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**Author:** Saez, Talens V.
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CHAPTER 4

Morphological transitions of a squaramide-based supramolecular polymer nanoparticle in water by modulating its monomer structure

This chapter was prepared as an original research paper: Victorio Saez Talens, D. M. M. Makurat, Tingxian Liu, Willem E. M. Noteborn, Wei Dai, Clément L. Guibert, Ilja K. Voets and Roxanne E. Kieltyka.
4.1 Abstract
We report the synthesis of a library of squaramide-based bolaamphiphiles with variable hydrophobic and hydrophilic domain sizes, consisting of varied aliphatic chains (n = 2 to 12 methylene repeat units) and linear oligo(ethylene glycol)(m = 11 to 36 repeat units), to probe their self-assembly into supramolecular polymer nanoparticles in water. Systematic variation of the hydrophobic chain length show that a minimum hydrophobic domain is required to shield the squaramide units from water when a constant hydrophilic domain is maintained for self-assembly. By contrast, an increase in the hydrophilic chain length of the bolaamphiphile, keeping the hydrophobic domain constant, results in a gradual transition from fibrillar to spherical nanoscale objects with an alteration in the aggregation mode of the monomers. By understanding the self-assembly space achievable for this bolaamphiphilic monomer through modulating its chemical structure, we show that supramolecular polymer nanoparticles of variable shape and size can be easily prepared.
4.2 Introduction

Supramolecular self-assembly has garnered much interest for the preparation of functional nanostructures due to its modular and expeditious nature. Anisotropic, shape-persistent nanostructures can be achieved through the supramolecular polymerization of designed monomer units by non-covalent interactions such as hydrogen-bonding, ionic, π-π and hydrophobic effects.\(^1\text{−}^{10}\) This approach can be especially useful in the development of therapeutic nanoparticle libraries for drug delivery, where size and shape among other physicochemical properties can be used to guide their biodistribution, uptake and clearance \textit{in vivo}.\(^{11}\text{−}^{17}\) However, control over nanoparticle shape and size still remains non-trivial, especially in aqueous media where the use of strong non-covalent interactions within the monomer are necessary to drive self-assembly, but can also result in kinetically trapped aggregates.\(^{18}\text{−}^{22}\) This challenge highlights that more insight into the monomer features that drive supramolecular polymerization is required to be able to exploit such nanostructures for future biomedical applications.

Bolaamphiphiles are highly attractive self-assembling modules because of their capacity to access a range of well-defined nanostructures in solution, such as micelles, vesicles and high aspect ratio assemblies, such as rods, helices or ribbons.\(^{23}\text{−}^{27}\) They consist of two hydrophilic domains with a central hydrophobic core to facilitate self-assembly in water. When rigid chains or hydrogen bonds are introduced into their structure, a transition from vesicular aggregates to elongated rod-like micelles or supramolecular polymers is observed.\(^{23,24}\) To this end, hydrogen-bonding units, such as amides\(^{28}\) or ureas\(^{27,29}\) are commonly incorporated into the hydrophobic domain, and recently, our group demonstrated the use of squaramides\(^{30,31}\) to elicit such morphological transitions.

Squaramides\(^{32}\text{−}^{35}\) are minimalistic ditopic hydrogen bonding units that provide unique opportunities for supramolecular polymer construction\(^{30,31,36,37}\) with two N-H hydrogen bond donors opposite two C=O hydrogen bond acceptors on a cyclobutenedione ring. Their high synthetic accessibility starting from commercially available squaric esters permits their facile incorporation into a wide range of synthetic scaffolds.\(^{31,38}\text{−}^{40}\) Previously, our group reported the incorporation of two squaramide synthons into an aliphatic core flanked by two undeca(ethylene glycol) chains to prepare supramolecular polymers in water (Figure 4.1, 1).\(^{30,31}\) I herein probe the self-assembly scope of this squaramide-based bolaamphiphile by modulating the size of the hydrophilic and hydrophobic
domains and examine their self-assembly into supramolecular polymers from the molecular to the nanoscale (Figure 4.1).

4.3 Results and discussion

The influence of the hydrophilic chains on the self-assembly of squaramide-based bolaamphiphiles was examined by synthesizing derivatives with 17 (1b), 24 (1c), and 36 (1d) and 7 (1e) oligo(ethylene glycol) repeat units while maintaining the aliphatic chain length constant (n=5) between this domain and the squaramide units (Figure 4.1). Secondly, the contribution of the hydrophobic domain of the bolaamphiphile was studied by modifying the aliphatic spacer between the hydrophilic chain and the squaramide motifs with 2 (2a), 6 (2b), 8 (2c) and 12 (2d) methylene repeat units, while keeping the remainder of the hydrophobic and hydrophilic undeca(ethylene glycol) chains lengths constant. An analogous synthetic approach to 1a was used to prepare the squaramide-based bolaamphiphile library with moderate yields over the various steps (see supporting information, section 4.6.3). Optically clear solutions (c = 580 µM) of
the various bolaamphiphiles were obtained when the compounds were dissolved in water, except for 1e that required sonication. This compound was abandoned
for further study and the remaining samples were left to stand 24 hours prior to measurement.

Cryogenic transmission electron microscopy (cryo-TEM) was used as a first approach to probe the effect of modulating the hydrophobic and hydrophilic domains of the bolaamphiphiles on the supramolecular polymer structure (Figure 4.2, see supporting information, section 4.6.4). Morphologically distinct self-assembled structures were obtained with systematic modification of the hydrophilic chain length. Samples of 1a displayed almost exclusively stiff high aspect ratio fibers, while 1b predominantly displayed elongated fibrillar constructs with a smaller population of shorter, sometimes spherical objects (Figure 4.2a and 4.2b). The fiber lengths were 234 ± 108 nm and 109 ± 44 nm for 1a and 1b, respectively. However, due to the high dispersity of these samples, fiber-like domains of up to a micron in length could be observed for 1a and half of this value for 1b, with comparable diameters of 6-7 nm. Self-assembly of 1c (Figure 4.2c) showed a mixture of spherical aggregates with a diameter of 6 ± 1 nm and rod-like aggregates with a length of 57 ± 24 nm, and is suggestive of kinetic trapping of the bolaamphiphile. For 1d, only spherical aggregates were found with a diameter of 9 ± 4 nm (Figure 4.2d). These results suggest that the steric bulk provided by the longer oligo(ethylene glycol) chain lengths drives the formation of spherical structures.

Subsequently, the effect of modulating the aliphatic chain length on the self-assembly of 2a-d was studied. No aggregates in solution were observed for 2a (Figure 4.2e). Alternatively, 2b (Figure 4.2f) displayed thin filamentous structures difficult to distinguish from the background, and 2c (Figure 4.2g) exhibited fibrillar objects on the order of 200 ± 93 nm and disperse in length with thickness of 5-6 nm. A combination of short rod-like objects and bundles thereof were found for 2d (Figure 4.2h). Thus, bolaamphiphile self-assembly into supramolecular polymers is observed when the aliphatic chains separating the hydrophilic side chains and the squaramide units have at least 8 to 10 methylene repeat units with a fixed undeca(ethylene glycol) chain length.

Small angle X-ray scattering (SAXS) experiments were performed to further characterize the differences in morphology between the various supramolecular polymers prepared from 1a-d, namely with respect to their size and shape (Figure 4.3a and supporting information). For 1a, we previously reported a SAXS profile
characteristic of rod-like structures with a length L outside the accessible q-range. It displays a $I \propto q^{-3}$ powerlaw regime at low q values, followed by a $I \propto q^{-4}$ regime at high q values.\textsuperscript{31} The experimental data was described with a form factor for rigid, homogenous cylinders yielding a cross-sectional radius of $\sim 3.5$ nm, which is comparable to the diameter measured by cryo-TEM. We further obtained a cross-sectional mass per unit length ($M_L$) of $\sim 5.3 \times 10^{20} \pm 0.6 \times 10^{20}$ g nm$^{-1}$ from the form factor modeling, which suggests approximately 18 - 21 squaramide bolaamphiphiles per nm along the fiber. The same model was applied to describe the SAXS profiles of 1b, providing an $\rho_{cs}$ of $\sim 3.7$ nm and a $M_L$ of $\sim 5.9 \times 10^{20} \pm 0.5 \times 10^{20}$ g nm$^{-1}$ that translates into a value of roughly 16 - 20 bolaamphiphiles per nm. In contrast, 1c could not be modeled satisfactorily in the same manner. Instead, two form factors (for homogeneous cylinders and spheres) were employed to take the coexistence between fibrils and spherical objects as revealed by cryo-TEM into account. Finally, the sample containing the longest hydrophilic chain, 1d, gave rise to scattering profiles characteristic of objects with a low aspect ratio. A plateau is found at low q values followed by an $I \propto q^{-4}$ powerlaw regime at high q-values. This data set was best modeled with form factors derived for homogeneous and fuzzy spheres,$^{41,42}$ yielding comparable quality of fit and radii of $\sim 5.5$ nm, providing an estimated overall aggregation number of 31-63 molecules. The small angle X-ray scattering experiments confirm the transition from predominantly fibrillar towards spherical morphologies upon increasing oligo(ethylene glycol) length as seen in the cryo-TEM images.
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The self-assembly of the various squaramide-based bolaamphiphiles was further probed at the molecular level by ultraviolet-visible spectroscopy (UV-Vis), fluorescence and IR spectroscopy. Earlier, we reported that self-assemblies of 1a in water showed UV-Vis maxima at 255 and 329 nm, corresponding to the HOMO-(LUMO+1) and HOMO-LUMO transitions of the squaramide synthon, respectively.31 These maxima were largely insensitive to changes in concentration, meanwhile heating of the bolaamphiphiles up to 85°C resulted in perturbation of these bands, thus suggestive of strong aggregation in water. Even with a slightly longer oligo(ethylene glycol) (OEG) chain, 1b showed a comparable absorption.

Figure 4.3. (a) SAXS profiles for 4 mg/mL samples of 1a, 1b, 1c and 1d in water (circular dots) modeled with a form factor describing a rigid homogenous cylinder for 1a and 1b (green and blue line), two form factors of a describing a homogenous cylinder and a homogenous sphere for 1c (red line) and a form factor of a fuzzy sphere for 1d (black line). The scattering profiles have been shifted vertically by multiplying by a factor of 2 (red line), 7 (blue line) and 20 (green line). (b, c) Normalized UV-Vis absorption traces of (b) 1a-d and (c) 2a-d (c = 30 µM) in water at room temperature after 24 hours of sample equilibration. d) UV-Vis spectrum of 1a at different temperatures: RT, Day 0 (black line), Heat to 85°C, Day 0 (red line), Cool to RT, Day 0 (blue line) and RT, Day 5 (green line).
spectrum in water, whereas in spectra of 1c and 1d the sharp bands at 255 and 329 nm were of decreased intensity with red and blue-shifts of these maxima, respectively, consistent with a lower degree of polymerization (Figure 4.3b). Conversely, decreasing the length of the aliphatic spacer between the squaramide motifs and the hydrophilic oligo(ethylene glycol) chains resulted in even more pronounced differences in the UV-Vis spectra (Figure 4.3c). The UV-Vis spectra for 1a-d and 2a-d corroborate the morphologies observed at the nanoscale by cryo-TEM and SAXS.

Temperature-dependent UV-Vis measurements (Figure 4.3d) were carried out to gain insight into supramolecular polymerization of 1a. Heating the fibrillar sample from 25 to 85 °C resulted in spectra consistent with depolymerization of the bolaamphiphiles. The absorbance at 329 nm, a maxima consistent with the aggregated state, as a function of increasing temperature showed an abrupt decrease in intensity around 60 °C. Upon cooling, this peak showed strong hysteresis and did not return after five days, suggesting the formation of a kinetically trapped aggregate distinct from the initial supramolecular polymer. However, it is unclear how much more time is required to return to the initial fibrillar aggregate state.

UV-Vis spectra of 2a and 2b bearing 2 and 6 methylenes, respectively, lacked features of squaramide self-assembly in water, consistent with cryo-TEM imaging. When the length of the aliphatic spacer is further increased to 8 methylene units, as in 2c, the onset of a spectral profile similar to that of 1a is observed. In the case of 2d, the spectral profile resembled that of 1a with peaks at 329 and 255 nm, on par with earlier findings of squaramide self-assembly. Thus, self-assembly of squaramide-based bolaamphiphiles is readily probed by UV-Vis spectroscopy, revealing distinct regimes of polymerization consistent with their molecular structure.

Aggregation of the squaramide-based bolaamphiphiles was further supported by fluorescence measurements using the Nile red dye (NR, see supporting information, section 4.6.8). NR is a hydrophobic dye that undergoes a blue shift in its emission maximum at 650 nm and an increase in fluorescence intensity when encapsulated in a hydrophobic environment. This shift thus provides a view into the contribution of the hydrophobic effect to the self-assembly process. A blue-shift of the emission wavelength and 4 to 5-fold increase in the peak intensity of
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NR were recorded for 1a-d when compared against the dye in water as a control (Figure 4.4a). The same shift was observed for 1a and 1b, Δλ<sub>max</sub> = -35 nm, while 1c presented a Δλ<sub>max</sub> = -26 nm and 1d a Δλ<sub>max</sub> = -21 nm, consistent with a decrease in the hydrophobic character of the bolaamphiphile with increasing oligo(ethylene glycol) length.

Conversely, for 2a-d a gradual increase in the fluorescence intensity is measured with the increased length of the aliphatic spacer, as expected (Figure 4.4b). An emission peak was recorded for 2a on par with that of the NR dye in water, whereas shifts of Δλ<sub>max</sub> of -10, -18 and -38 nm were measured for 2b, 2c and 2d, respectively, with a 6-fold increase in the intensity for the latter. The fluorescence

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Figure 4.4. Fluorescence spectra of NR (ex. = 550 nm) with 1a-e (a) and 2a-d (b) in aqueous solution (c = 30 µM).
results support the UV-Vis measurements and trends when altering the hydrophobic and hydrophilic domains of the squaramide-based bolaamphiphile.

Figure 4.5. FTIR spectra of 1a-d (a) and 2a-d (b) in D$_2$O (c = 5.8 mM). N-H (inset), amide I and amide II regions are plotted.

Fourier transform infrared (FTIR) spectroscopy was used to investigate the hydrogen bonding in the various squaramide-based supramolecular polymer nanoparticles. For compound 1a, we earlier reported the N-H stretch at 3162 cm$^{-1}$ and C=O stretches at 1687, 1676 and 1642 cm$^{-1}$ for the squaramide and carbamate moieties of the squaramide-based bolaamphiphile when self-assembled in water, while a small broad peak at 1796 cm$^{-1}$ was assigned to the ring breathing of the squaramide moiety. The features of the IR spectrum were largely unchanged with decreasing concentration, except for its intensity. When the temperature was increased from 25 to 65 °C (Figure S4.13) in water, a shift to higher
wavenumbers of the N-H peak of 1a of 3 cm\(^{-1}\) with a decrease in its intensity with concomitant changes in the carbonyl region were observed, indicative of hydrogen-bonding between bolaamphiphiles. Through increasing the oligo(ethylene glycol) chain length (1b to 1d), the N-H stretch peak becomes bimodal and decreases in intensity towards the baseline. In the carbonyl region, similar patterns of bands are observed for 1a and 1b, and 1c and 1d (Figure 4.5a). The spectral differences between these two sets of bolaamphiphiles in the C=O and N-H regions are thus suggestive of a distinct mode of aggregation between both pairs. Subsequently, we measured samples 1b-d in HFIP-d\(_2\) (see supporting information, section 4.6.7). As expected, loss of the N-H stretch peak was observed for 1b-d due its likely superposition with the O-H stretch. The ring breathing peak and the C=O stretches of the squaramide synthon in the amide I region were shifted to higher wavenumbers by 10-15 cm\(^{-1}\) and decreased in intensity with the addition of HFIP-d\(_2\), consistent with disassembly of the supramolecular polymer. The IR results suggest a distinct aggregation mode between 1a and 1b, and 1c and 1d due to the increased length of the oligo(ethylene glycol) chains that imposed sterically on the formation of supramolecular polymers.

IR studies were also carried out on 2a-d where the eventual formation of fibrillar aggregates in water was observed. The gradual appearance of a vibration at 3162 cm\(^{-1}\) was observed when comparing the N-H stretches (inset Figure 4.5b) of 2a and 2d. This result is consistent with the increased hydrophobic shielding of the squaramide units from their aqueous surroundings with lengthening of the aliphatic spacer. In the amide II region, 2a showed two sharp peaks at 1578 cm\(^{-1}\) and 1530 cm\(^{-1}\), similar to those found for 2a when dissolved in HFIP-d\(_2\) and absent in 2b-d. Moreover, a gradual increase in intensity was observed for the carbonyl stretch of the squaramide at 1643 cm\(^{-1}\) and the ring breathing peak moved to lower wavenumbers (from 1801 cm\(^{-1}\) for 2a to 1797 cm\(^{-1}\) for 2d), consistent with their polymerization upon increasing aliphatic chain length. Thus, lesser degree of aggregation is observed when increasing the length of the hydrophilic oligo(ethylene glycol) chains from 11 to 36 repeat units, while 8 methylene repeat units are optimal in the aliphatic spacer within the bolaamphiphile to facilitate the interaction between the squaramides by hydrogen bonding.
4.4 Conclusions

In conclusion, I have synthesized a library of squaramide-based bolaamphiphiles where we systematically modify the hydrophilic side chains (1a-e) and the hydrophobic core (2a-d) independently to study their consequence on self-assembly of the supramolecular polymers. A minimum hydrophobic chain length of 8 carbons between the squaramide and a fixed undeca(ethylene glycol) chain length was required to ensure the formation of fibrillar aggregates. On the other hand, further increasing the oligo(ethylene glycol) chain length (up to m=36), while maintaining the same aliphatic chain length constant of 10 carbons, was found to provide a handle to guide their morphological transition from fibrillar to spherical aggregates due to increasing steric demand of the hydrophilic domains. Moreover, spectroscopic studies propose a distinct mode of bolaamphiphile self-assembly when spherical aggregates are formed; changes to the hydrogen-bonding configuration observed in comparison to the fiber structures. However, the precise nature of their self-assembly in these spherical aggregates is unknown, namely whether they occupy a bent or straight configuration and if they are being encountered during thermal depolymerization of the supramolecular polymer fibers. Thus, studies are underway to further characterize their nature and whether the various morphologies are kinetically trapped or thermodynamic assemblies, and the potential to control their supramolecular polymerization.

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4.6 Supporting Information

4.6.1 Materials
All solvents and reagents were obtained from commercial suppliers and were used without further purification. The oligo(ethylene glycol) methyl ether(s) were obtained from Polypure and Broadpharm. Deuterated dimethyl sulfoxide and chloroform were purchased from Euriso-top. Palladium on matrix activated carbon, triethylsilane, deuterium oxide, hexafluoroisopropanol and all other commercial grade reagents and chemicals were purchased from Sigma Aldrich and used as received. Milli-Q water was used for all the studies.

4.6.2 General 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, a Bruker DMX-400, a Bruker AV-II-600 MHz and a 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$_2$O-CH$_3$CN 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 $\alpha$-cyano-4-hydroxycinnamic acid as a matrix on a ground steel target plate. Cryogenic transmission electron microscopy (cryo-TEM) samples were applied to freshly glow-discharged Lacey Carbon Film (300 mesh Cu grids) and plunge-frozen in liquid ethane using a Leica EM GP. Images were recorded with a Tecnai F20 FEG (FEI company, The Netherlands), equipped with a field emission gun at 200 kEV using a Gatan UltraScan camera (Gatan company, Germany). Small angle X-ray scattering (SAXS) measurements were carried on a 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 $\lambda = 1.54$ Å at a flux of 1x10$^8$ ph/s and a Pilatus 300K silicon pixel detector with 487 x 619 pixels of 172 µm x 172 µm in size placed at two sample-to-detector distances of 713 and 1513 mm respectively to access a $q$-range of $0.006 \leq q \leq 0.44$ Å$^{-1}$ with $q = 4 \pi/\lambda (\sin \theta/2)$. 

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Silver behenate was used for calibration of the beam center and the \( q \) range. Samples were contained at room temperature 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 self-assembled structures were obtained by subtraction of the scattering contribution of the solvent and quartz capillary. The small angle X-ray scattering profiles were analyzed using the software package SasView (http://www.sasview.org/). Absorption spectra were obtained on a Cary 300 UV-Vis spectrophotometer. All measurements were carried out using a quartz cuvette with a 1 cm path length. Fluorescence spectra were acquired on a Tecan Plate Reader Infinite M1000 using 96 well plates (PP Microplate, solid F-bottom (flat), chimney well). Transmission FTIR spectra were measured using a Bio-Rad Excalibur spectrometer equipped with a nitrogen cooled MCT detector. A liquid transmission cell with CaF\(_2\) windows and a fixed nominal path length of 50 mm was used. Sample spectra were recorded in deuterated solvents at room temperature, with a resolution of 1 cm\(^{-1}\). For each spectrum 128 scans were averaged. The final absorbance spectra was expressed in terms of absorbance and corrected by manual subtraction of a water vapor spectrum. Baseline was subtracted and brought to a zero value by using Origin 9.1 software.
4.6.3 Synthetic routes
Synthesis of squaramide-based bolaamphiphiles

Scheme S4.1. Synthetic route of 1a-e and 2a-d.

The synthesis of 5d, 6a, 7a and 1a were reported in chapter 2. A similar synthetic approach was followed for the synthesis of the rest of the monomers, which is reported below.
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**Synthesis of 5a**

1,n-alkyldiamine (a: n=2, 1.8 g, 30 mmol) was dissolved in 50 mL CH₂Cl₂ and cooled to 0°C. Benzyl chloroformate (2.6 g, 15 mmol) was dissolved in 75 mL CH₂Cl₂ 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 MgSO₄, and the CH₂Cl₂ was evaporated under reduced pressure. The crude product was purified by normal phase chromatography by using CH₂Cl₂/CH₃OH/Et₃N (99/0/1-75/24/1 v/v/v).

5a: Yield: 2.47 g, 85.0 % ¹H-NMR (δH[ppm], DMSO-d₆, 300 MHz): 7.38-7.27 (m, 5H), 5.00 (s, 2H), 3.08-3.02 (m, 2H), 2.66-2.61 (t, 2H). ¹³C-NMR (δC[ppm], DMSO-d₆, 75 MHz) 156.71, 137.56, 128.78, 128.20, 128.16, 65.72, 42.79, 41.11.

**Synthesis of 5b-5e**

A similar synthetic approach to 5d was carried out for the synthesis of 5b, 5c and 5e. N-(Benzylxycarbonyloxy)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 5b 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 MgSO₄ and evaporated to yield compound 5b as a white crystalline solid.

For 5c and 5e, 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.

5b: Yield: 1.69 g, 76.5 % ¹H-NMR (δH[ppm], DMSO-d₆, 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). ¹³C-NMR
(δC[ppm], DMSO-d6, 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.

5c: Yield: 0.68 g, 67.6 % 1H-NMR (δH[ppm], DMSO-d6, 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) 13C-NMR (δC[ppm], DMSO-d6, 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.

5e: Yield: 1.73 g, 71.7 % 1H-NMR (δH[ppm], DMSO-d6, 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) 13C-NMR (δC[ppm], DMSO-d6, 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 6b-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, 5d (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 CH3CN/H2O 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.

6b: Yield: 0.35 g, 49.1 % 1H-NMR (δH[ppm], CDCl3, 600 MHz): 7.35-7.29 (m, 5H), 5.08 (s, 2H), 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). 13C-NMR (δC[ppm], CDCl3, 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]+.
6c: Yield: 0.30 g, 76.6 % \[^1H\text{-NMR}\] (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), 1.50-1.47 (m, 4H), 1.28 (m, 12H). \[^{13}C\text{-NMR}\] (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]^+.

6d: Yield: 0.26 g, 71.9 % \[^1H\text{-NMR}\] (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). \[^{13}C\text{-NMR}\] (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: 6.61min, m/z: 1950.47 [M+H]^+. MALDI-TOF-MS: m/z calc: 1949.18; found: 1972.32 [M+Na]^+.

6e: Yield: 0.62 g, 62.7 % \[^1H\text{-NMR}\] (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). \[^{13}C\text{-NMR}\] (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 7b-e

Compound 6 (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\(_3\)SiH 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\(_3\)CN/H\(_2\)O over 30-45 minutes on a
C18 silica column. The product was concentrated by evaporation and lyophilized overnight to obtain compound 7b-e as a white solid.

7b: Yield: 143 mg, 58.6 % ¹H-NMR (δH[ppm], CDCl₃, 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). ¹³C-NMR (δC[ppm], CDCl₃, 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]⁺.

7c: Yield: 164 mg, 54.0 % ¹H-NMR (δH[ppm], CDCl₃, 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), 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). ¹³C-NMR (δC[ppm], CDCl₃, 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]⁺⁺. MALDI-TOF-MS: m/z calc: 1438.88; found: 1462.09 [M+Na]⁺.

7d: Yield: 160 mg, 61.0 % ¹H-NMR (δH[ppm], CDCl₃, 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). ¹³C-NMR (δC[ppm], CDCl₃, 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]⁺.

7e: Yield: 179 mg, 87.1 % ¹H-NMR (δH[ppm], CDCl₃, 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). ¹³C-NMR (δC[ppm], CDCl₃, 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 8a-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, 5a (0.23 g, 1.18 mmol), 5b (0.5 g, 2 mmol), 5c (0.56 g, 2 mmol) and 5e (0.41 g, 1.22 mmol) were added respectively, followed by the addition of a few drops of DIPEA and left to reflux overnight. The product was purified by flash column chromatography using a gradient of 10-90% CH$_3$CN/H$_2$O over 30-45 minutes on a C18 silica column. The product was concentrated by evaporation and lyophilized overnight to obtain a white solid.

**8a:** 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]$^+$. 

**8b:** 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]$^+$. 

**8c:** 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]$^+$. 

**8d:** 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 9a-d**

Compound 8 (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 7. The Cbz-deprotection of the amine moiety was achieved by the dropwise addition of Et₃SiH 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% CH₃CN/H₂O over 30-45 minutes on a C18 silica column. The product was concentrated by evaporation and lyophilized overnight to obtain compound 9a-d as a white solid.

**9a:** Yield: 240 mg, 42.6 % 
1H-NMR (δH[ppm], CDCl₃, 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). 13C-NMR (δC[ppm], CDCl₃, 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]⁺.

**9b:** Yield: 200 mg, 65.2 % 
1H-NMR (δH[ppm], CDCl₃, 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). 13C-NMR (δC[ppm], CDCl₃, 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]⁺.
9c: Yield: 48.33 mg, 63.1 % $^1$H-NMR ($\delta_{\text{H}}$[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 ($\delta_{\text{C}}$[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]$^+$.

9d: Yield: 310 mg, 69% $^1$H-NMR ($\delta_{\text{H}}$[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 ($\delta_{\text{C}}$[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 7 (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) were added until the starting material 7 disappeared. The completion of the reaction was verified by LC-MS, and purified by flash column chromatography using a gradient of 10-90% CH$_3$CN/H$_2$O 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 ($\delta_{\text{H}}$[ppm], CDCl$_3$, 400 MHz): 5.05 (br s, 1H), 4.21-4.20 (m, 4H), 3.73-3.65 (m, 136 H), 3.56-3.55 (t, 4H), 3.39 (s, 6H), 3.15-3.13 (m, 4H), 2.85-1.65-1.63 (m, 8H), 1.48-1.26 (m, 34H). $^{13}$C-NMR ($\delta_{\text{C}}$[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]$^+$. 
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), 1.92 (br s, 10H), 1.65-1.64 (m, 8H), 1.50-1.27 (m, 36H). $^{13}$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, 70.44, 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]$^+$. 

1d: Yield: 59.0 mg, 46.3 % $^1$H-NMR (δ$_H$[ppm], CDCl$_3$, 850 MHz): 5.07 (br s, 1H), 4.21 (m, 4H), 3.74-3.55 (m, 286 H), 3.38 (s, 6H), 3.14-3.13 (m, 4H), 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]$^+$. 

1e: Yield: 76.2 mg, 42.9 % $^1$H-NMR (δ$_H$[ppm], CDCl$_3$, 400 MHz): 4.23 (m, 4H), 3.68-3.66 (m, 52H), 3.59-3.56 (t, 4H), 3.40 (s, 6H), 3.18-3.15 (m, 4H), 2.96-2.86 (br s, 6H), 1.71-1.64 (m, 6H), 1.52-1.27 (m, 36H). $^{13}$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: 1385.91 [M+Na]$^+$. 

**Synthesis of 2a-d**

Compound 9 (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) were added until the starting material 7 disappeared. The product was purified by flash column chromatography using a gradient of 10-90% CH$_3$CN/H$_2$O 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): 7.41 (s, 1H), 6.21 (s, 1H) 4.21-4.17 (m, 4H), 3.76-3.53 (m, 92H), 3.41 (s, 4H), 3.37 (s, 6H), 1.66-1.65 (m, 4H), 1.44-1.40 (m, 6H). $^{13}$C-NMR (δ$_C$[ppm], CDCl$_3$, 212.5 MHz): 182.87, 182.05,
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168.80, 167.67, 156.78, 71.91, 71.89, 70.61, 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]+.

2b: Yield: 90.1 mg, 45.5 % 1H-NMR (δH[ppm], CDCl3, 850 MHz): 8.45 (s, 2H), 5.36 (s, 2H), 4.24-4.21 (m, 4H), 3.73-3.66 (m, 84H), 3.58-3.56 (t, 4H), 3.40 (s, 6H), 3.20-3.16 (m, 2H). 13C-NMR (δC[ppm], CDCl3, 212.5 MHz): 192.44, 186.56, 181.92, 169.17, 157.75, 72.91, 72.87, 71.57, 71.56, 71.50, 71.47, 71.45, 71.43, 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]+.

2c: Yield: 19.4 mg, 48 % 1H-NMR (δH[ppm], CDCl3, 400 MHz): 8.38 (s, 2H), 5.20 (s, 2H), 4.23 (m, 4H), 3.70-3.66 (m, 84H), 3.57-3.56 (t, 4H), 3.39 (s, 6H), 3.16-3.14 (m, 2H). 13C-NMR (δC[ppm], CDCl3, 100 MHz): 181.50, 169.10, 168.55, 157.48, 72.92, 72.88, 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]+.

2d: Yield: 53 mg, 50.5 % 1H-NMR (δH[ppm], CDCl3, 400 MHz): 7.85 (s, 2H), 7.56 (s, 2H), 5.20 (s, 2H), 4.23-4.20 (m, 4H), 3.70-3.55 (m, 92H), 3.39 (s, 6H), 3.17-3.13 (m, 4H), 2.20 (s, 4H), 1.66 (m, 8H), 1.49-1.26 (m, 42H). 13C-NMR (δC[ppm], CDCl3, 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]+.
4.6.4 Cryogenic transmission electron microscopy (cryo-TEM)

Cryogenic transmission electron microscopy (cryo-TEM) samples were prepared by applying three microliters of sample solution (580 µM) to a freshly glow-discharged Lacey Carbon Film (300 mesh Cu grids). The excess of liquid was blotted away for 1 second (95% humidity, RT, Whatman No.4 filter paper) and plunge-frozen in liquid ethane at -183 °C using a Leica EM GP. Images of the vitrified samples were recorded with a Tecnai F20 FEG (FEI company, The Netherlands), equipped with a field emission gun at 200 kEV using a Gatan UltraScan camera (Gatan company, Germany) with a defocus between -3 and -9 µm. The obtained cryo-TEM images were analyzed using the "Fiji" image processing software. Samples 1a, 1b, 1c, 1d and 2c were analyzed. The length and width of 50 aggregates were measured per sample. Values are expressed as the average ± their standard deviation.

Figure S4.1. Histograms of length (234 ± 108 nm) and width (5 ± 1 nm) distributions measured for 1a.
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Figure S4.2. Histograms of length (109 ± 44 nm) and width (5 ± 1 nm) distributions measured for 1b.

Figure S4.3. Histograms of length (57 ± 24 nm) and width (5 ± 1 nm) distributions measured for 1c.

Figure S4.4. Histograms of diameter distributions measured for spherical aggregates of 1c (6 ± 1 nm) (left) and 1d (9 ± 4 nm) (right).
Figure S4.5. Histograms of length (200 ± 93 nm) and width (4 ± 1 nm) distributions measured for 2c.
4.6.5 Small angle X-ray scattering (SAXS)

Small angle X-ray scattering (SAXS) experiments were performed on aqueous solutions of the monomers 1a, 1b, 1c and 1d to study their progressive transition from fiber-like to spherical aggregates upon increasing the length of the hydrophilic segment. All SAXS measurements were carried out in MQ water at room temperature. The SAXS profiles of the 4 and 5 mg/mL samples are given in Figure S4.7 for the molecules above. In the low-\( q \) regime, the scattering profiles in log-log representation of 1a and 1b exhibited a powerlaw slope of \(-1\), which is typical for cylindrical objects. A low-\( q \) plateau followed by a steep powerlaw 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 S4.6) 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 S4.6. 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)).
Appropriate form factors were selected to model the SAXS profiles in Figure S4.7. 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 $\rho_{\text{solvent}} = 9.37 \times 10^6 \text{ Å}^{-2}$ was used. The values obtained from modeling the various curves are reported in table S4.1.

To extract the cross-sectional mass per unit length, $M_L$, from the scattering profiles for 1a and 1b, two equations were used:
\[ I(q) = \frac{\pi}{q} I_{cs}(q) \] (1)

\[ M_L = \frac{I_{cs}(0)}{c\Delta \rho_M^2} \] (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 S4.8 (a and b). For all samples, the specific volume, \( \upsilon = 0.83 \text{ cm}^3 \text{ g}^{-1} \), was estimated based on the reciprocal density of oligo(ethylene glycol) \( (M_w > 600 \text{ g mol}^{-1}, \rho = 1.2 \text{ g cm}^{-3}) \), on par with an earlier publication.\(^1\)

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

\[ I(0) = N(\Delta \rho V)^2 = \frac{C\Delta \rho^2 \upsilon^2 M W}{N_A} \] (3)

\( I(0) \) was obtained from equation (2). The molecular weight of the aggregate \( (M_w) \) was calculated from the mass per unit volume \( (C) \), contrast \( (\Delta \rho_M) \), specific volume \( (\upsilon) \) of 1d, and Avogadro’s number \( (N_A) \).\(^3\)

**Table S4.1.** Structural parameters extracted from the SAXS profiles of the squaramide-based bolaamphiphiles 1a, 1b and 1d.

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

* Estimated overall aggregation number.
Figure S4.8: Casassa–Holtzer plot of the scattering profiles in Figure S4.7 for 1a (a) and 1b (b). The Holtzer plateaus (0.0065 ≤ q ≤ 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 ≤ q ≤ 0.0245 Å⁻¹) is indicated by the red and black lines.
4.6.6. UV-vis spectroscopy

UV-Vis samples were prepared from stock solutions of the various squaramide-based bolaamphiphiles 1a-d and 2a-d (5.8 mM) equilibrated overnight prior to their dilution at the measuring concentration (30 μM).

Figure S4.9. Normalized UV-Vis spectra of 1a (a), 1b (b), 1c (c) and 1d (d) in water (blue) and HFIP (green) at 30 μM.
Figure S4.10. Normalized UV-Vis spectra of 2a (a), 2b (b), 2c (c) and 2d (d) in water (blue) and HFIP (green) at 30 μM.
4.6.7. Fourier transform infrared (FTIR)

Solutions of the various squaramide-based bolaamphiphiles (5.8 mM) were prepared in D₂O and HFIP-d₂ and left to equilibrate overnight before measurement.

Figure S4.11. FTIR spectra recorded for 1a (a), 1b (b), 1c (c) and 1d (d) in D₂O and HFIP-d₂ 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.
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Figure S4.12. FTIR spectra for 2a (a), 2b (b), 2c (c) and 1d (d) in D$_2$O 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 S4.13. FTIR spectrum of 1a in D$_2$O with increasing temperature from 25 ºC (blue line) to 65 ºC (red line). Inset: NH region (3200-3120 cm$^{-1}$).
4.6.8. Fluorescence spectroscopy

Examination of hydrophobic domains: 7 µL of a 15 µM (0.005 mg/mL) stock solution of Nile Red in CH$_3$OH was spotted in the wells of a 96-well plate (PP Microplate, solid F-bottom (flat), chimney well) and was placed under vacuum for at least four hours. Once the solvent was completely removed, 100 µL of the various squaramide-based bolaamphiphiles (stock solution: 30 µM) were added to the wells pre-spotted with Nile Red. The solutions were shaken vigorously in the Tecan plate reader (300 seconds, 654 rpm, linear mode) and then allowed to equilibrate overnight at room temperature. Fluorescence measurements were collected at excitation wavelength of 550 nm and an emission wavelength from 570 to 700 nm.

![Molecular structure of Nile Red (NR).](image)

Figure S4.14. Molecular structure of Nile Red (NR).
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4.6.9. References

