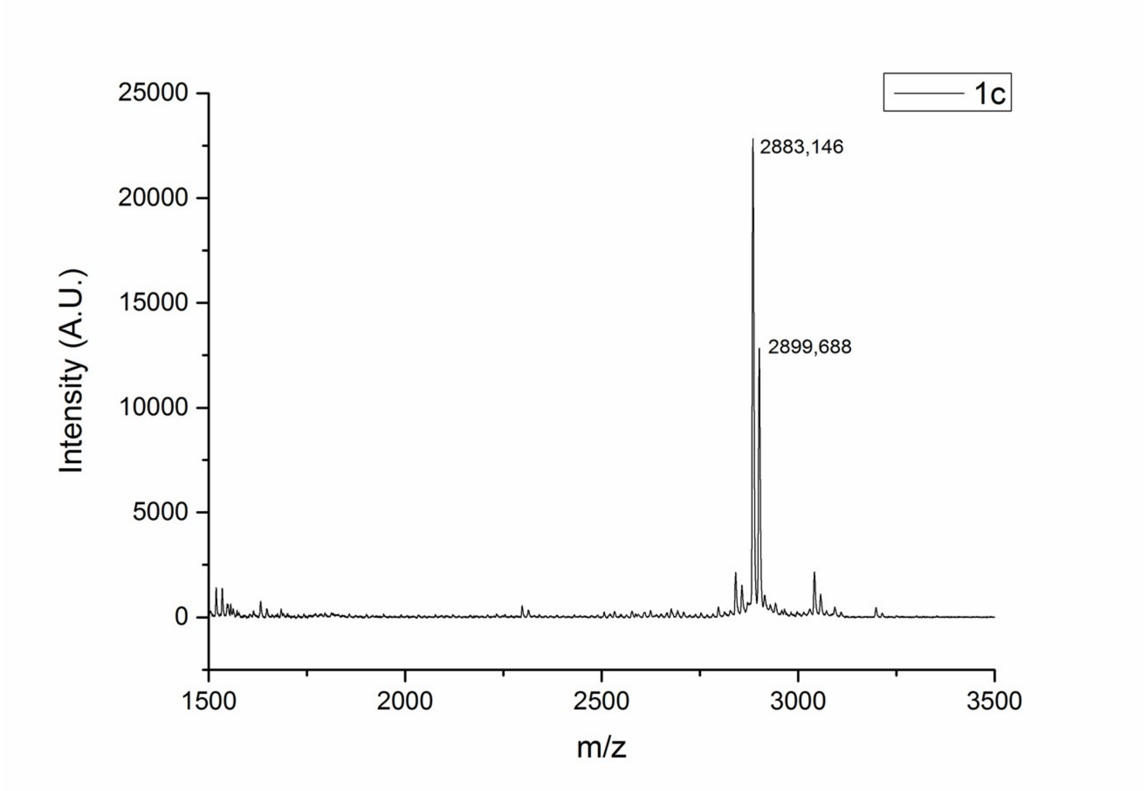


Figure S6. MALDI-TOF-MS spectrum of compound **1c**.

1d: Yield: 59.0 mg, 46.3% ^{1}H-NMR (δ_H[ppm], $CDCl_3$, 850 MHz): 7.88 (br s, 2H), 7.64 (br s, 2H), 5.07 (br s, 1H), 4.21 (m, 4H), 3.74-3.48 (m, 292 H), 3.38 (s, 6H), 3.14-3.13 (m, 4H), 2.44 (br s, 8H), 1.63 (m, 8H), 1.47-1.25 (m, 34H). ^{13}C-NMR (δ_C[ppm], $CDCl_3$, 212.5 MHz): 71.90, 70.67, 70.56, 70.52, 70.51, 70.48, 70.45, 70.42, 69.70, 63.72, 59.07, 44.65, 43.21, 41.07, 41.05. LC-MS: 6.91 min, m/z: 1968.33 $[M+2H]^{2+}$,985.80 $[M+4H]^{4+}$. MALDI-TOF-MS: *m/z* calc: 3916.38; found: 3939.67 $[M+Na]^{+}$.

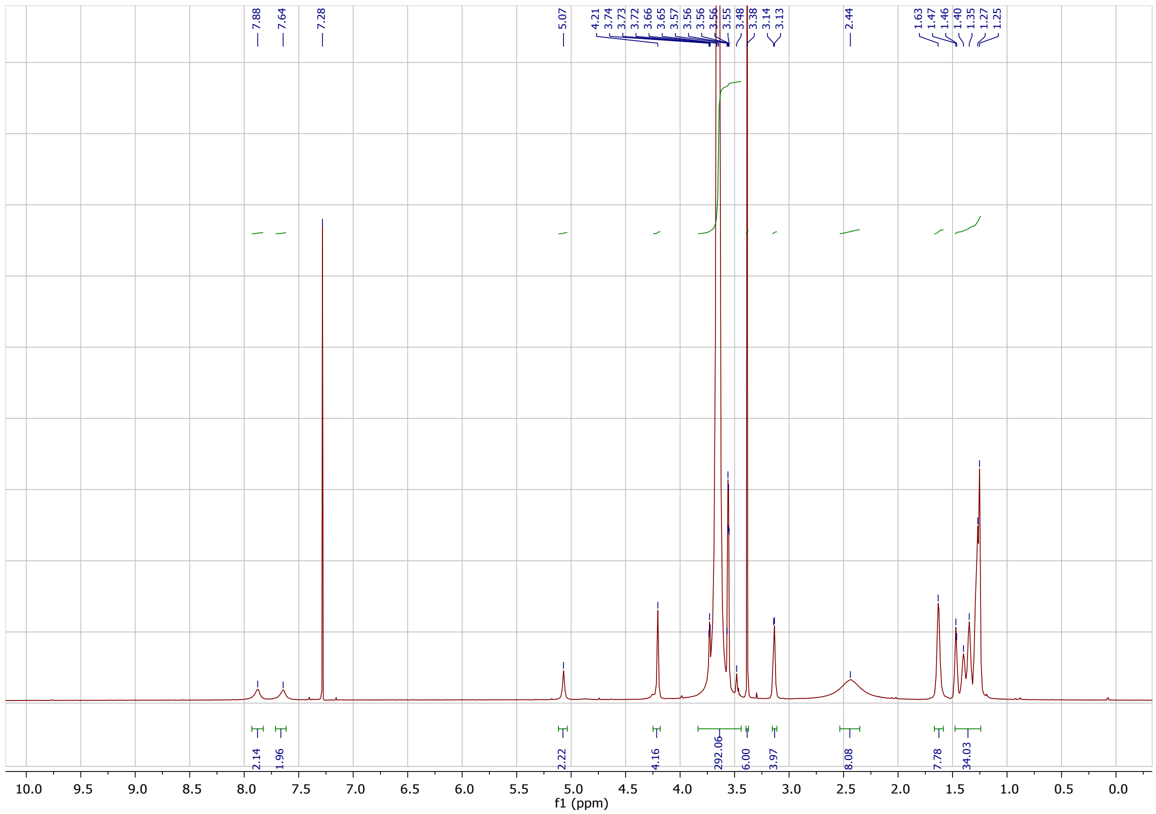


Figure S7. ^{1}H NMR of compound **1d** in $CDCl_3$.

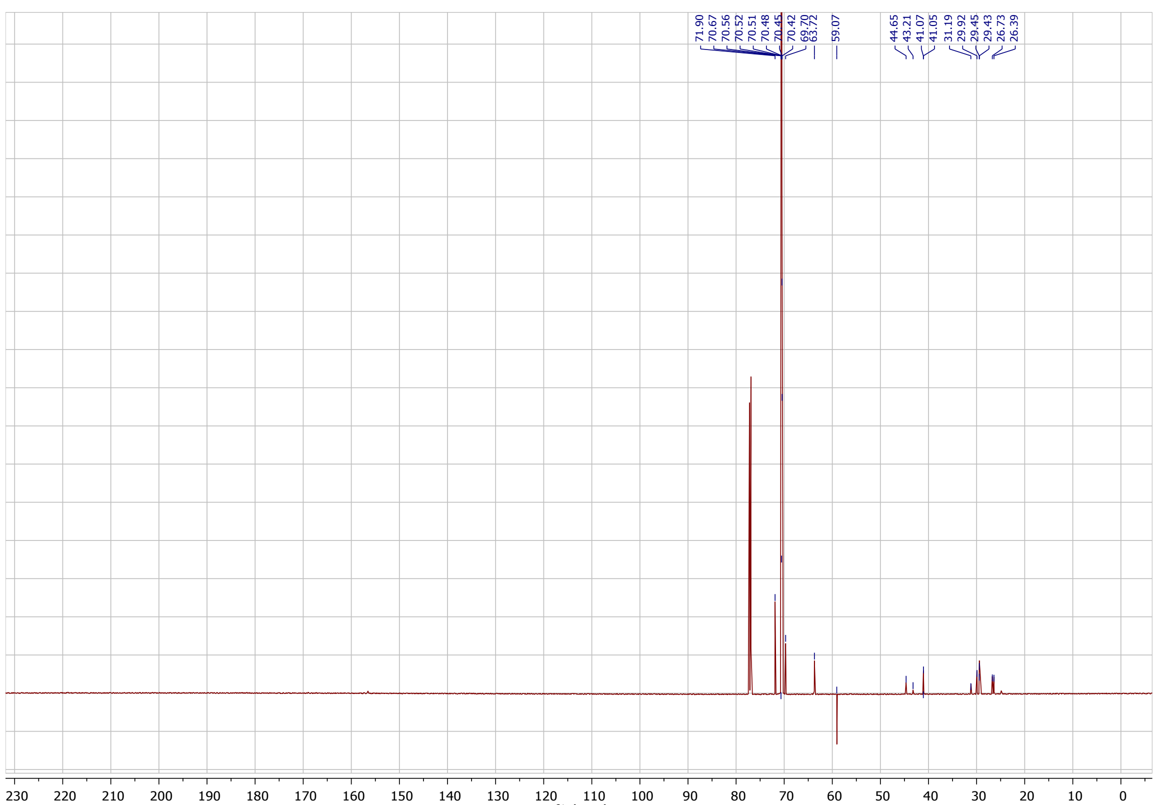


Figure S8. ^{13}C NMR of compound **1d** in $CDCl_3$.

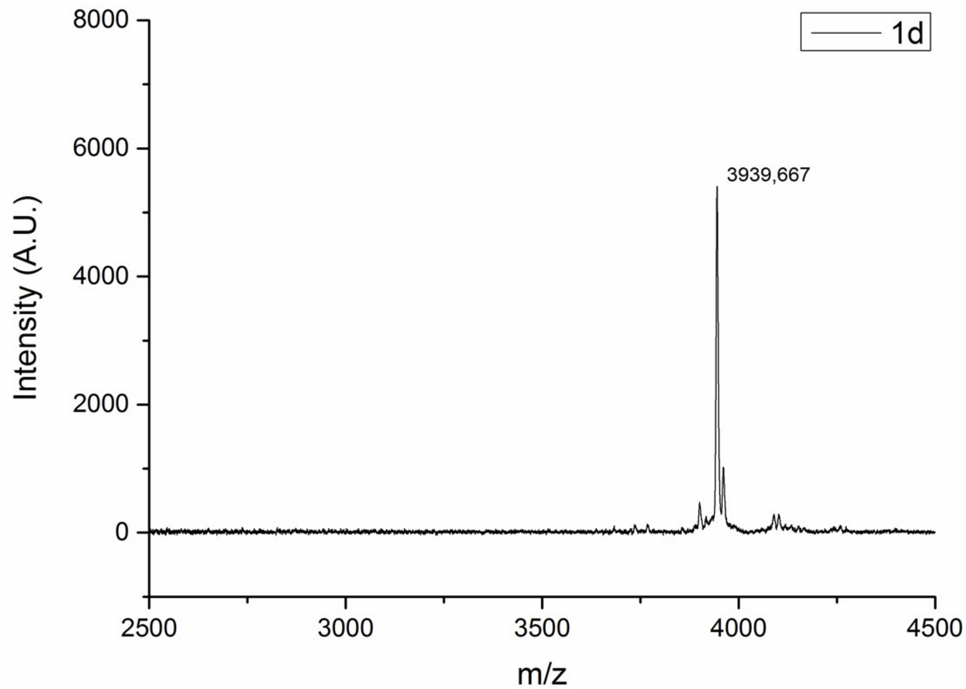


Figure S9. MALDI-TOF-MS spectrum of compound **1d**.

1e: Yield: 76.2 mg, 42.9% ^{1}H-NMR (δ_H[ppm], $CDCl_3$, 400 MHz): 4.24-4.22 (m, 4H), 3.71-3.66 (m, 52H), 3.58-3.57 (t, 4H), 3.40 (s, 6H), 3.19-3.15 (m, 4H), 2.98-2.86 (m, 6H), 1.71-1.63 (m, 6H), 1.51-1.27 (m, 34H).^{13}C-NMR (δ_C[ppm], $CDCl_3$, 100 MHz). 181.61, 168.71, 157.55, 72.90, 72.79, 71.53, 71.44, 70.74, 64.68, 60.04, 45.87, 42.08, 31.35, 30.90, 30.81, 30.39, 30.23, 30.15, 30.04, 27.73, 27.41, 27.73 LC-MS: 7.64 min, m/z: 1363.60 $[M+H]^+$. MALDI-TOF-MS: *m/z* calc: 1362.86; found: 1385.97 $[M+Na]^+$, 1401.94 $[M+K]^+$.

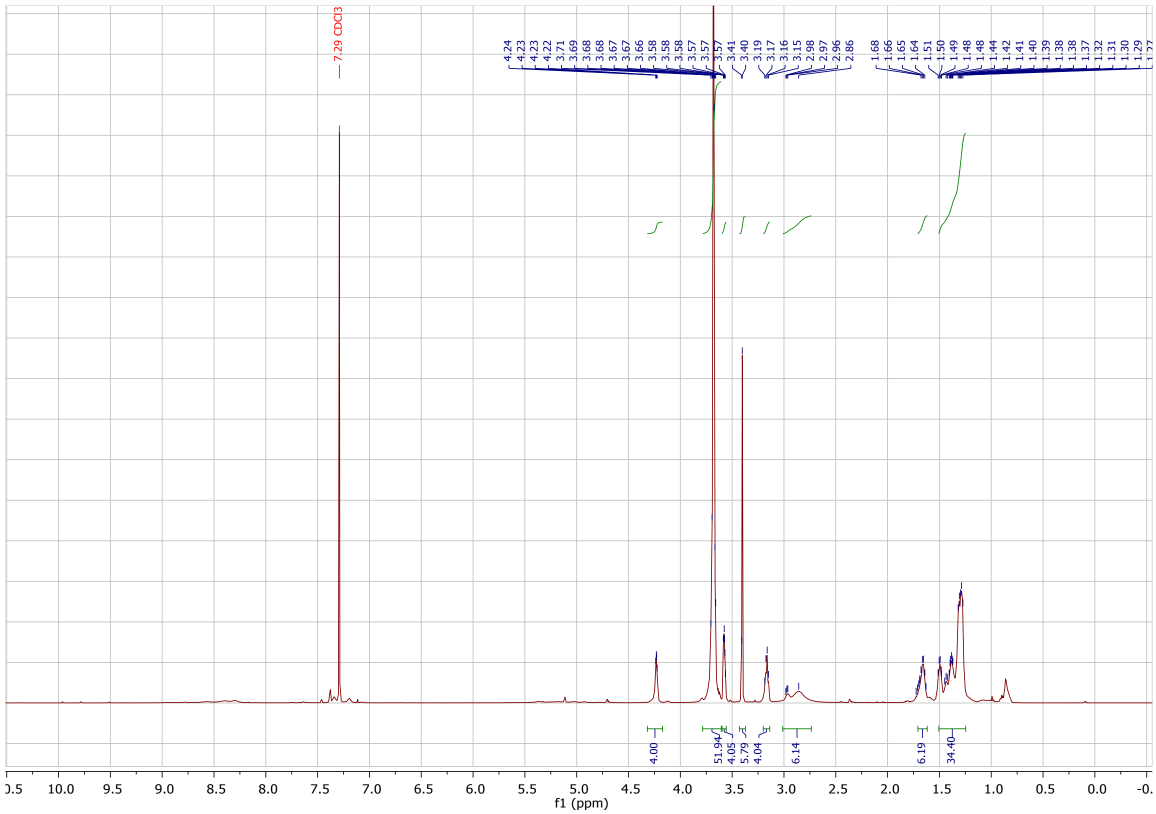


Figure S10. ^{1}H NMR of compound **1e** in $CDCl_3$.

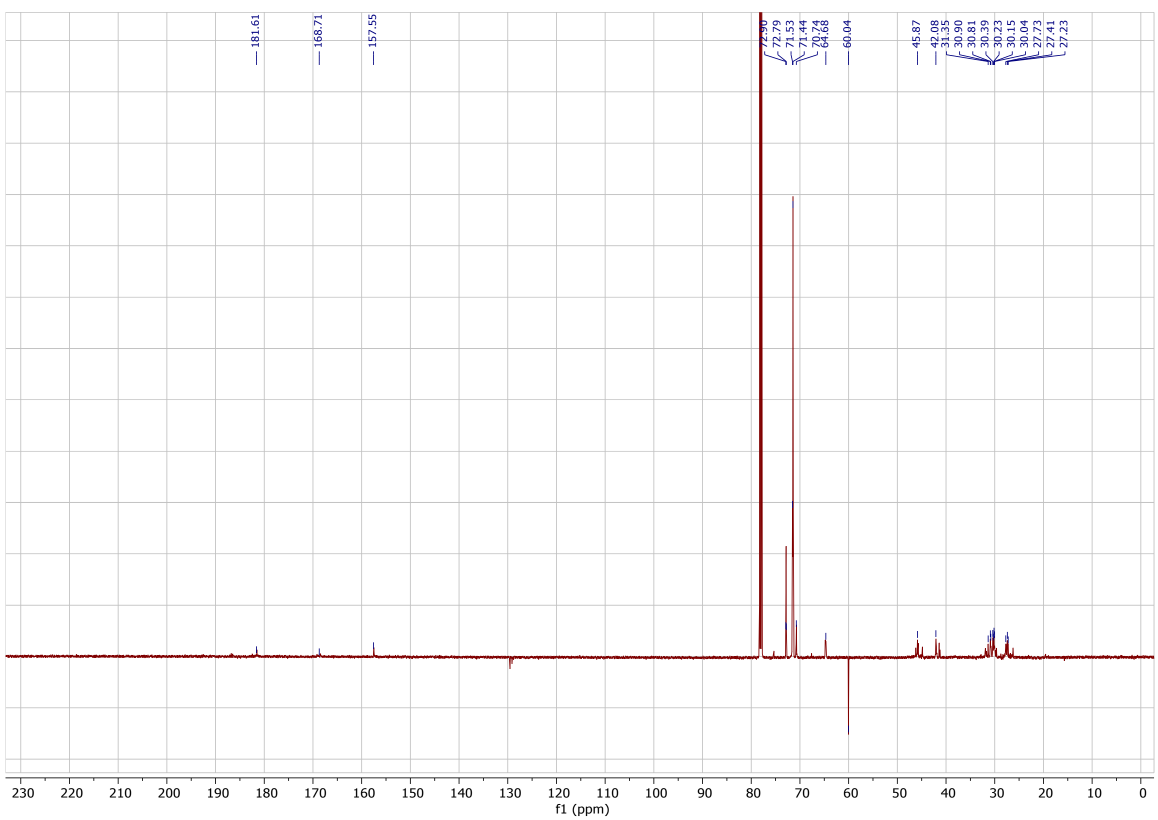


Figure S11. ^{13}C NMR of compound **1e** in $CDCl_3$.

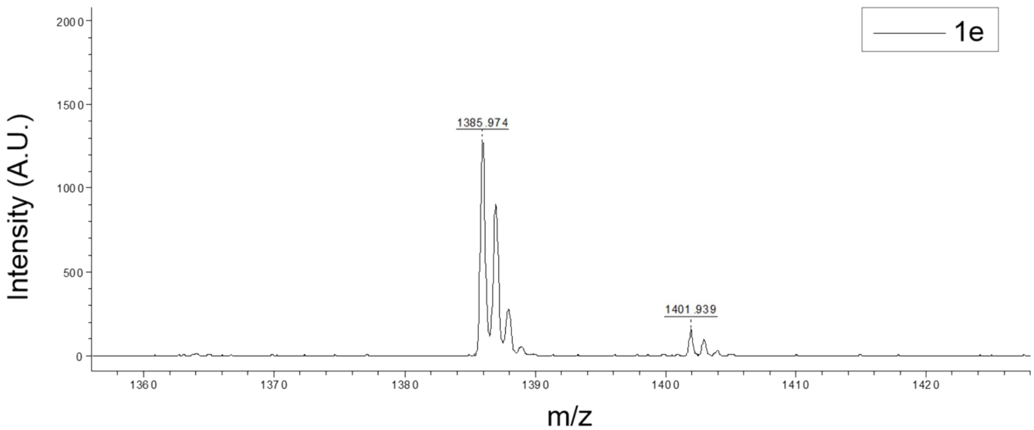


Figure S12. MALDI-TOF-MS spectrum of compound **1e**.

Synthesis of 2a-d

Compound **7** (**a:** 118 mg, 0.16 mmol; **b:** 200 mg, 0.25 mmol; **c:** 40 mg, 0.05 mmol; **d:** 106 mg, 0.11 mmol) was dissolved in 10 mL chloroform with a few drops of DIPEA. 1,7-heptanediamine (**a:** 11.8 µL, 0.078 mmol; **b:** 18.9 µL, 0.125 mmol; **c:** 3.8 µL, 0.025 mmol; **d:** 8.9 µL, 0.059 mmol) was added to the mixture and refluxed overnight. If necessary, an additional amount of 1,7-heptanediamine (up to a maximum of 2 equivalents) was added until the starting material **7** disappeared. The product was purified by flash column chromatography using a gradient of 10-90% CH_3CN/H_2O over 30-45 minutes on a C18 silica column. The product was concentrated by evaporation and lyophilized overnight to obtain a white solid.

2a: Yield: 66.0 mg, 56.6% ^{1}H-NMR (δ_H[ppm], $CDCl_3$, 850 MHz): 7.58 (s, 1H), 7.32 (s, 1H), 6.14 (s, 1H) 4.21-4.17 (m, 4H), 3.82-3.53 (m, 92H), 3.41 (s, 4H), 3.37 (s, 6H), 1.66-1.66 (m, 4H), 1.44-1.40 (m, 6H). ^{13}C-NMR (δ_C[ppm], $CDCl_3$, 212.5 MHz): 182.87, 182.05, 168.80, 167.67, 156.78, 71.91, 71.89, 70.61, 70.60, 70.58, 70.55, 70.52, 70.50, 70.48, 70.44, 70.43, 70.40, 70.38, 69.70, 69.39, 68.93, 68.86, 67.03, 66.81, 63.81, 59.05, 43.79, 43.06, 42.14, 29.81, 24.43. LC-MS: 4.56 min, m/z: 1491.17 $[M+H]^+$. MALDI-TOF-MS: *m/z* calc: 1490.82; found: 1513.927 $[M+Na]^+$.

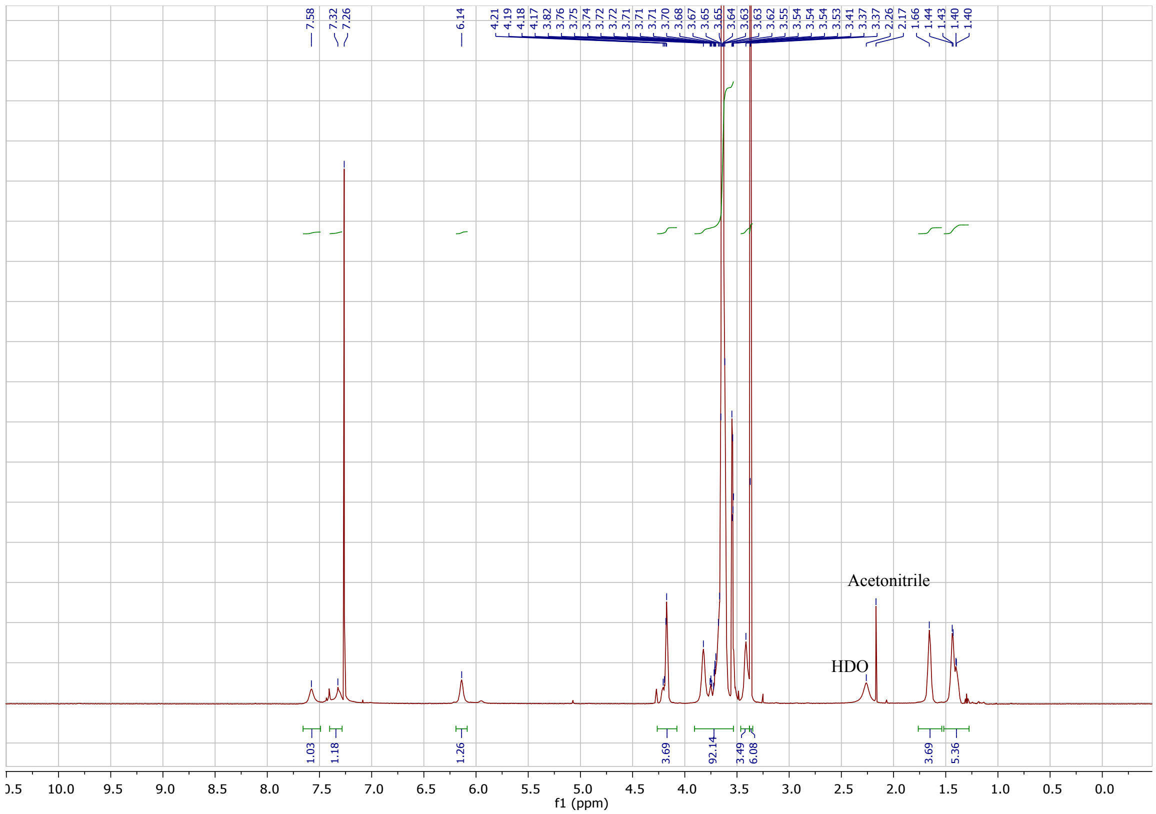


Figure S13. ^{1}H NMR of compound **2a** in $CDCl_3$.

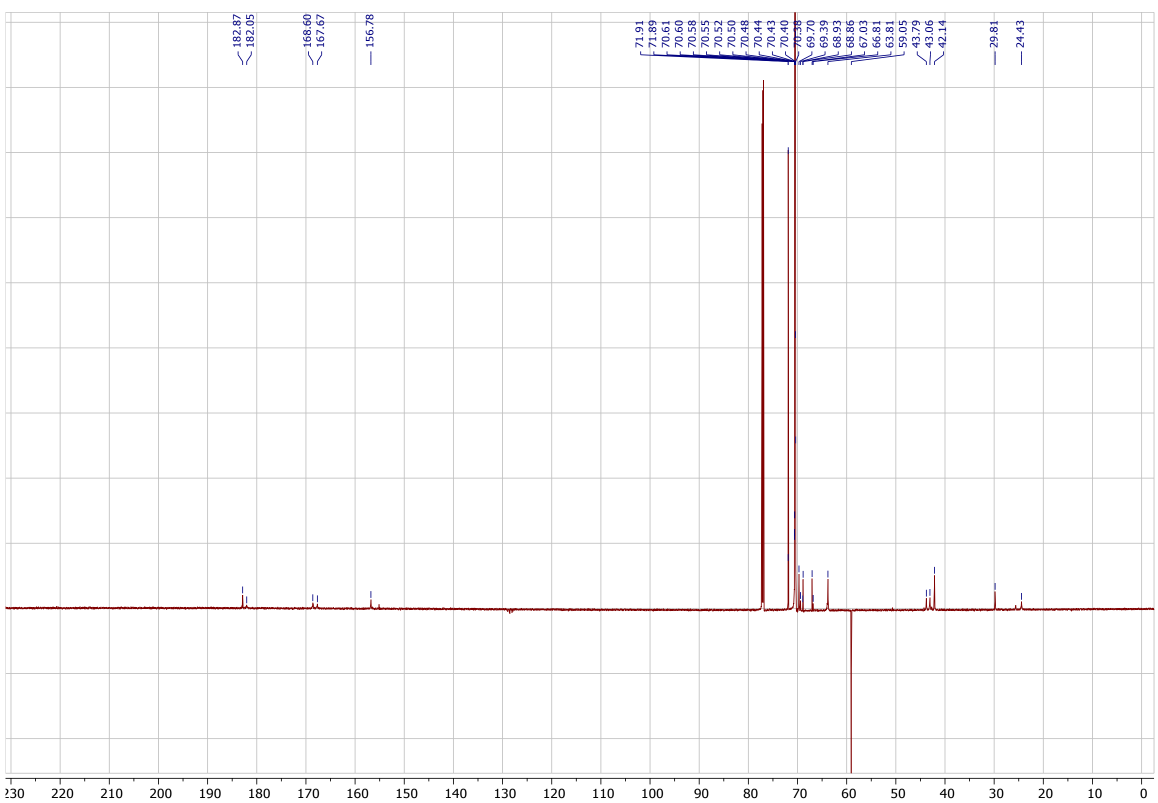


Figure S14. ^{13}C NMR of compound **2a** in $CDCl_3$.

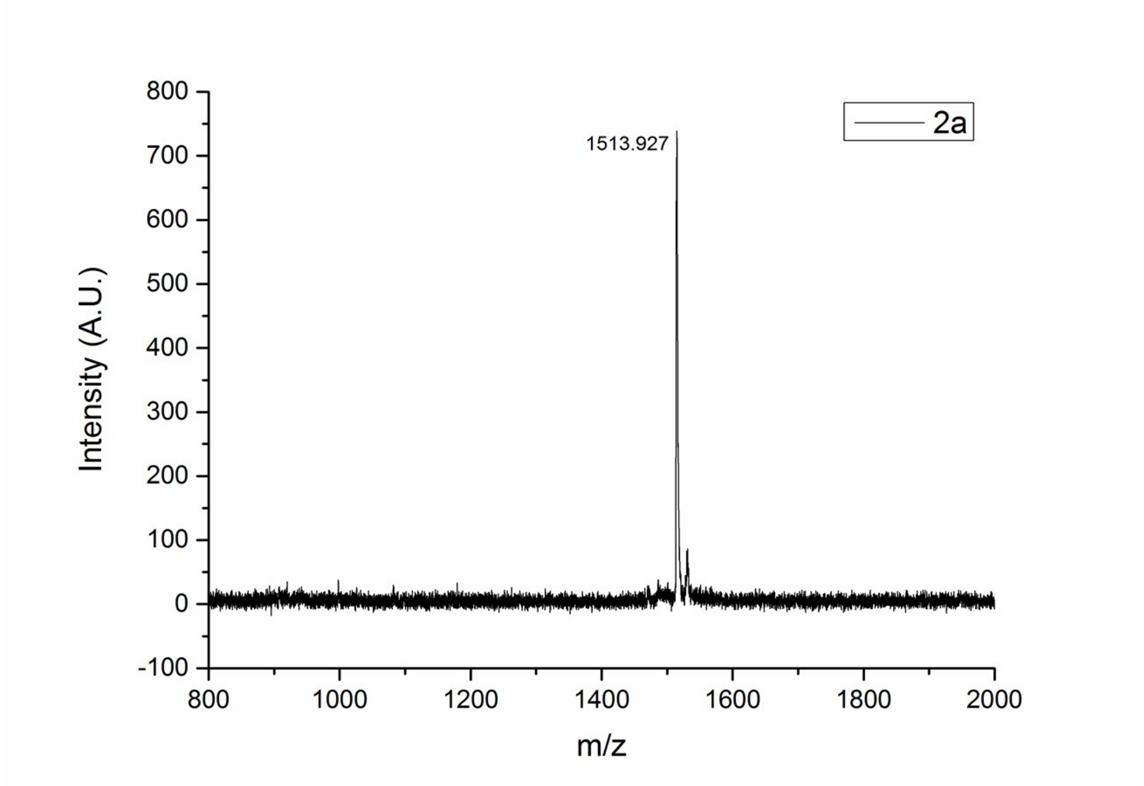


Figure S15. MALDI-TOF-MS spectrum of compound **2a**.

2b: Yield: 90.1 mg, 45.5% ^{1}H-NMR (δ_H[ppm], $CDCl_3$, 850 MHz): 8.45-8.35 (m, 2H), 5.47-5.21 (m, 2H), 4.27-4.21 (m, 4H), 3.69-3.66 (m, 84H), 3.58-3.56 (t, 4H), 3.40 (s, 6H), 3.21-3.15 (m, 4H), 2.98-2.87 (m, 4H), 1.74-1.63 (m, 8H), 1.56-1.27 (m, 18H). ^{13}C-NMR (δ_C[ppm], $CDCl_3$, 212.5 MHz): 192.44, 186.56, 181.92, 169.17, 157.75, 72.91, 72.87, 71.57, 71.56, 71.53, 71.50, 71.47, 71.45, 71.43, 71.41, 71.39, 71.37, 71.34, 71.31, 70.76, 70.68, 64.89, 64.68, 60.11, 45.38, 44.77, 41.83, 31.69, 30.75, 30.61, 27.05, 26.83. LC-MS: 5.85 min, m/z: 1604.67 $[M+H]^+$. MALDI-TOF-MS: *m/z* calc: 1602.95; found: 1603.97 $[M+H]^+$, 1625.93 $[M+Na]^+$, 1641.87 $[M+K]^+$.

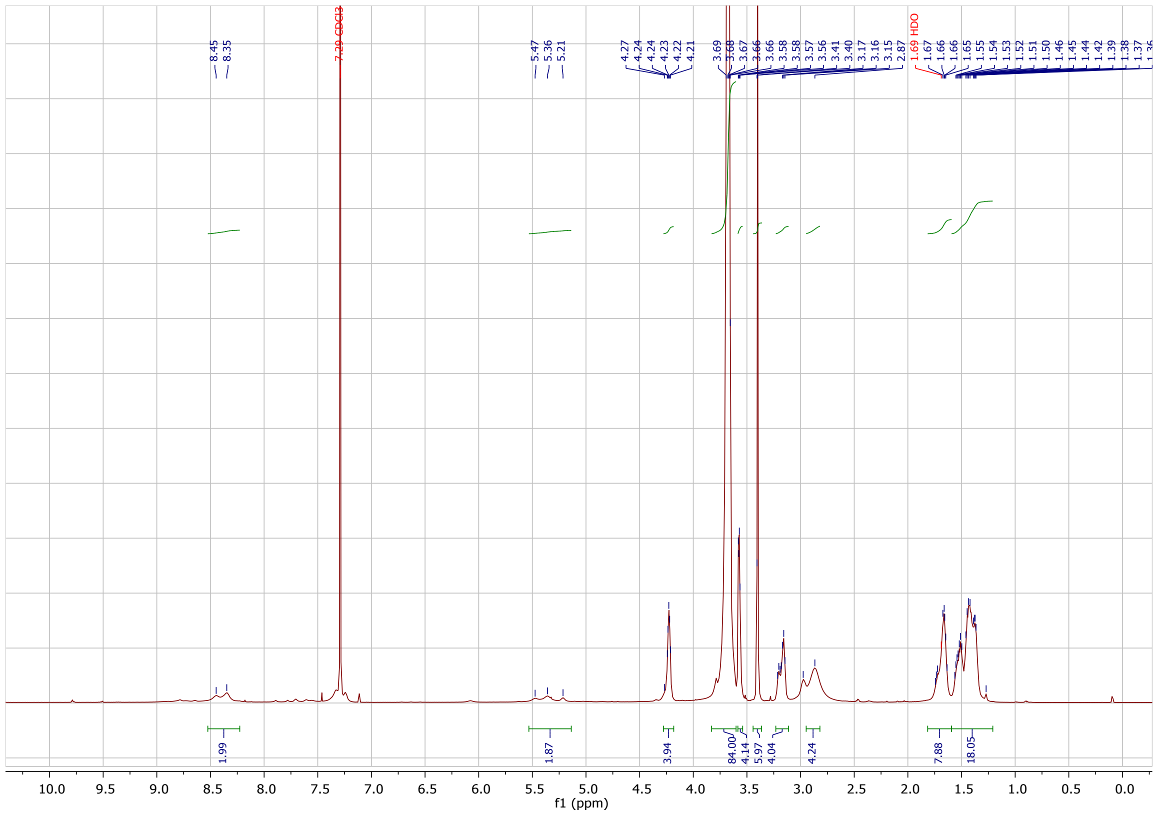


Figure S16. ^{1}H NMR of compound **2b** in $CDCl_3$.

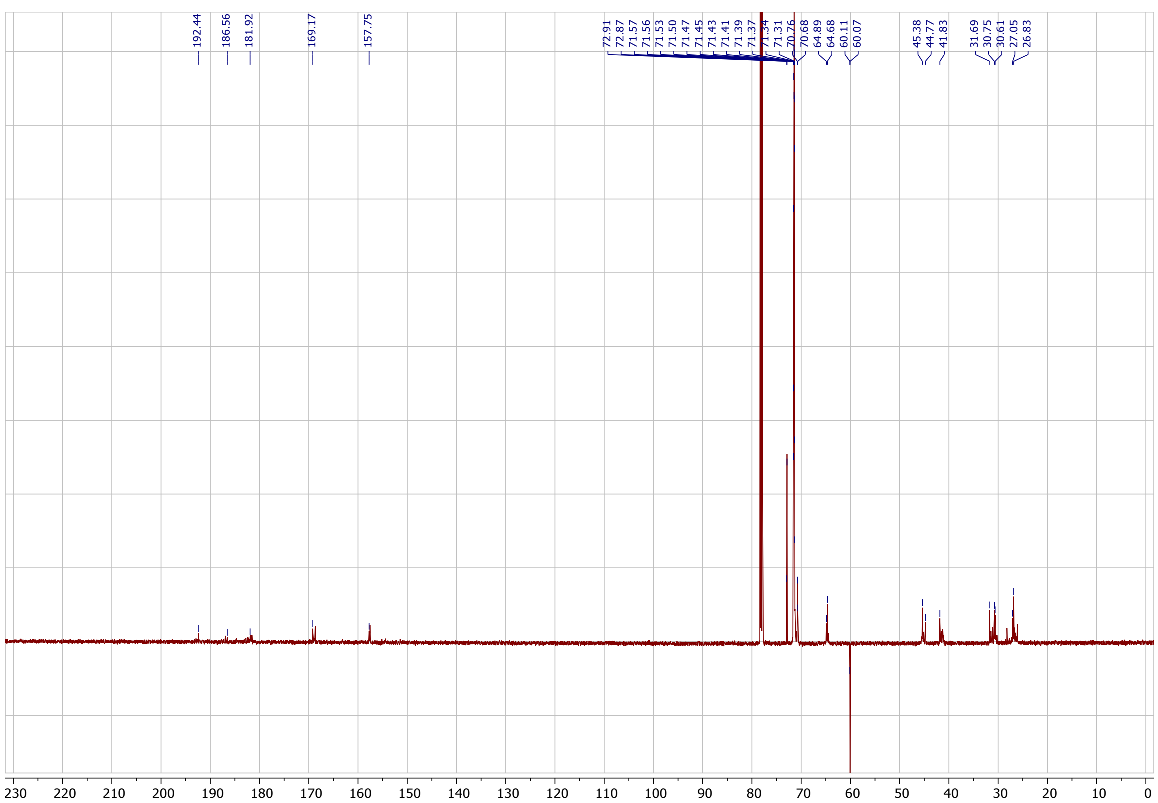


Figure S17. ^{13}C NMR of compound **2b** in $CDCl_3$.

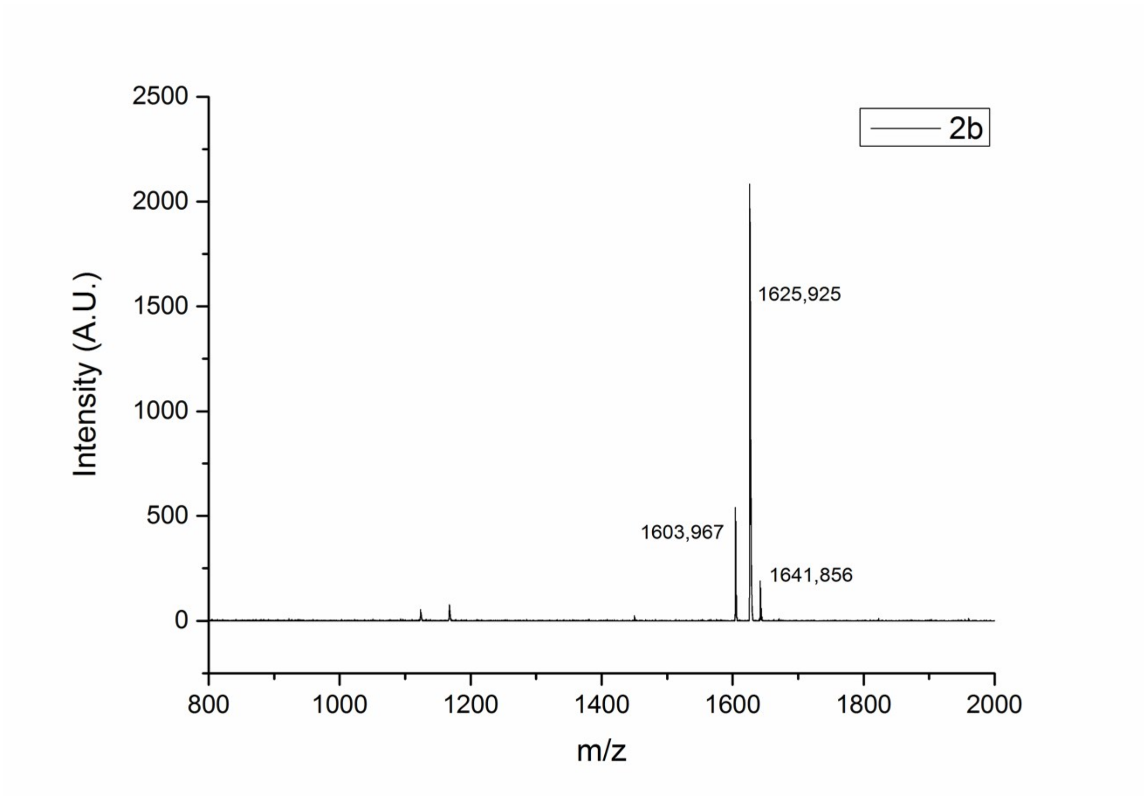


Figure S18. MALDI-TOF-MS spectrum of compound **2b**.

2c: Yield: 19.4 mg, 48% ^{1}H-NMR (δ_H[ppm], $CDCl_3$, 400 MHz): 7.76 (s, 2H), 7.52 (s, 2H), 5.07-5.03 (m, 2H), 4.22-4.18 (m, 4H), 3.71-3.53 (m, 84H), 3.54-3.53 (m, 4H), 3.36 (s, 6H), 3.12-3.10 (m, 4H), 2.23 (s, 4H), 1.62-1.58 (m, 8H), 1.45-1.27 (m, 26H). ^{13}C-NMR (δ_C[ppm], $CDCl_3$, 100 MHz): 181.50, 169.10, 168.55, 157.48, 72.92, 72.88, 72.86, 72.83, 71.57, 71.54, 71.52, 71.51, 71.48, 71.46, 71.44, 71.42, 71.41, 71.39, 71.37, 71.34, 70.75, 70.71, 64.83, 64.70, 60.06, 45.77, 45.60, 44.85, 42.03, 41.34, 31.86, 30.85, 30.77, 30.74, 30.07, 30.01, 29.78, 27.64, 27.31, 26.15. LC-MS: 6.38 min, m/z: 1659.73 $[M+H]^+$. MALDI-TOF-MS: *m/z* calc: 1659.01; found:1660.14 $[M+H]^+$, 1682.07 $[M+Na]^+$, 1698.02 $[M+K]^+$.

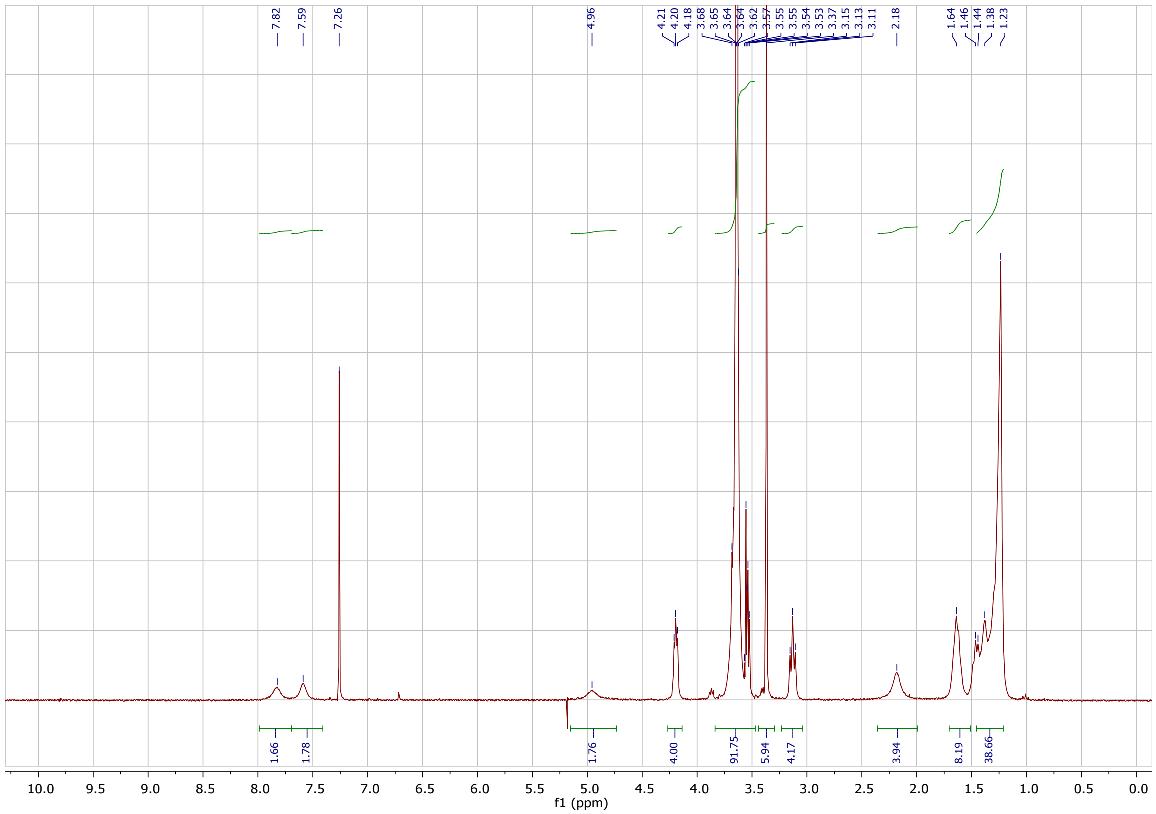


Figure S19. ^{1}H NMR of compound **2c** in $CDCl_3$.

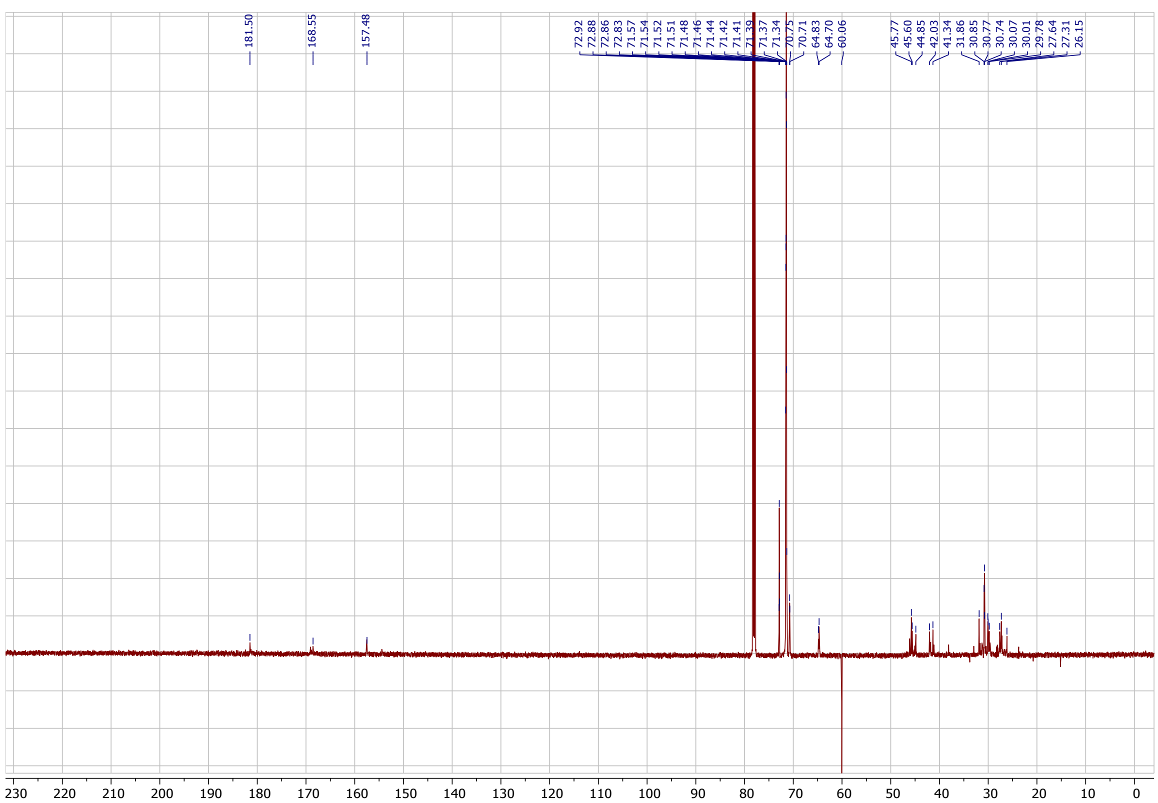


Figure S20. ^{13}C NMR of compound **2c** in $CDCl_3$.

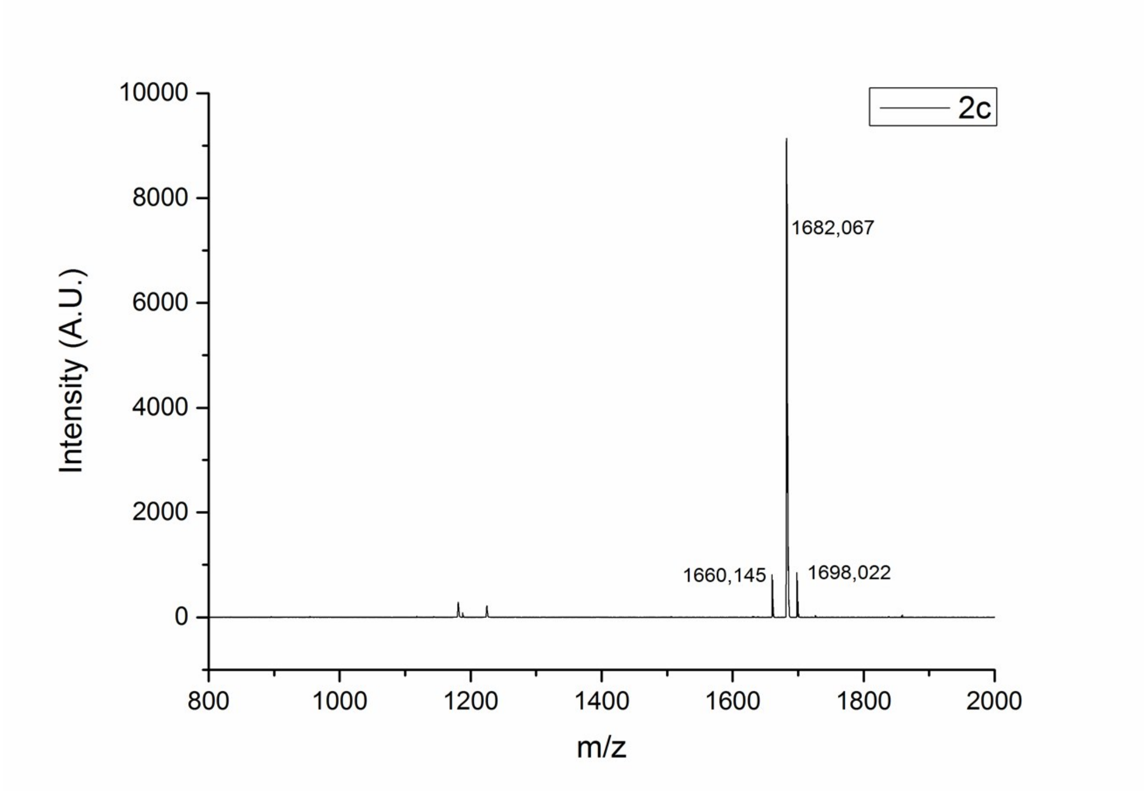


Figure S21. MALDI-TOF-MS spectrum of compound **2c**.

2d: Yield: 53 mg, 50.5% ^{1}H-NMR (δ_H[ppm], $CDCl_3$, 400 MHz): 7.82 (s, 2H), 7.59 (s, 2H), 4.96 (s, 2H), 4.21-4.18 (m, 4H), 3.68-3.53 (m, 92H), 3.37 (s, 6H), 3.15-3.11 (m, 4H), 2.18 (s, 4H), 1.64 (m, 8H), 1.46-1.23 (m, 38H). ^{13}C-NMR (δ_C[ppm], $CDCl_3$, 100 MHz): 182.21, 168.91, 167.05, 156.50, 71.91, 70.57, 70.53, 70.49, 69.66, 63.81, 59.03, 44.84, 43.11, 41.09, 31.19, 29.97, 29.57, 29.29, 26.77, 26.47, 24.66. LC-MS: 7.10 min, m/z: 1773.11 $[M+H]^+$. MALDI-TOF-MS: *m/z* calc: 1771.13; found: 1794.886 $[M+Na]^+$.

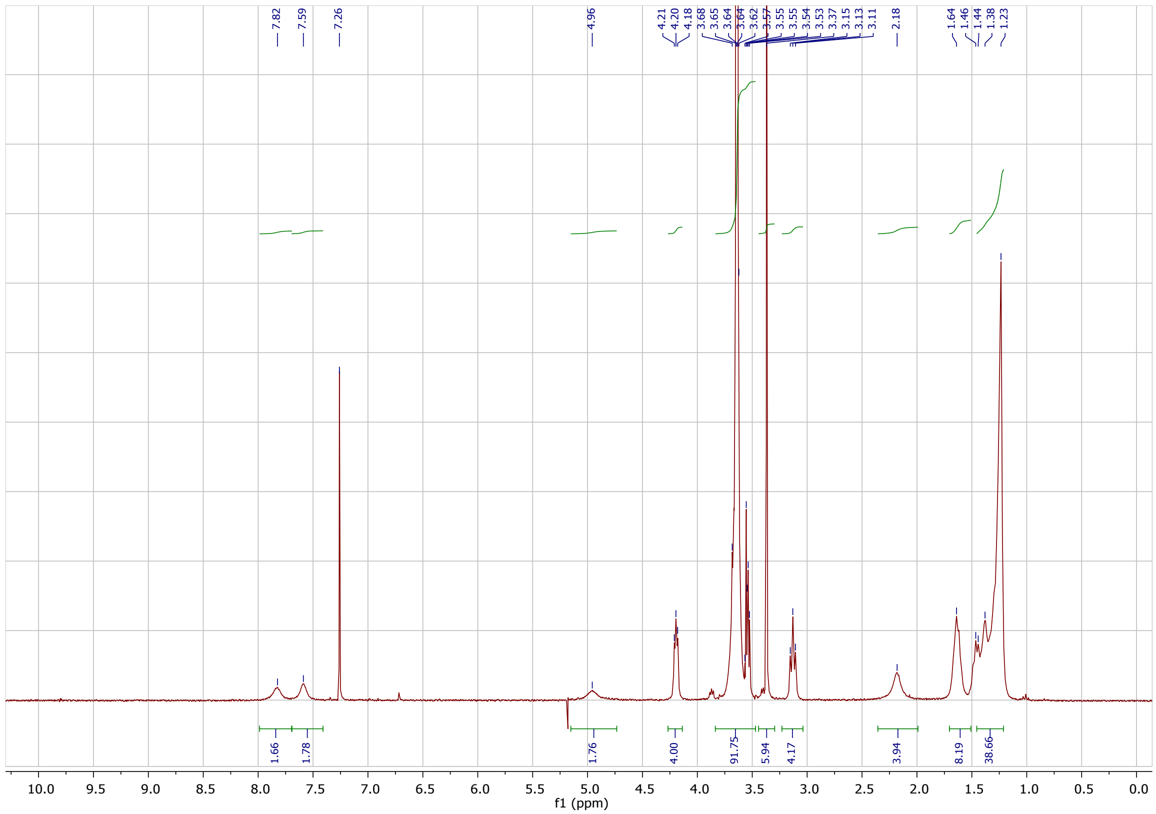


Figure S22. ^{1}H NMR of compound **2d** in $CDCl_3$.

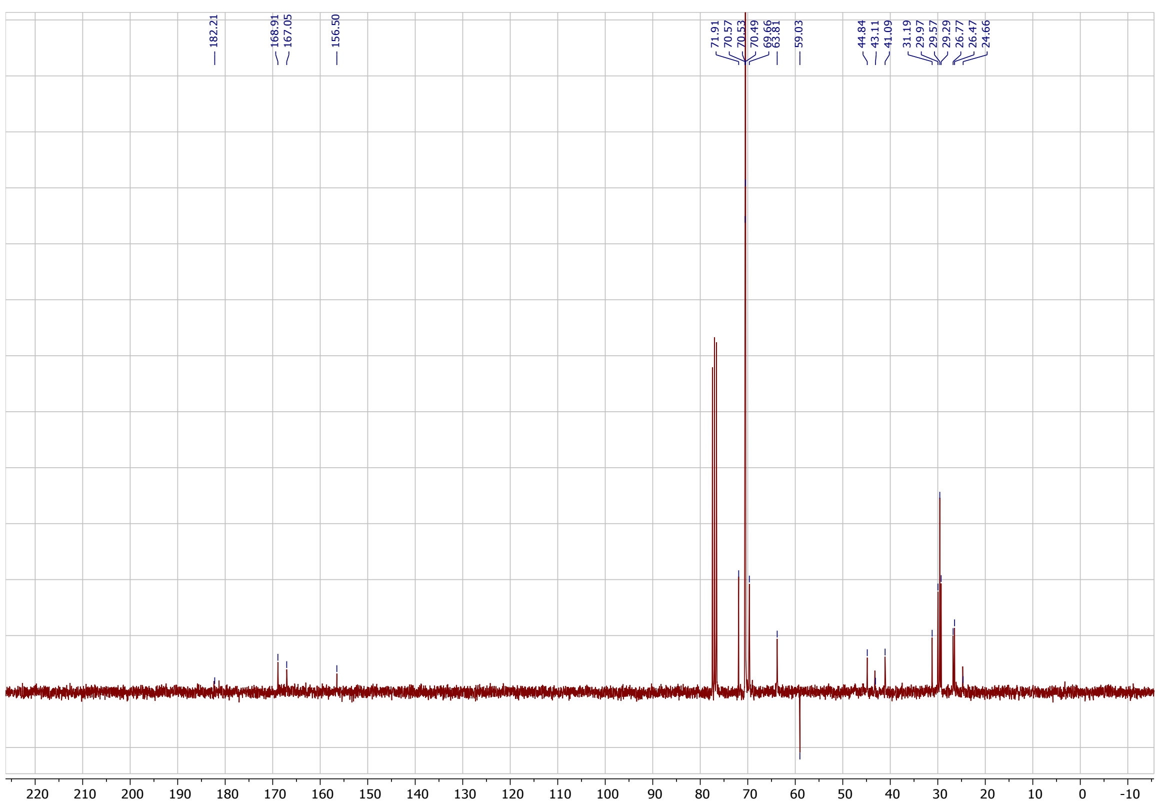


Figure S23. ^{13}C NMR of compound **2d** in $CDCl_3$.

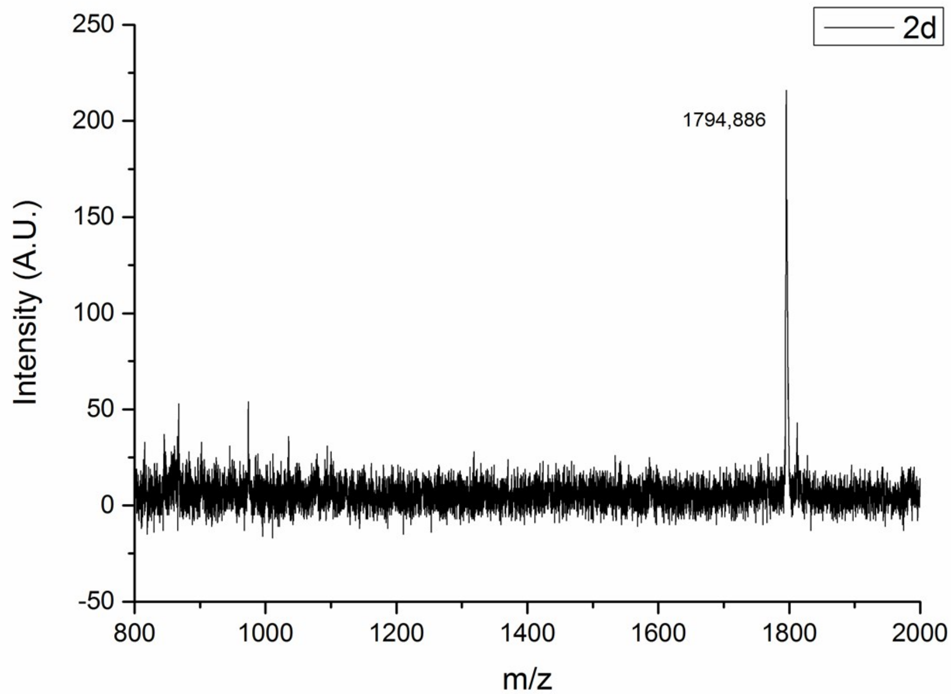


Figure S24. MALDI-TOF-MS spectrum of compound **2d**.

3. Cryogenic transmission electron microscopy (cryo-TEM)

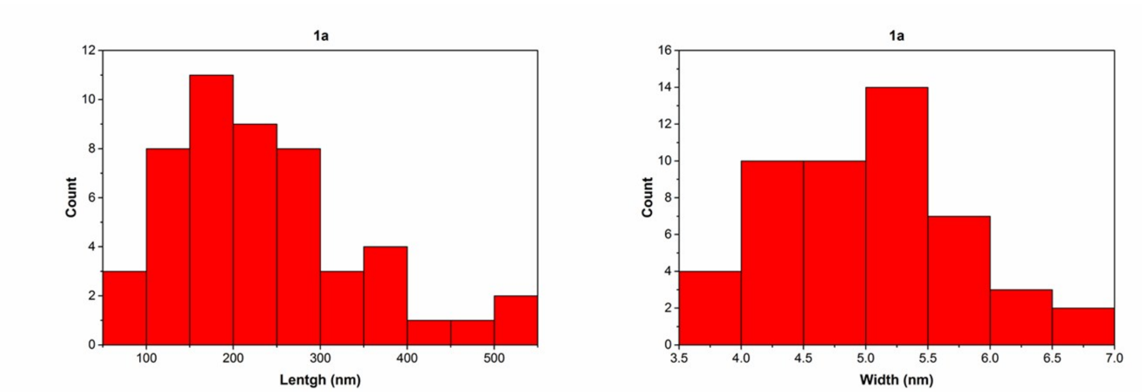


Figure S25. Histograms of length (234 ± 108 nm) and width (5 ± 1 nm) distributions measured for **1a**.

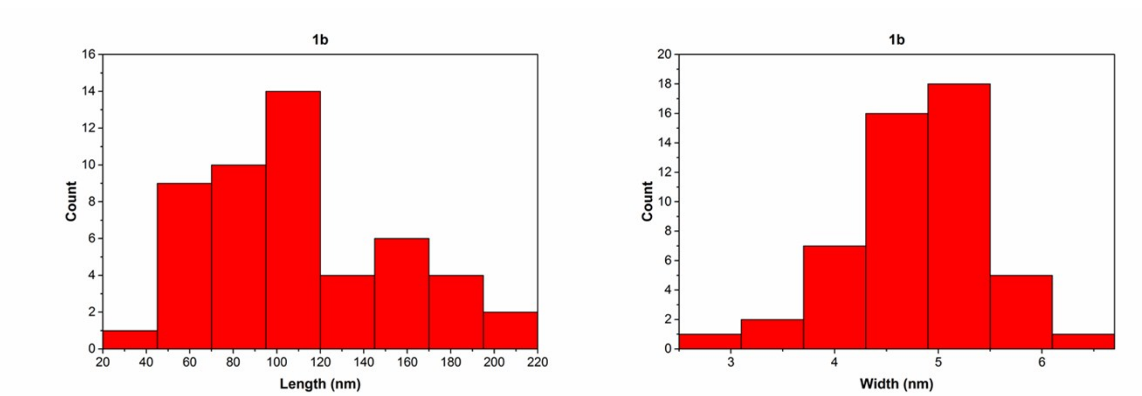


Figure S26. Histograms of length (109± 44 nm) and width (5 ± 1 nm) distributions measured for **1b**.

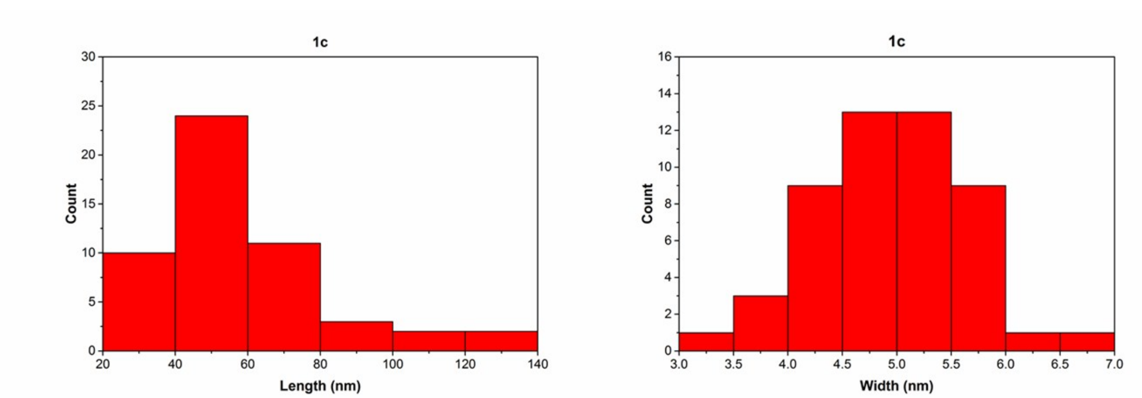


Figure S27. Histograms of length (57 ± 24 nm) and width (5 ± 1 nm) distributions measured for **1c**.

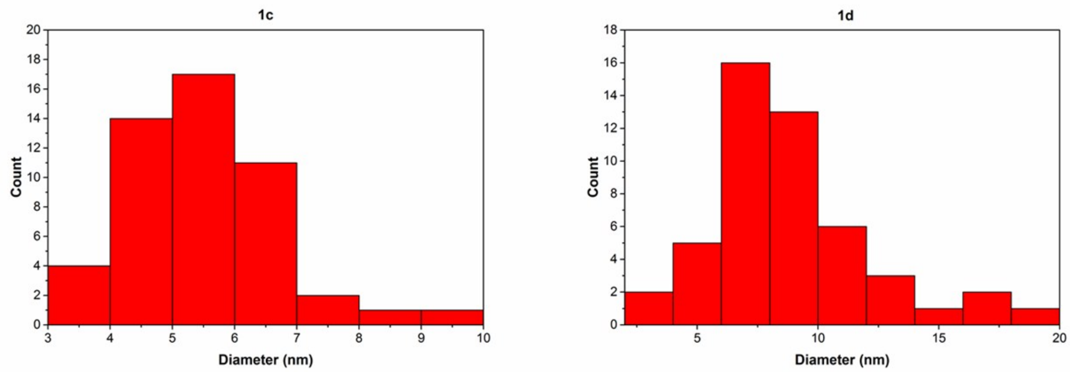


Figure S28. Histograms of diameter distributions measured for spherical aggregates of **1c** (6 ± 1 nm) (*left*) and **1d** (9 ± 4 nm) (*right*).

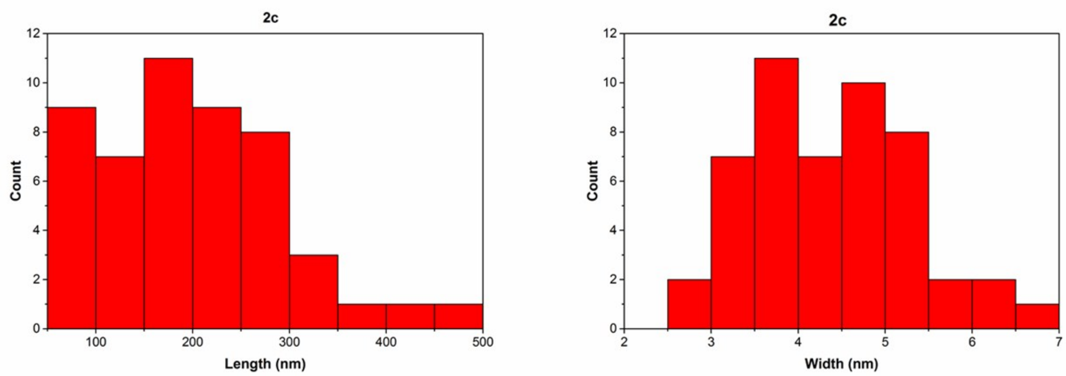


Figure S29. Histograms of length (200 ± 93 nm) and width (4 ± 1 nm) distributions measured for **2c**.

4. Small angle X-ray scattering (SAXS)

The SAXS profiles of the 4 and 5 mg/mL samples are given in **Figure S7** for the molecules above. In the low-*q* regime, the scattering profiles in log-log representation of **1a** and **1b** exhibited a power law slope of -1, which is typical for cylindrical objects. A low-*q* plateau followed by a steep power law decay with a slope of approximately -4 in the high-*q* regime is observed for sample **1d** with the longest oligo(ethylene glycol) chain, which is typical for low aspect ratio aggregates, such as spherical objects. Sample **1c** displays a profile where a moderate slope between 0 and -1 is observed in the low-to-intermediate *q* regime due to the coexistence of fibrillar and spherical aggregates. Upon normalization to 1 mg mL^{-1} (**Figure S6**) the SAXS profiles collected at 4 and 5 mg mL^{-1} superpose for monomers **1a**, **1b** and **1d**. We can safely neglect interspecies interactions and model these data sets exclusively using a form factor model. However, the 4 and 5 mg mL^{-1} spectra of **1c** do not superpose, as the coexistence is concentration-dependent, favoring a different equilibrium between fibrillar and spherical aggregates at 4 versus 5 mg mL^{-1}.

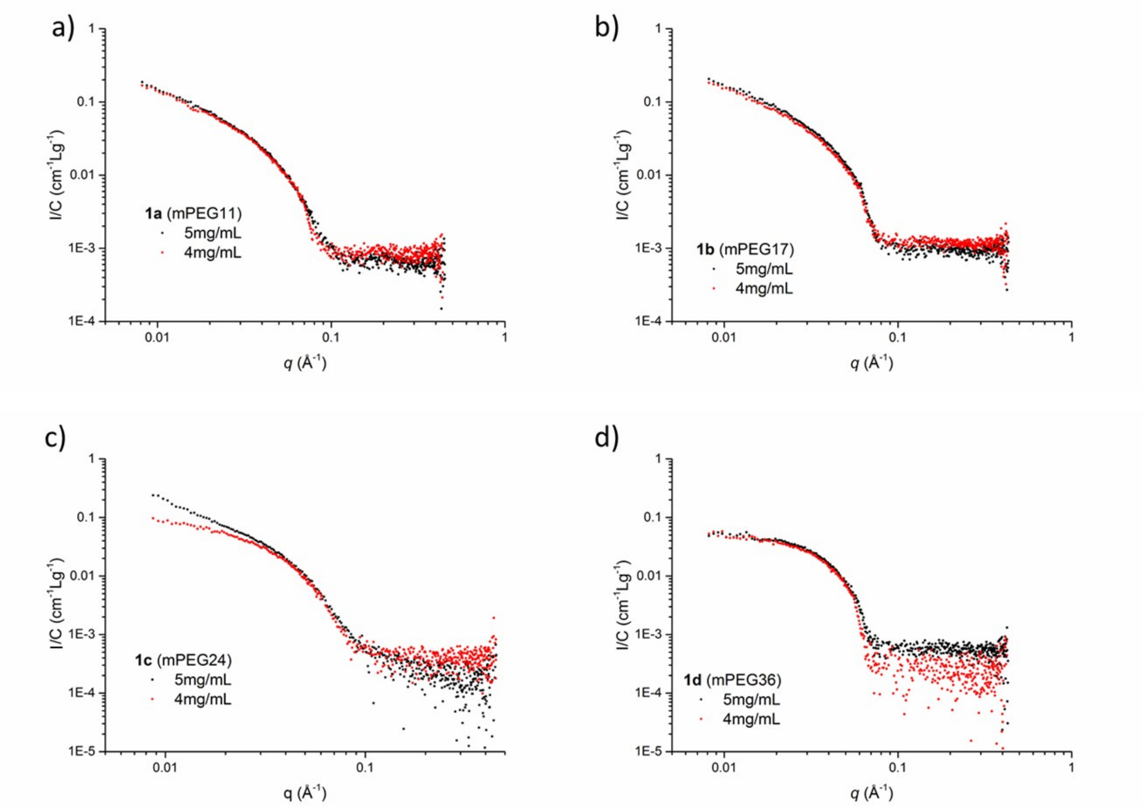


Figure S30. SAXS profiles of squaramide-based supramolecular polymers collected at a concentration of 4 and 5 mg mL^{-1} normalized by weight concentration for a) **1a**, b) **1b**, c) **1c**, and d) **1d** (i.e., the symbols correspond to experimental data (I/c vs. q)).

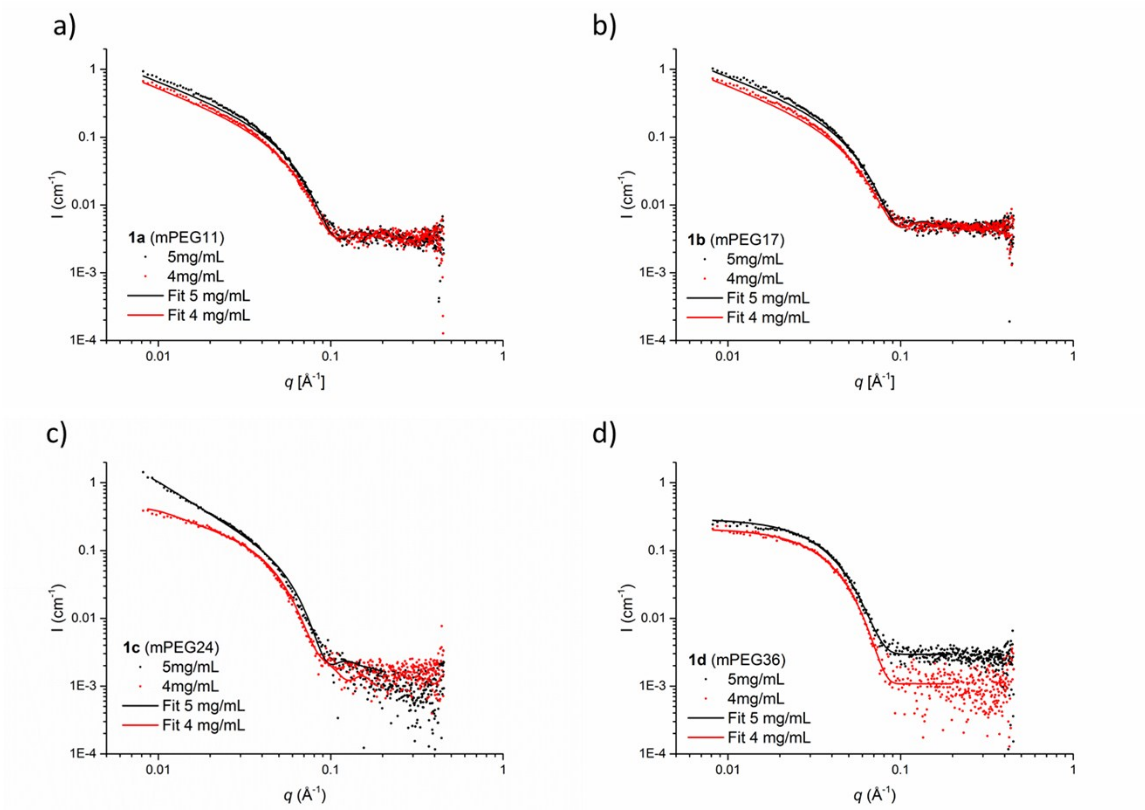


Figure S31. SAXS profiles of a) **1a**, b) **1b**, c) **1c**, and d) **1d**. Symbols represent experimental data; lines represent data modeled with a form factor for rigid homogeneous cylinder form molecules **1a** (a) and **1b** (b), two form factors describing a homogenous cylinder and a homogeneous sphere for **1c** (c) and fuzzy spheres for monomer **1d** (d).

Appropriate form factors were selected to model the SAXS profiles in Figure S7. The SAXS profiles of **1a** and **1b** were modeled with a form factor of homogeneous cylinders, **1d** with a form factor for fuzzy spherical objects, while for **1c**, two form factors describing a homogenous cylinder and a homogeneous sphere were utilized to model the data. Other form factors such as flexible or core shell homogenous cylinders were tested for the monomers **1a** and **1b**, while a homogenous sphere form factor was also tested for **1d**, resulting in modeling with a lower level of accuracy. In all cases, a fixed $\rho_{solvent} = 9.37 \times 10^6$ Å^{-2} was used. The values obtained from modeling the various curves are reported in table S1.

To extract the cross-sectional mass per unit length, M_L, from the scattering profiles for **1a** and **1b**, two equations were used:

$$I\,(q) = \frac{\pi}{q} I_{cs}\,(q) \qquad (1)$$

$$M_L = \frac{I_{cs}(0)}{c\Delta\rho_M^2} \qquad (2)$$

The electron length density difference per mass, $\Delta\rho_M$, was extracted from modeling of the curves and the height of the Holtzer plateau, I_{cs}, which is indicated by the solid lines in Figure S8 (a and b). For all samples, the specific volume, υ = 0.83 cm^3 g^{-1},was estimated based on the reciprocal density of oligo(ethylene glycol) (M_w> 600 g mol^{-1}, ρ = 1.2 g cm^{-3}),[2]on par with an earlier publication.[1]

In order to estimate the number of monomers in spherical aggregates of **1d**, we used:

$$I(0) = N(\Delta\rho V)^2 = \frac{C\Delta\rho^2 v^2 MW}{N_A} \quad (3)$$

I (0) was obtained from equation *(2)*. The molecular weight of the aggregate (Mw) was calculated from the mass per unit volume (C), contrast ($\Delta\rho_M$), specific volume (υ) of **1d**, and Avogadro's number (N_A).[3]

Table S1. Structural parameters extracted from the SAXS profiles of the squaramide-based bolaamphiphiles **1a**, **1b** and **1d**.

Sample	$\Delta\rho_{cyl}$ (A^{-2})	$\Delta\rho_M$ (cm g^{-1})	I (cm^{-2} L g^{-1})	I_{cs} (0) (cm^{-2})	M_L (g nm^{-1})	Mw (g mol^{-1})	molec/nm	r_{cs} (nm)
1a (4 mg/mL)	10.44×10^6	8.91×10^9	1.48×10^5	1.88×10^5	5.93×10^{20}	-	21	3.4
1a (5 mg/mL)	10.46×10^6	9.07×10^9	1.37×10^5	2.18×10^5	5.30×10^{20}	-	18	3.5
1b (4 mg/mL)	10.40×10^6	8.54×10^9	1.70×10^5	2.17×10^5	7.44×10^{20}	-	20	3.8
1b (5 mg/mL)	10.46×10^6	9.06×10^9	1.53×10^5	2.44×10^5	5.95×10^{20}	-	16	3.8
1d (4 mg/mL)	10.44×10^6	1.37×10^{10}	8.09×10^5	1.03×10^6	-	1.20×10^{12}	31 *	3.6
1d (5 mg/mL)	10.55×10^6	9.77×10^9	8.49×10^5	1.35×10^6	-	2.48×10^{12}	63 *	4.5

*Estimated overall aggregation number.

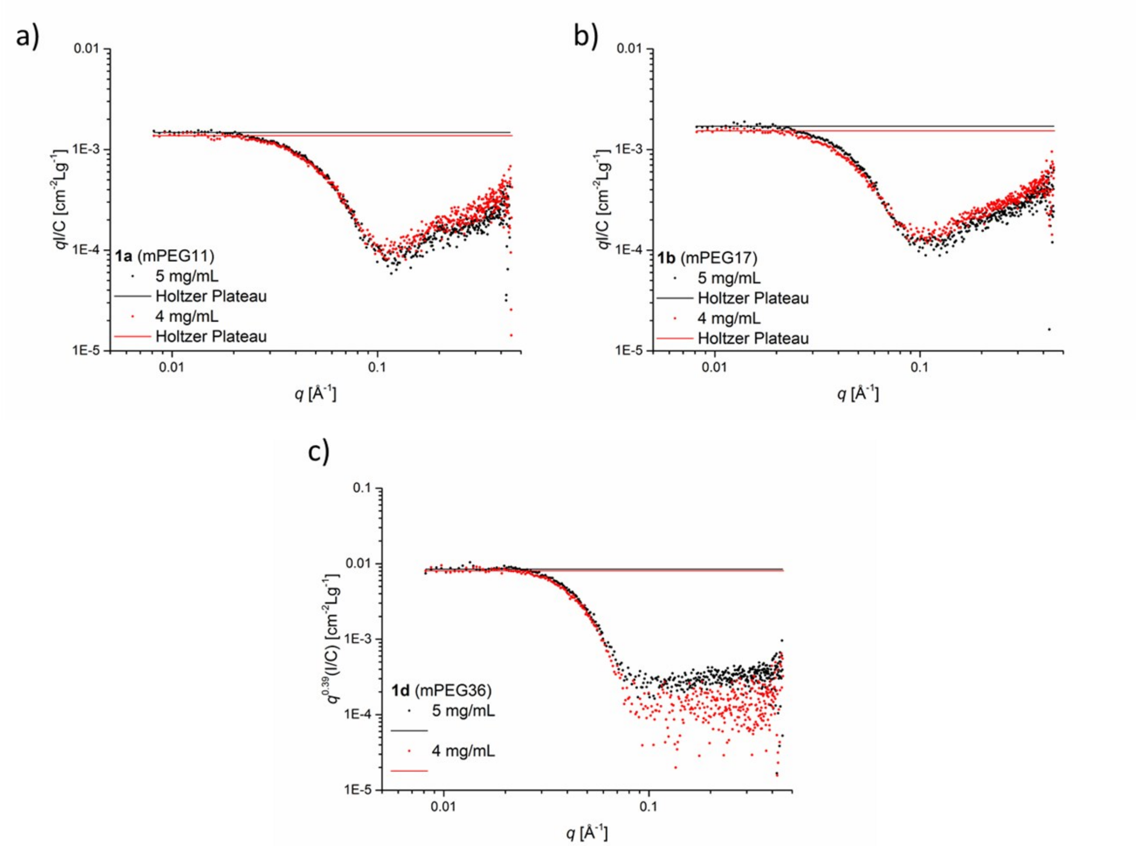


Figure S32: Casassa–Holtzer plot of the scattering profiles in Figure S6 for **1a** (a) and **1b** (b).The Holtzer plateaus ($0.0065 \leq q \leq 0.0197$ Å-1) are indicated by solid red and black lines. $I_{cs}(q)$ determination plot of the scattering profile for **1d** (c). The $I_{cs}(q)$ plateau ($0.0086 \leq q \leq 0.0245$ Å^{-1}) is indicated by the red and black lines.

5. UV-Vis spectroscopy

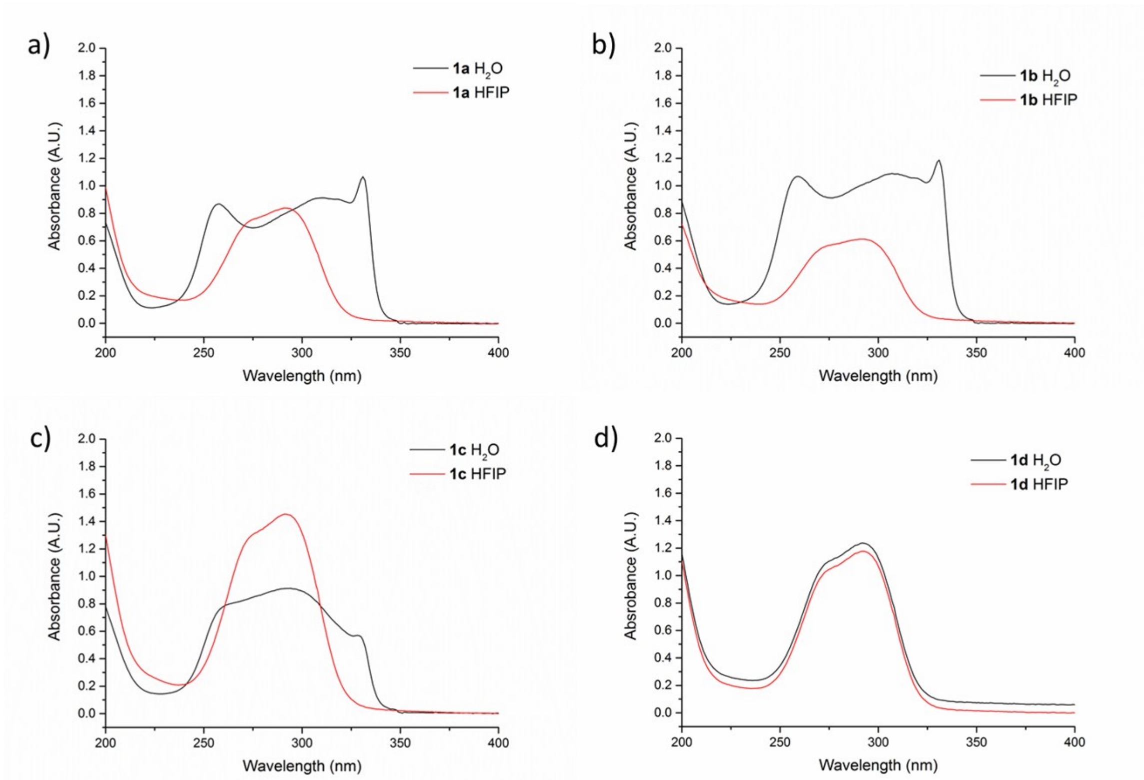


Figure S33. UV-Vis spectra of **1a** (a), **1b** (b), **1c** (c) and **1d** (d) in water (*blue*) and HFIP (*green*) at 30 μM.

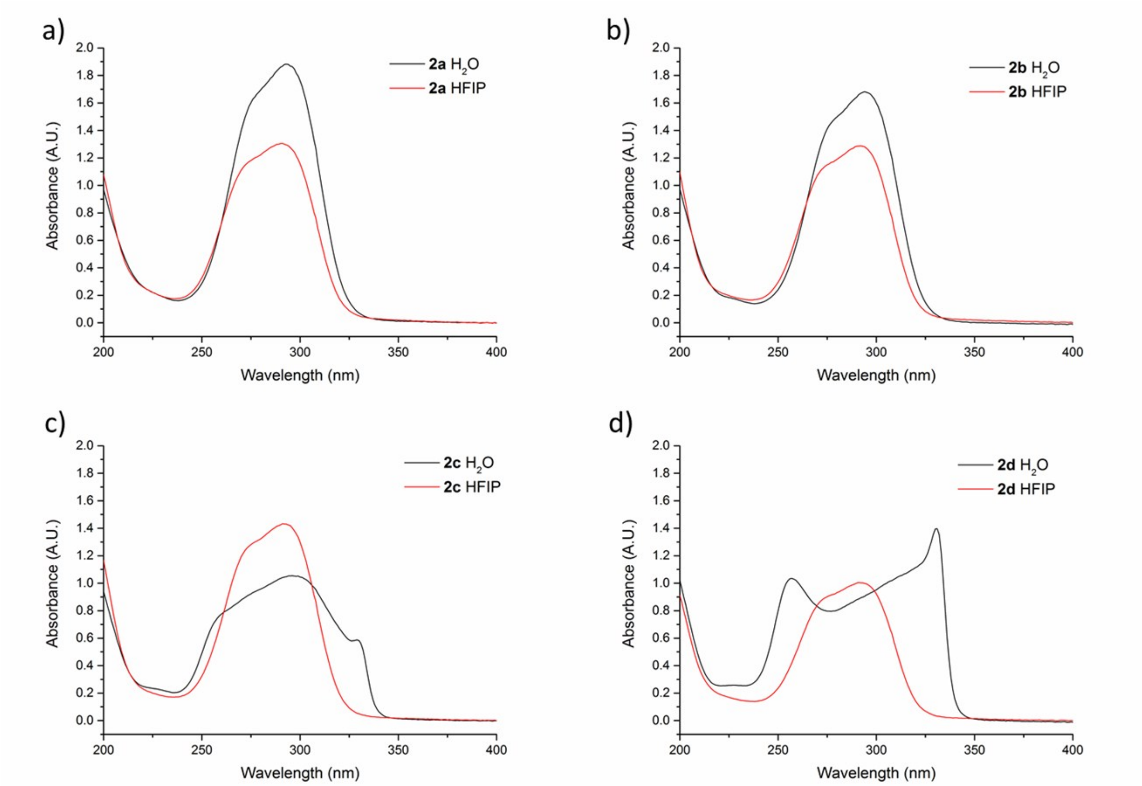


Figure S34. UV-Vis spectra of **2a** (a), **2b** (b), **2c** (c) and **2d** (d) in water (*blue*) and HFIP (*green*) at 30 μM.

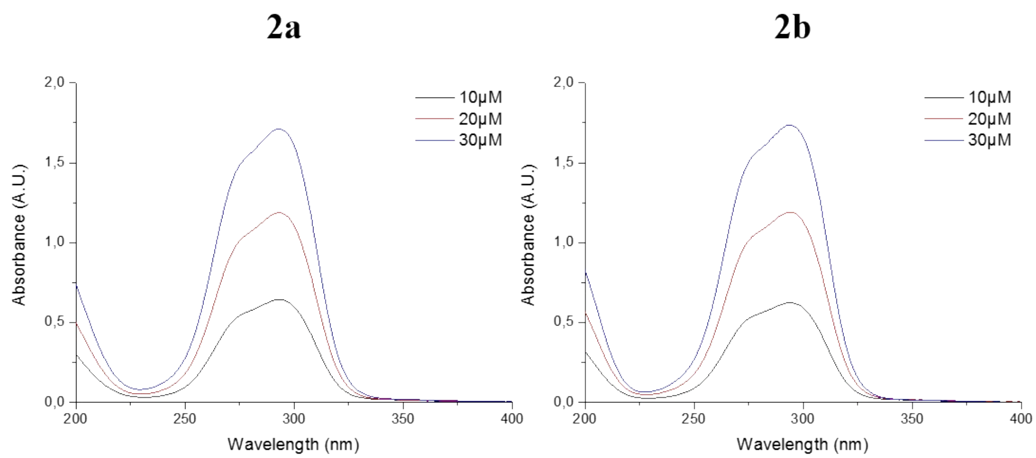


Figure S35. UV-Vis spectra of **2a** (*left*) and **2b** (*right*) in water at 30 μM (*blue*), 20 μM (*red*) and 10 μM (*black*).

6. Fourier transform infrared (FTIR)

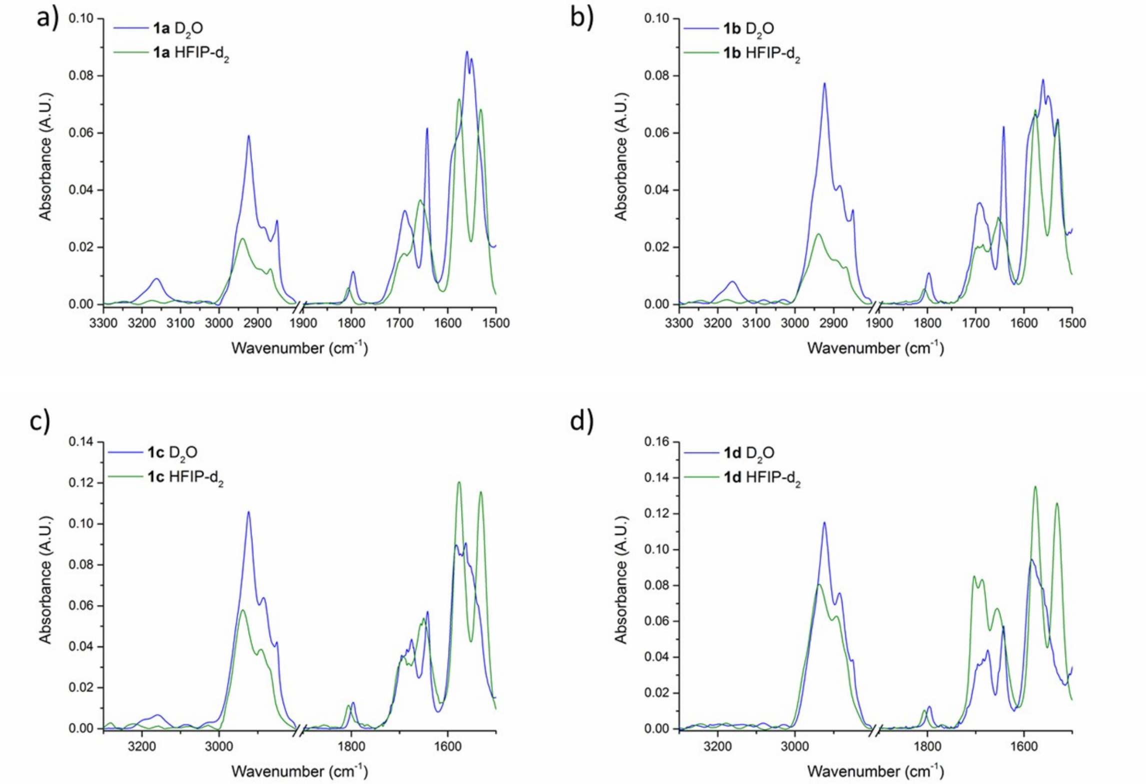


Figure S36. FTIR spectra recorded for **1a** (a), **1b** (b), **1c** (c) and **1d** (d) in D_2O and HFIP-*d_2* in N-H and C-H stretch regions above 2800 cm^{-1}, and the amide I and amide II region between 1900-1500 cm^{-1}.

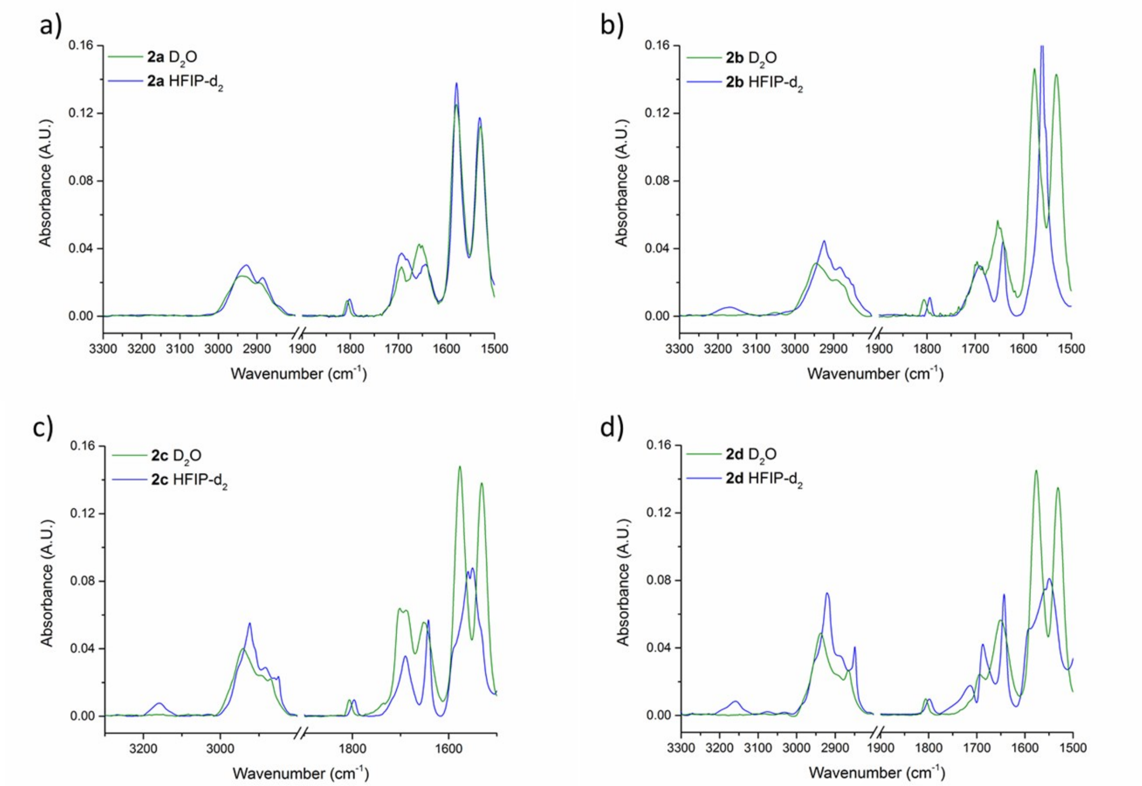


Figure S37. FTIR spectra for **2a** (a), **2b** (b), **2c** (c) and **1d** (d) in D_2O and HFIP-d_2 in N-H and C-H stretch regions above 2800 cm^{-1}, and the amide I and amide II regions between 1900-1500 cm^{-1}.

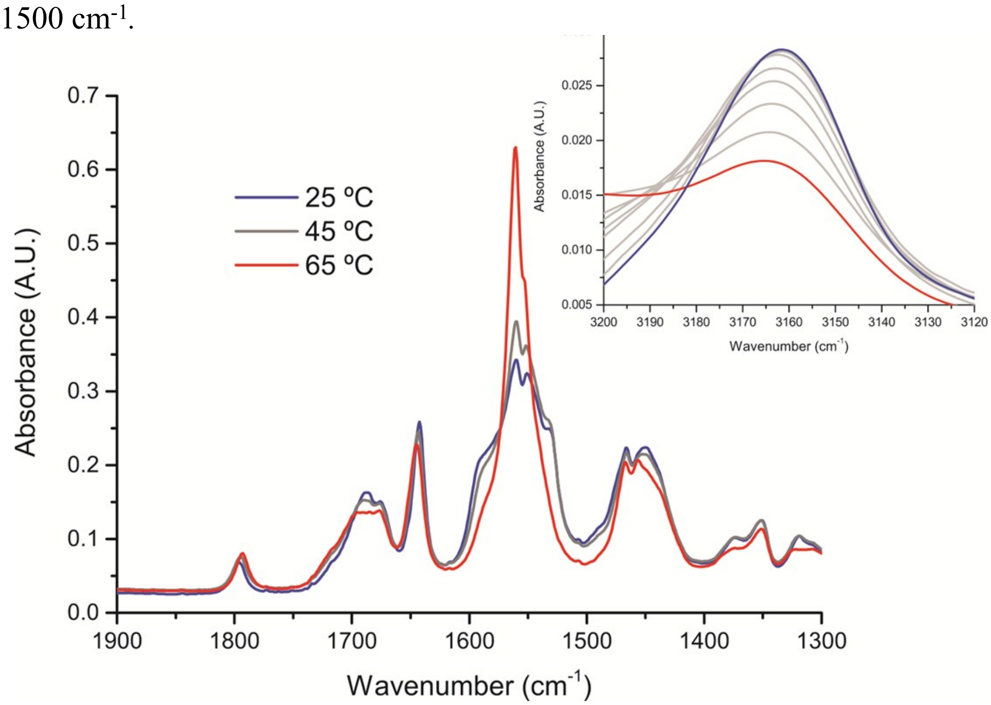


Figure S38. FTIR spectrum of **1a** in D_2O with increasing temperature from 25 ºC (*blue line*) to 65 ºC (*red line*). Inset: NH region (3200-3120 cm^{-1}).

7. References

(1) Saez Talens, V.; Englebienne, P.; Trinh, T. T.; Noteborn, W. E. M.; Voets, I. K.; Kieltyka, R. E. *Angew. Chemie - Int. Ed.* **2015**, *54* (36), 10502–10506.

(2) Lewalter, J.; Skarping, G.; Ellrich, D.; Schoen, U. *MAK Collect. Occup. Heal. Saf.* **2012**, *10*, 248–270.

(3) Jacques, D. A.; Trewhella, J. *Protein Sci.* **2010**, *19* (4), 642–657.