ELECTRONIC SUPPLEMENTARY INFORMATION FOR:

Tuning the aqueous self-assembly of multistimuli-responsive polyanionic peptide nanorods

Maximilian von Gröning,[a] Isja de Feijter,[b,c] Marc C.A. Stuart,[d] Ilja K. Voets[b] and Pol Besenius*[a]

[a] *Organic Chemistry Institute and CeNTech, Westfälische Wilhelms-Universität Münster, Corrensstrasse 40, 48149 Münster, Germany. Tel: +49 251 83 63928.*

[b] *Institute for Complex Molecular Systems and Laboratory of Macromolecular and Organic Chemistry, Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven, The Netherlands.*

[c] *Laboratory of Chemical Biology, Department of Biomedical Engineering, Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven, The Netherlands.*

[d] *Department of Biophysical Chemistry, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands.*

p.besenius@uni-muenster.de

Figures S1 – S5

CD

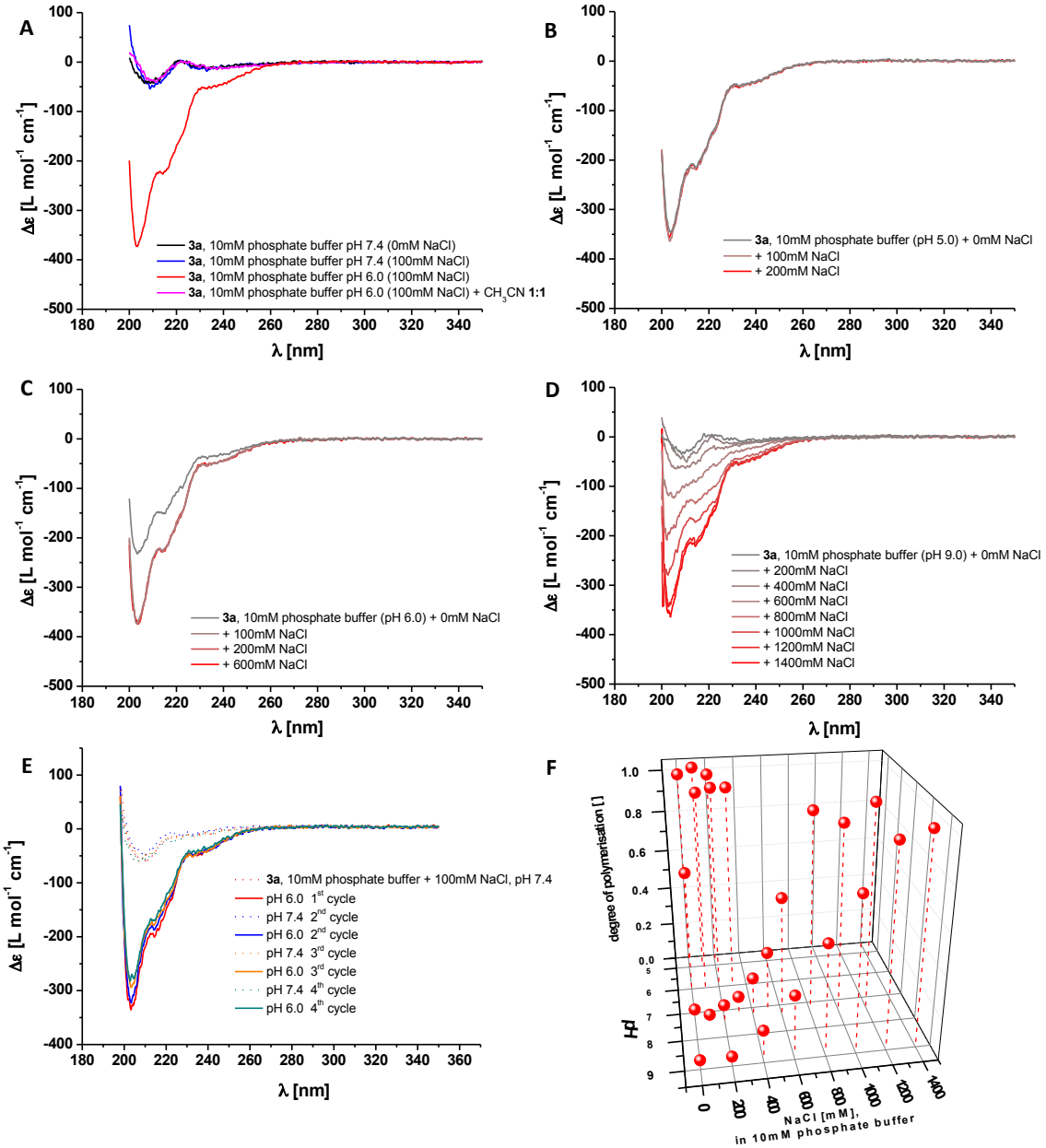


Figure S1. CD spectra for dendritic peptide amphiphile **3a** ($2x10^{-5}$ M) in 10 mM phosphate buffer at 293 K: **A)** 0 mM NaCl and 100 mM NaCl at pH 7.4, 100 mM NaCl at pH 6.0 and in a mixture of buffer (10 mM phosphate, 100 mM NaCl at pH 6.0) with CH_3CN (1:1), all at 293 K; **B)** ionic strength dependent CD spectra at pH 5.0 in 10 mM phosphate buffer; **C)** ionic strength dependent CD spectra at pH 6.0 in 10 mM phosphate buffer; **D)** ionic strength dependent CD spectra at pH 9.0 in 10 mM phosphate buffer; **E)** pH dependent CD spectra in 10 mM phosphate buffer, switched four times between pH 7.4 and pH 6.0. **F)** The dimensionless degree of aggregation (0 referring to the molecularly dissolved state and 1 to a fully polymerized system, monitored at the CD band of **λ=**204 nm) of **3a** (2×10^{-5} M)**,** in 10 mM phosphate buffer at 293 K, plotted as a function of the pH and the concentration of added NaCl.

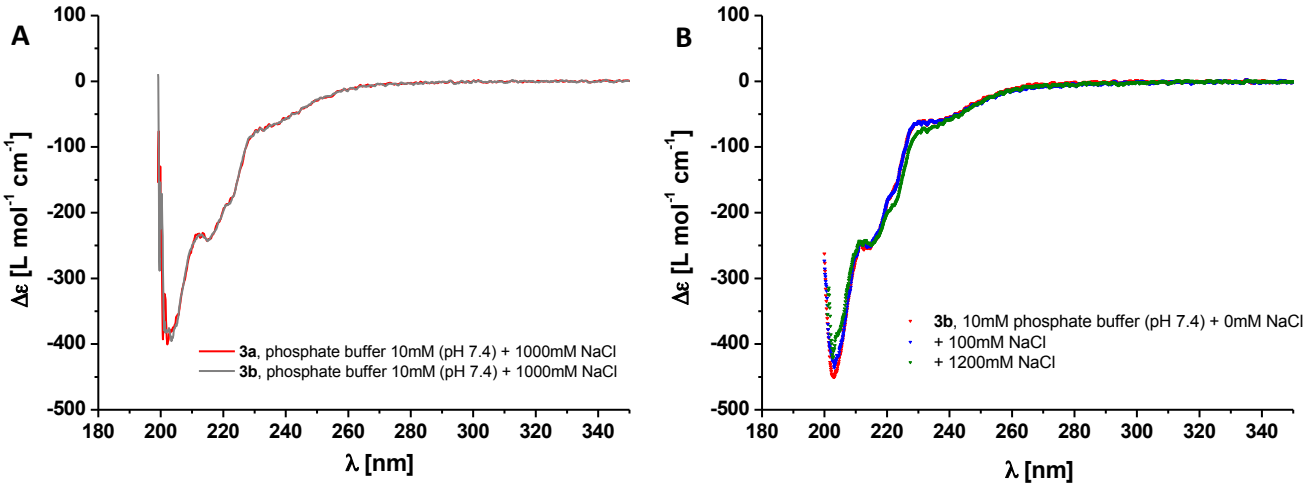


Figure S2. **A)** CD spectra for dendritic peptide amphiphiles **3a** and **3b** at pH 7.4 in 10 mM phosphate buffer, at 1000 mM NaCl; **B)** Ionic strength dependent CD spectra for the neutral dendritic peptide amphiphile **3b** at pH 7.4 in 10 mM phosphate buffer at 293 K, 0 mM NaCl, 100 mM NaCl and 1200 mM added NaCl.

SAXS

The *q*-dependence of the scattering intensity is given by the general equation:

$$I(q) = \frac{c\Delta\rho^2 \nu^2 M_W}{N_{AV}} P(q) S(q) \qquad (0.0)$$

with the weight concentration, *c* in g/l[3], the contrast, Δρ in cm^{-2}, the partial specific volume in cm^3/g, the molecular weight, M_W in g/mol, Avogadro's number, N_{AV} in mol^{-1}, the form factor, $P(q)$ and the structure factor, $S(q)$, and the magnitude of the scattering vector, q/Å^{-1}, defined as

$$q = \frac{4\pi}{\lambda} \sin\left(\frac{\vartheta}{2}\right) \qquad (0.0)$$

with the wavelength of the incident X-rays, λ in Å, and the scattering angle θ.

As discussed in the main text, the small angle X-ray scattering (SAXS) profiles are characteristic of one-dimensional rod-like objects with length, *L*, and cross-sectional radius, R_{cs}. The SAXS profiles are well described with a form factor $P(q)$ for rigid cylinders in the intermediate to high *q*-regime where we assume $S(q) = 1$ (Fig. S3), which allows us to extract a value for R_{CS} = 2.8 nm. However, the length of the nanorods cannot be quantified based on these fits, as it is larger than the experimental resolution of roughly $1/q_{min} \sim 1/0.06$ nm$^{-1} \sim 17$ nm and moreover, $S(q) \neq 1$ in the low-*q* regime.
To rationalize the non-monotonic dependence of the forward scattering intensity, $I(0)$ on the ionic strength, we need to take into account the effect of the NaCl concentration on the average M_w of the self-assembled nanorods and on the rod-rod interactions described by $S(q)$. Scattering experiments on other self-assembled one-dimensional objects, such as wormlike micelles, generally reveal two different regimes as a function of an external parameter – typically monomer or salt concentration - that controls the average length of the self-assemblies.[1, 2] For relatively short objects, the forward scattering intensity increases with increasing concentration as the average M_w increases as the self-assemblies grow in length. Above a given threshold concentration, the structure factor depresses the forward scattering intensity as the assemblies start to overlap and form a transient network. Figures S3 and S5 show very similar behaviour for our charged peptide nanorods **3a**. At low NaCl concentrations, c_{NaCl}, the forward scattering intensity $I(0)$ increases with increasing c_{NaCl} reflecting the increase in the average M_W of the aggregates, while $I(0)$ decreases with increasing c_{NaCl} at high c_{NaCl} as the rods start to touch and overlap. The threshold NaCl concentration above which $I(0)$ decreases with increasing c_{NaCl} is much lower for the neutral peptides **3b** which are already rather long at low c_{NaCl}.

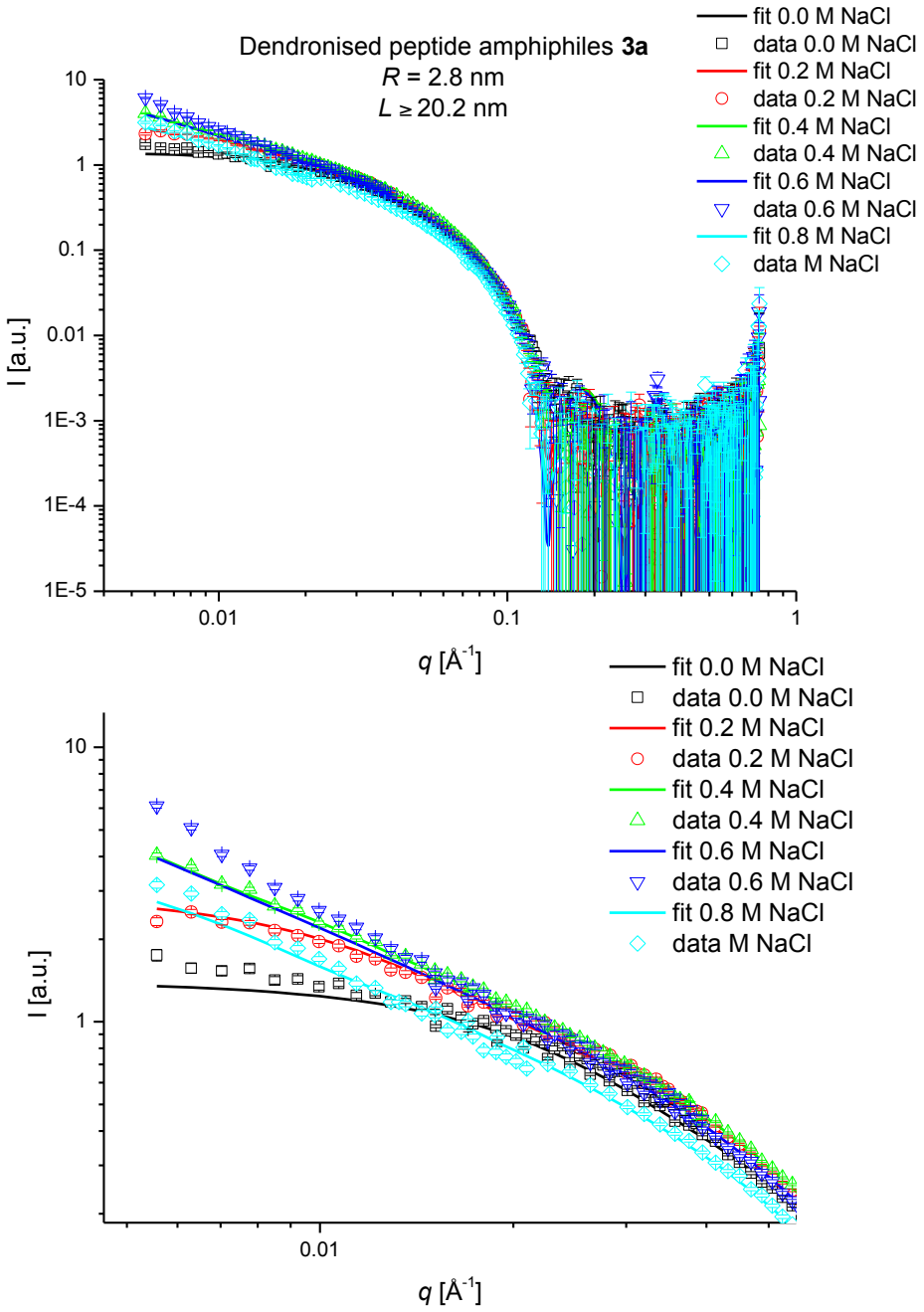


Figure S3. Small Angle X-Ray Scattering profiles (symbols) and fits using a form factor describing a rigid cylinder (lines) for the dendritic peptide amphiphile **3a** (10 mg/ml), in 20 mM phosphate buffer (pH 7.4) at 293 K, and variable NaCl concentrations: 0 M NaCl, 0.2 M NaCl, 0.4 M NaCl, 0.6 M NaCl and 0.8 M added NaCl; lower graph is zoomed in on the low-*q* portion of the scattering profiles.

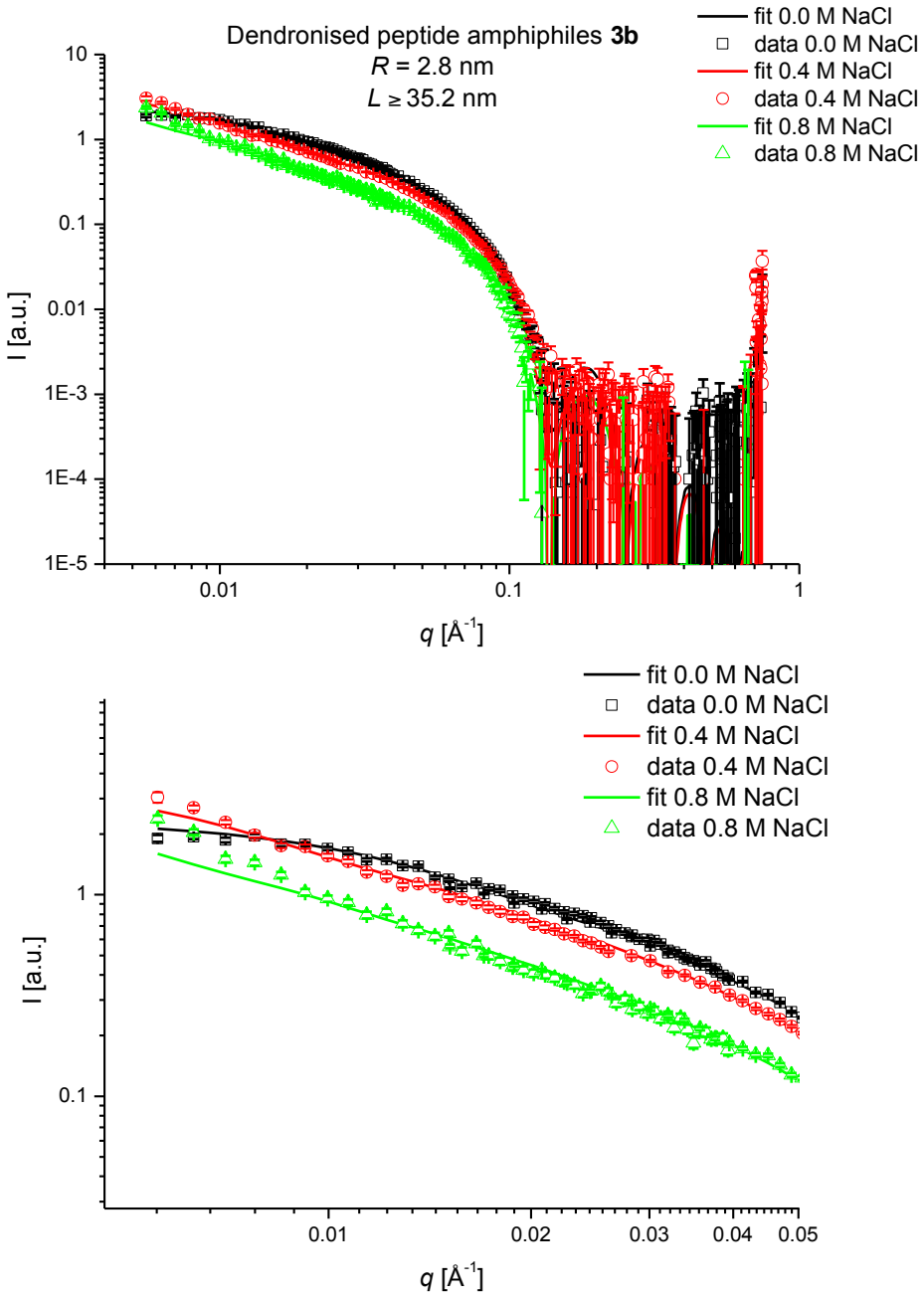


Figure S4. Small Angle X-Ray Scattering profiles (symbols) and fits using a form factor describing a rigid cylinder (lines) for the dendritic peptide amphiphile **3b** (10 mg/ml), in 20 mM phosphate buffer (pH 7.4) at 293 K, and variable NaCl concentrations: 0 M NaCl, 0.4 M NaCl and 0.8 M added NaCl; lower graph is zoomed in on the low-*q* portion of the scattering profiles.

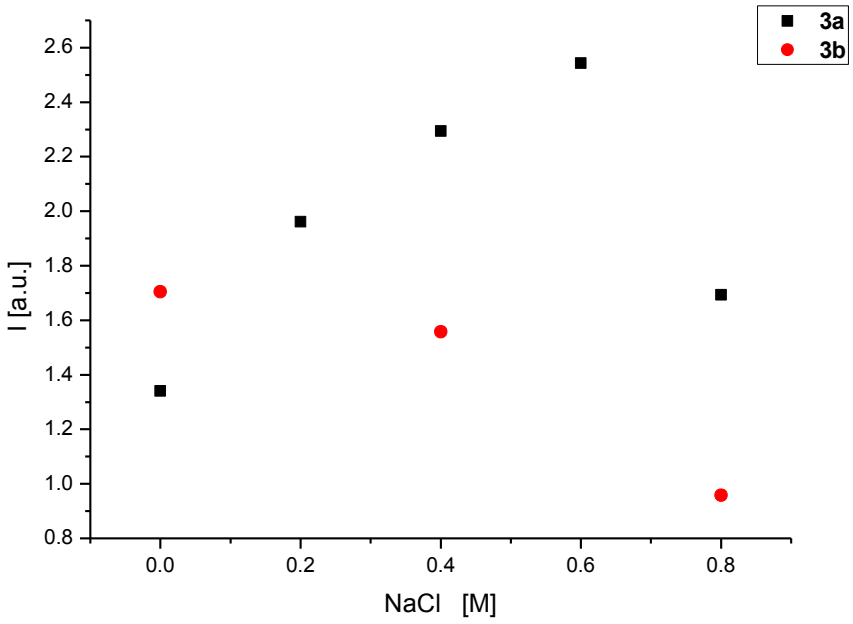


Figure S5. Forward scattering intensity (here approximated by the intensity at q = 0.01) as a function of NaCl concentration for **3a** and **3b**.

Experimental Section

Materials

Unless stated otherwise, all reagents and chemicals were obtained from commercial sources at the highest purity available and used without further purification. Water was demineralised prior to use. Some solvents were dried using the following drying agents: dichloromethane over sodium hydride, tetrahydrofurane over sodium and benzophenone, Methanol over molecular sieves 3 Å, *N,N*-dimethylformamide over molecular sieves 3 Å.
Purification via preparative column chromatography was carried out using silica-gel with an average grain size of 15-40 µm (MERCK). Solvents which were used as a mobile phase were used without further purification. Analysis of the collected fractions was performed via TLC on silica coated aluminum sheets (60 F254, MERCK).
The Solid phase peptide synthesis (SPPS) was carried out on a Peptide Synthesizer SP 4000 (LABORTEC AG) using 2 chloro-tritylchloride resin and SPPS-grade reagents and solvents.

Instrumentation

The NMR-spectra were recorded on the spectrometer ARX 300 (BRUKER). All measurements were carried out in deuterated solvents. The chemical shift (δ) is recorded in parts per million (ppm) and relative to the residual solvent protons. The measured coupling constants were calculated in Hertz (Hz). To analyse the spectra the software MESTRENOVA 6.2.1 was used. The description of the signals was done as follows: s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet and m = multiplet.

Mass spectra were recorded on the electronspray ionization spectrometers (ESI) Micro Tof (BRUKER) and Orbi-Trap LTQ-XL (THERMO SCIENTIFIC) using methanol as solvent.

CD-spectra were recorded on a J-815 (JASCO) using the software Spectra Manager 2.08.04 and processed with Origin Pro.

X-Ray scattering measurements were performed on a Ganesha lab instrument equipped with a GeniX-Cu ultra low divergence source producing X-ray photons with a wavelength of 1.54 Å and a flux of $1x10^8$ ph/s. Scattering patterns were collected using a Pilatus 300K silicon pixel detector with 487 x 619 pixels of 172 μm^2 in size placed at a sample-to-detector distance of 480 and 1080 mm respectively. . The beam centre and the *q* range were calibrated using the diffraction peaks of silver behenate. The liquid samples were contained in 1 mm quartz capillaries.

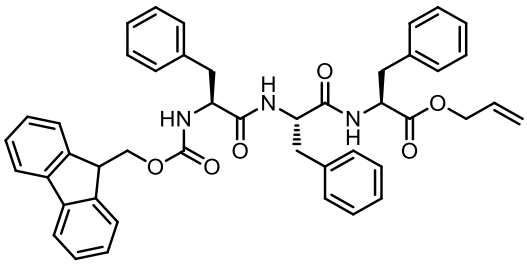
Samples for cryogenic Transmission Electron Microscopy (Cryo-TEM) were prepared by deposition of a few microliters of 5 mg/ml solutions of dendritic peptide amphiphiles on glow-discharged holey carbon-coated grids (Quantifoil 3.5/1, Quantifoil Micro Tools, Jena, Germany). After the excess liquid was blotted at 100% humidity and 22 °C, the grids were vitrified in liquid ethane (Vitrobot, FEI, Eindhoven, The Netherlands). The vitrified specimens were mounted in a liquid nitrogen-cooled Gatan 626 cryo-holder (Gatan Inc., Pleasanton, CA) and inserted in the electron microscope. Low-dose images were recorded with a Gatan 4K slow-scan CCD camera (Pleasanton, CA) on a Philips CM 120 electron microscope (FEI, Eindhoven, The Netherlands) equipped with a LaB_6 tip operated at 120 kV.

Synthetic procedures

Synthesis of the 2-chlorotrityl resin bound triphenylalanine via SPPS

The loading of the resin was performed according to a procedure described in literature.[3-5] The appropriate Fmoc-protected amino acid (9 mmol, 3.00 eq.) was dissolved in 20 mL DCM-DMF (4:1) and added to the 2-chlorotrityl-chloride resin (2.00 g, 3 mmol) followed by the addition of 1.20 mL DIPEA. After stirring for 5 min at room temperature an additional 6 mL of DIPEA was added. The reaction mixture was stirred for 1 h at room temperature and afterwards treated with 2 mL MeOH. The vessel was drained and the beads were washed consecutively three times each with DCM, DMF, DCM and MeOH. Afterwards the beads were dried under vacuum over night. The following step-wise chain elongation was performed according to literature.[6]
The dried beads were swollen in DMF p.a. for 45 min while shaking the reaction vessel. After draining the solution, piperidine (20% in DMF) was added and the vessel was shaken for 20 min. After draining of the vessel the beads were washed four times with DMF and twice with DCM and isopropanol. To investigate the success of the deprotection, free amine groups were detected via the KAISER-test. Equal amounts of a 5% ninhydrin solution in EtOH, 80% phenole in EtOH and 0.001 M KCN in pyridine were mixed with a few beads and heated for 1 min to 100 °C. A blue color of the beads indicated free amine functionalities on the resin.
The resin was treated with a solution of the corresponding protected amino acid (9 mmol, 3.0 eq.), DIPCDI (1.60 mL, 9.9 mmol, 3.3 eq.) and ethyl-2-cyano-hydroxyiminacetat (Oxyma pure®) (1.44 g, 10.8 mmol, 3.6 eq.), all dissolved in DMF. After shaking for 45 min the solution was removed and the resin was washed five times with DMF, before another KAISER-test was carried out. A negative KAISER-test indicated the complete conversion of the amine groups, therefore a positive KAISER-test required a repetition of the coupling process, starting with the addition of the amino acid solution. This procedure was repeated with the Fmoc-Phe-OH for every coupling process, starting with the Fmoc deprotection on the resin.

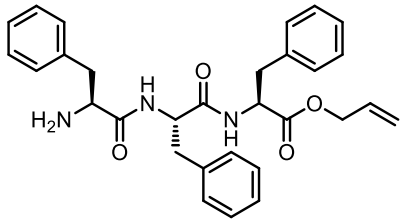
Fmoc-$(Phe)_3$-allylester 1[7]



Acetyl chloride (50 mL; 42.5 g; 0.73 mol) was added slowly to allyl alcohol (50 mL; 55.0 g; 0.70 mol) at 0 °C and the resulting solution was stirred for 2 h. Fmoc-protected triphenylalanine bound to the 2-chlorotrityl resin (1.9 g; 2.8 mmol) was swollen in about 10 mL DCM and then added to 40 mL of the HCl/allyl alcohol solution. After stirring for 12 h at room temperature the solution was drained and the resin washed three times with DCM. The organic solvent was removed under reduced pressure. The resulting sticky oil was purified via flash chromatography over SiO_2 [DCM/THF (0-50%)]; R_f [DCM/EtOAc (25%)] = 0.71.

Yield: 1,632 g; 2.26 mmol (81 %), $C_{45}H_{43}N_3O_6$, white solid.
^{1}H-NMR (300 MHz, DMSO-d_6, 298 K): δ [ppm]= 2.60-3.10 (m, 6H, CH_2^{benzyl}); 3.85-4.30 (m, 4H, $CH^{fluorenyl}CH_2$, $CH_2CH{=}CH_2$); 4.44-4.68 (m, 4H, NCHCO, $CH^{fluorenyl}$); 5.13-5.20 (m, 1H, CH=C*H*^{trans}); 5.20-5.31 (m, 1H, CH=C*H*^{cis}); 5.73-5.89 (m, 1H, C*H*=CH_2); 6.90-7.48/7.50-7.66/7.79-7.94 (m, 23H, $CH^{aromatic}$); 7.97-8.41 (m, 2H, NHCO); 8.58-8.69 (m, 1H, $NHCO_2$)
ESI-MS (positive mode): *m/z* calcd. for $[M+Na]^+$ 744.3044; found: 744.3031 $[M+Na]^+$.

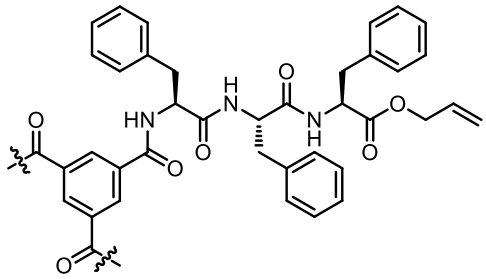
H_2N-(Phe)$_3$-allylester 1.2



1 (879 mg; 1.52 mmol) was dissolved in ACN (40 mL). Piperidin (6 mL) was added slowly at 0°C, and the solution was stirred for 1 h at room temperature. The solvent was removed under reduced pressure and the residue purified via flash chromatography over SiO_2 [EtOAc/DCM/MeOH (4:2:0 – 4:2:1)], R_f (EtOAc/DCM/MeOH 4:2:1) = 0.68.

Yield: 378 mg; 0.76 mmol (50 %), $C_{27}H_{29}N_3O_4$, white solid.
^{1}H-NMR (300 MHz, $CDCl_3$, 298 K): δ [ppm]= 2.62-3.19 (m, 6H, CH_2^{benzyl}); 3.62-3.80 (m, 1H, H_2N-C*H*); 4.47-4.84 (m, 4H, NCHCO, $CH_2CH{=}CH_2$); 5.18-5.28 (m, 1H, CH=C*H*^{cis}); 5.70-5.91 (m, 1H, CH=C*H*^{trans}); 6.55-6.69 (m, 1H, C*H*=CH_2); 6.98-7.40 (m, 15H, $CH^{aromatic}$)
ESI-MS (positive mode): *m/z* calcd. for $[M+H]^+$ 500,2544; found: 500,2546 $[M+H]^+$; *m/z* calcd. for $[M+Na]^+$ 522.2363; found: 522.2363 $[M+Na]^+$.

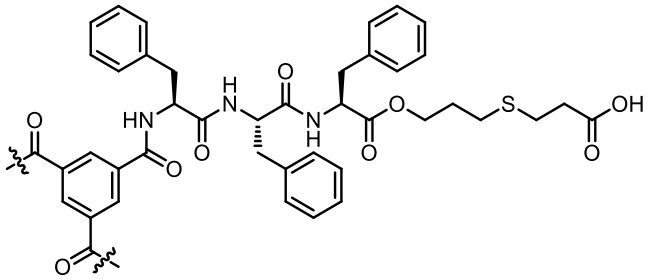
BTA–[(Phe)$_3$-allylester)]$_3$ 1.3



1.2 (362 mg; 0.68 mmol; 4 eq.) and benzene-*1,3,5*-tricarbonylchloride (45 mg; 0.17 mmol; 1 eq.) were disolved in dry DCM (10 mL) under Argon atmosphere. DIPEA (1.6 mL; 6.8 mmol; 40 eq.) was added, as well as 1 mL of dry DMF in order to avoid precipitation of the product, and the reaction mixture stirred for 18 h at room temperature. The solvent was removed under reduced pressure and the residue purified via precipitation from THF into diethylether, isolated via centrifugation, followed by diethylether and ACN washing steps. The product was purified further by flash chromatography over SiO_2 [DCM/MeOH (0-5%)], R_f (DCM/5% MeOH) = 0.78.

Yield: 176 mg; 0.11 mmol (65 %), $C_{99}H_{99}N_9O_{15}$, white solid.
^{1}H-NMR (300 MHz, DMSO-d_6, 298 K): δ [ppm]= 2.70-3.08 (m, 18H, CH_2^{benzyl}); 4.47-4.78 (m, 15H, $OCH_2CH=CH_2$, NCH); 5.11-5.20 (m, 3H, $CH=CH^{cis}$); 5.20-5.30 (m, 3H, $CH=CH^{trans}$); 5.72-5.87 (m, 3H, $CH=CH_2$); 6.94-7.33 (m, 45H, CH^{phenyl}); 8.15 (s, 3H, CH^{BTA}); 8.17-8.23 (m, 3H, NH); 8.50-8,72 (m, 6H, NH)
ESI-MS (positive mode): *m/z* calcd. for $[M+Na]^+$ 1676.7153; found: 1676.7122 $[M+Na]^+$.

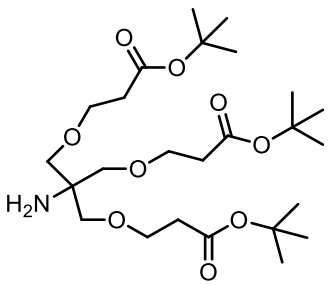
BTA-[(Phe)$_3$-3-((3-carboxypropyl)thio)propionic acid]$_3$ 2



1.3 (166 mg; 0.11 mmol; 1 eq.) and 2,2-dimethoxy-2-phenylacetophenone (DMPA) (41 mg; 0.16 mmol; 1.5 eq.) were dissolved in freshly distilled, inhibitor free, THF (10 mL) under argon atmosphere. 1 mL of dry DMF (SPPS grade) was added in order to avoid precipitation of the product. Mercaptopropionic acid (0.14 mL; 1.59 mmol; 15 eq.) was added and the reaction mixture illuminated with UV light (λ=365 nm) under stirring in a UV chamber for 48 h. The solvent was removed under reduced pressure and the residue purified via precipitation from THF into diethylether, isolated via centrifugation, followed by diethylether and ACN washing steps.

Yield: 172 mg; 0.09 mmol (82 %), $C_{108}H_{117}N_9O_{21}S_3$, white powder.
^{1}H-NMR (300 MHz, DMSO-d_6, 298 K): δ [ppm]= 1.60-1.80 (m, 6H, OCH_2CH_2); 2.32-2.48 (m, 12H, $OCH_2CH_2CH_2S$, SCH_2CH_2COOH); 2.58-2.68 (m, 6H, SCH_2CH_2COOH); 2.76-3.12 (m, 18H, CH_2^{benzyl}); 4.03 (t, ^{3}J=4.0 Hz, 6H, OCH_2); 4.47-4.76 (m, 9H, NCHCO); 6.80-7.43 (m, 45H, CH^{phenyl}); 8.16 (s, 3H, CH^{BTA}); 8.20-8.33 (m, 3H, NH); 8.577 (d, ^{3}J=8.6 Hz, 3H, NH); 8.66-8.81 (m, 3H, NH)
ESI-MS (positive mode): *m/z* calcd. for $[M+Na]^+$ 1995.7502; found: 1995.7467 $[M+Na]^+$.

Tris{[2-(*tert*-butoxycarbonyl)ethoxy]methyl}methylamine 4.1[8]

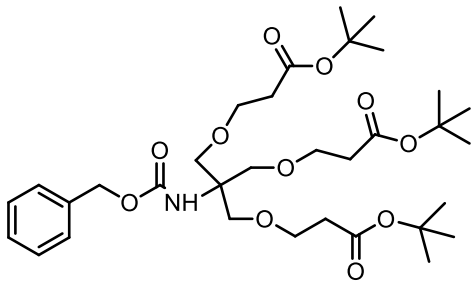


Tris(hydroxymethyl)aminomethane (Tris) (1.21g; 10 mmol; 1eq.) was dissolved in DMSO (2 mL) and cooled to 15°C under Argon atmosphere. Under stirring 5 M NaOH (0.2 mL)

was added first, followed by dropwise addition of *tert*-butyl acrylate (5.0 mL; 34 mmol; 3.4 eq.). The reaction mixture was stirred at room temperature for 24 h. The solvents (except DMSO) were then removed under reduced pressure and the residue purified via flash chromatography over SiO_2 [EtOAc/cyclohexane (2:1) + 0,05 vol% NH_4OH], R_f [EtOAc/Cyclohexane (2:1) + 0,05 vol% NH_4OH] = 0.43.

Yield: 1.646 g; 3.26 mmol (33 %), $C_{25}H_{47}NO_9$, colourless oil.
^{1}H-NMR (300 MHz, $CDCl_3$, 298 K): δ [ppm]= 1.39 (s, 27H, -CH_3^{tBu}); 2.40 (t, ^{3}J=6.3 Hz, 6H, OCH_2CH_2); 3.26 (s, 6H, CCH_2O); 3.59 (t, ^{3}J=6.3 Hz, 6H, OCH_2CH_2).
^{13}C-NMR (75 MHz, $CDCl_3$, 298 K): δ [ppm]= 28.20 ($(CH_3)C$); 36.40 (CH_2CH_2O); 56.12 (CCH_2O); 67.22 (OCH_2CH_2); 72.86 (CCH_2O); 80.52 ($C(CH_3)_3$); 171.02 (*C*O*t*Bu).
ESI-MS (positive mode): *m/z* calcd. for $[M+H]^+$ 506.3324; found: 506.3322 $[M+H]^+$.

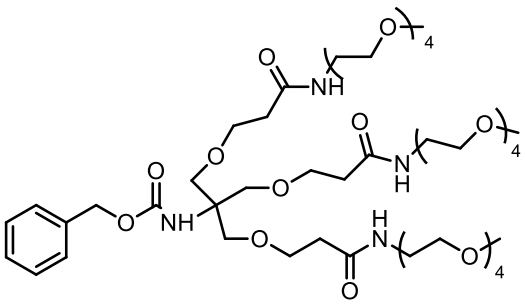
Benzyl *N*-Tris{[2-(*tert*-Butoxycarbonyl)ethoxy]methyl}methyl-carbamate 4.2



The amine **4.1** (1.000 g; 1.98 mmol; 1 eq.) was dissolved in CH_2Cl_2 (15 mL) and 25% aqueous Na_2CO_3 (7.5 mL) was added while stirring. Then, the benzyl chloroformate (0.88 mL; 6.19 mmol; 3.1 eq.) was added dropwise and the reaction mixture was stirred for 48 h. The product was extracted with CH_2Cl_2, dried over $MgSO_4$ and the solvent removed under reduced pressure. The residue was purified by flash column chromatography over SiO_2 (cyclohexane/EtOAc 2:1, R_f= 0.56).

Yield: 1.097 g; 1.71 mmol (86 %), $C_{33}H_{53}NO_{11}$, colourless oil.
^{1}H-NMR (300 MHz, $CDCl_3$, 298 K): δ [ppm]= 1.36 (s, 27H, -CH_3^{tBu}); 2.36 (t, ^{3}J=6.3 Hz, 6H, OCH_2CH_2); 3.54-3.59 (m, 12H, OCH_2CH_2, CCH_2O); 4.96 (s, 2H, CH_2^{benzyl}); 7.20-7.32 (m, 5H, $CH^{aromatic}$).
^{13}C-NMR (75 MHz, $CDCl_3$, 298 K): δ [ppm]= 28,19 ($(CH_3)C$); 36.32 (CH_2CH_2O); 56.82 (CCH_2O); 66.21 (CH_2^{benzyl}); 67.18 (CH_2CH_2O); 69.47 (CCH_2O); 80.58 ($C(CH_3)_3$); 127.99, 128.08, 128.50, 135.20 ($CH^{aromat.}$); 155.20 (CONH); 170.94 (COO*t*-Bu).
ESI-MS (positive mode): *m/z* calcd. for $[M+Na]^+$ 662.3511; found: 662.3509 $[M+Na]^+$.

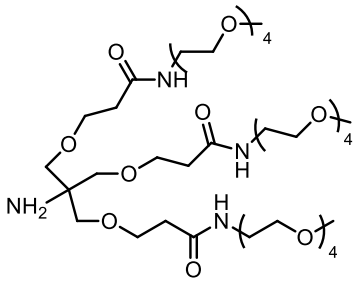
Benzyl *N*-Tris{[2-(tetraethyleneglycol amido)ethoxy]methyl}methyl-carbamate 4.3



4.2 (1.072 g; 1.67 mmol) was dissolved in DCM (5 mL) under Argon. TFA (5 mL) was added and the reaction stirred for 1 h at room temperature. The solvents were then removed under reduced pressure. The reaction was repeated in DCM-TFA 1:1 (10 mL), followed by evaporation under reduced pressure.
After drying the deprotected product under high vacuum, the residue was dissolved in SPPS grade DMF (15 mL) under argon, followed by the addition of PyBOP (4.538 g; 8.72 mmol; 6 eq.), DIPEA (15.0 mL; 87 mmol; 60 eq.) and tetraethyleneglycol (TEG) amine (1.68 g; 8.11 mmol; 5.6 eq.) dissolved in SPPS grade DMF (5 mL). The reaction mixture was stirred for 24 h at room temperature. The solvent was removed under reduced pressure and the residue purified via flash chromatography over SiO_2 [EtOAc/MeOH (0-30%)], R_f [EtOAc/MeOH (7:3)] = 0.10.

Yield: 619 mg; 0.56 mmol (34 %), $C_{48}H_{86}N_4O_{20}$, pale yellow oil.
^{1}H-NMR (300 MHz, $CDCl_3$, 298 K): δ [ppm]= 2.29-2.53 (t, ^{3}J=6.2 Hz, 6H, CH_2C=O); 3.29-3.46 (m, 15H, CH_3, NC*H*$_2$CH$_2$); 3.46-3.78 (m, 54H, CH_2O); 5.01 (s, 2H, CH_2^{benzyl}); 7.27-7.35 (m, 5H, $CH^{aromatic}$).
^{13}C-NMR (75 MHz, $CDCl_3$, 298 K): δ [ppm]= 36.62 (OCH$_2$*C*H$_2$C=O); 39.29 (NCH$_2$*C*H$_2$O); 59.09 (CH_3); 66.30 (N*C*(CH$_2$)$_3$); 67.47 (CH_2^{benzyl}); 69.45 (NC(*C*H$_2$)$_3$); 70.04, 70.18, 70.42, 70.49 (OCH$_2$*C*H$_2$O); 155.30 (COO); 125.95, 128.10, 128.54, 136.66 ($C^{aromatic}$); 171.69 (NC=O).
ESI-MS (positive mode): *m/z* calcd. for $[M+Na]^+$ 1061.5776; found: 1061.5728 $[M+Na]^+$.

Tris{[2-(tetraethyleneglycol amido)ethoxy]methyl}methylamine 4.4

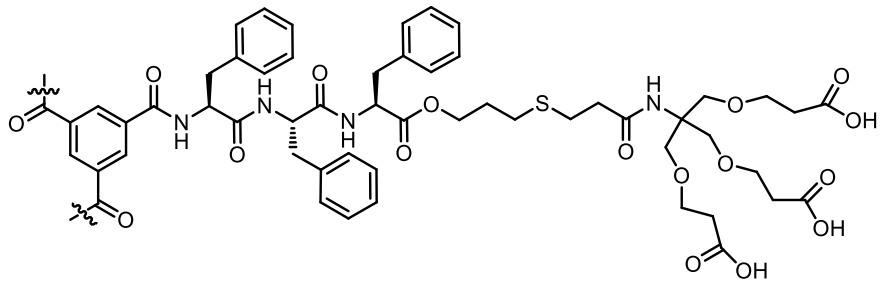


4.3 (619 mg; 0.60 mmol) was dissolved in MeOH (10 mL) and Pd/C (62 mg; 10 wt%) was added. The reaction mixture was stirred under a H_2-atmosphere for 12 h. The catalyst was

removed via filtration over celite, washed thoroughly with MeOH and the filtrate evaporated under reduced pressure.

Yield: 506 mg; 0.56 mmol (93 %), $C_{40}H_{80}N_4O_{18}$, pale yellow oil.
^{1}H-NMR (300 MHz, $CDCl_3$, 298 K): δ [ppm]= 2.41 (t, ^{3}J=5.8 Hz, 6H, CH_2C=O); 3.33 (s, 9H, CH_3); 3.36-3.45 (m, 12 H, NC*H*$_2$C*H*$_2$O); 3.45-3.62 (m, 42 H, NC(CH_2)$_3$, CH_2^{TEG}); 3.67 (t, ^{3}J= 5.8 Hz, 6H, , OC*H*$_2$$CH_2C$=O).
^{13}C-NMR (75 MHz, $CDCl_3$, 298 K): δ [ppm]= 36.55 (*C*H_2CO); 39.24 (NCH_2); 50.52 (N*C*(CH_2)$_3$; 59.00 (CH_3); 67.66 (O*C*$H_2$$CH_2C$=O); 69.99, 70.38, 70.42, 70.47 (O*C*H_2*C*H_2O); 71.88 (NC(*C*H_2)$_3$); 171.72 (C=O).
ESI-MS (positive mode): *m/z* calcd. for $[M+H]^+$ 905.5546; found: 905.5534 $[M+H]^+$, *m/z* calcd. for $[M+Na]^+$ 927.5360; found: 927.5355 $[M+Na]^+$.

BTA-{(Phe)$_3$-3-[(3-carboxypropyl)thio] propyl (tris[(2-carboxyethoxy)methyl]methylamide)}$_3$ 3a



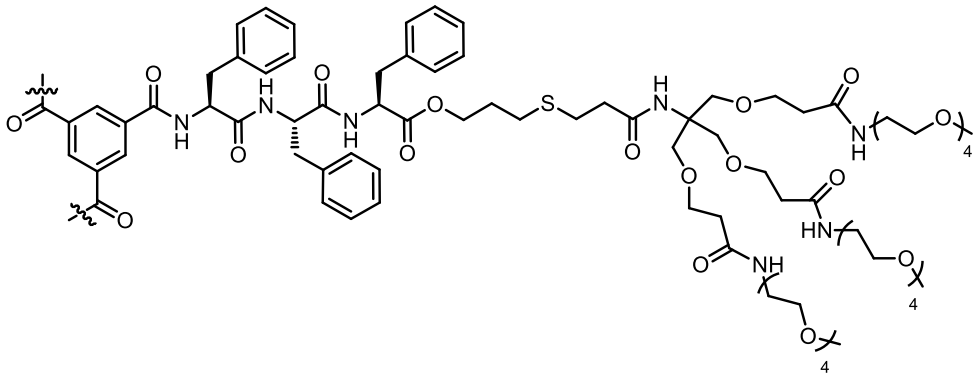
2 (46 mg; 0.02 mmol; 1 eq.), PyBOP (48 mg; 0.09 mmol; 4.5 eq.) and **4.1** (47 mg; 0.09 mmol; 4.5 eq.) were dissolved under argon in SPPS grade DMF (5 mL) and DIPEA (3 mL; 0.17 mmol; 8.5 eq.) were then added. The reaction mixture was stirred at room temperature for 18 h. The solvent was removed under reduced pressure and the residue purified via precipitation from THF into diethylether, isolated via centrifugation, followed by two washing steps with diethylether. The product was purified further by flash chromatography over SiO_2 (EtOAc), R_f= 0.86.
ESI-MS (positive mode): *m/z* calcd. for $[M+4Na]^{4+}$ 881.5416; found: 881.9156 $[M+4Na]^{4+}$; *m/z* calcd. for $[M+3Na]^{3+}$ 1167.8896; found: 1168.2244 $[M+3Na]^{3+}$; *m/z* calcd. for $[M+2Na]^{2+}$ 1740.3396; found: 1740.8414 $[M+2Na]^{2+}$.

The *tert*-butyl protecting groups were then removed by dissolving the purified product in DCM (3 mL), followed by the addition of TFA (3 mL) and stirring at room temperature for and 1 h. The solvents were then removed under reduced pressure and the deprotection reaction repeated in DCM-TFA 1:1 (6 mL), followed by evaporation under reduced pressure. The residue was purified via precipitation from DMF into diethylether, isolated via centrifugation, followed by two washing steps with diethylether and drying under high vacuum.

Yield: 67 mg; 0.02 mmol (95 %), $C_{147}H_{180}N_{12}O_{45}S_3$, white solid.
^{1}H-NMR (300 MHz, DMSO-d_6, 298 K): δ [ppm]= 1.54-1.89 (m, 6H, OCH_2C*H*$_2$$CH_2S$); 2.29-2.37 (m, 6H, OCH_2CH_2C*H*$_2$S); 2.37-2.45 (m, 18H, OCH_2C*H*$_2$COOH); 2.55-2.64 (m, 6H, SCH_2C*H*$_2$CON); 2.70-3.19 (m, 24H, 6H S*C*H_2CH_2CON, 18H C*H*$_2^{benzyl}$); 3.50-3.66 (m, 36H, C*H*$_2$O*C*H_2); 4.04 (t, ^{3}J=6.2 Hz, 6H, O*C*$H_2CH_2CH_2S$); 4.44-4.48 (m, 9H, α-CH); 6.95-7.40 (m,

48, 45 $H^{aromatic}$, 3 NH); 8.15 (s, 3H, CH^{BTA}); 8.25 (d, ^3J=8.1 Hz, 3H, NH); 8.57 (d, ^3J=7.4 Hz, 3H, NH); 8.65 (d, ^3J=8.2 Hz, 3H, NH).

BTA-{(Phe)$_3$-3-[(3-carboxypropyl)thio]propyl (tris{[2-(tetraethyleneglycol amido)ethoxy]methyl}methylamide)}$_3$ 3b

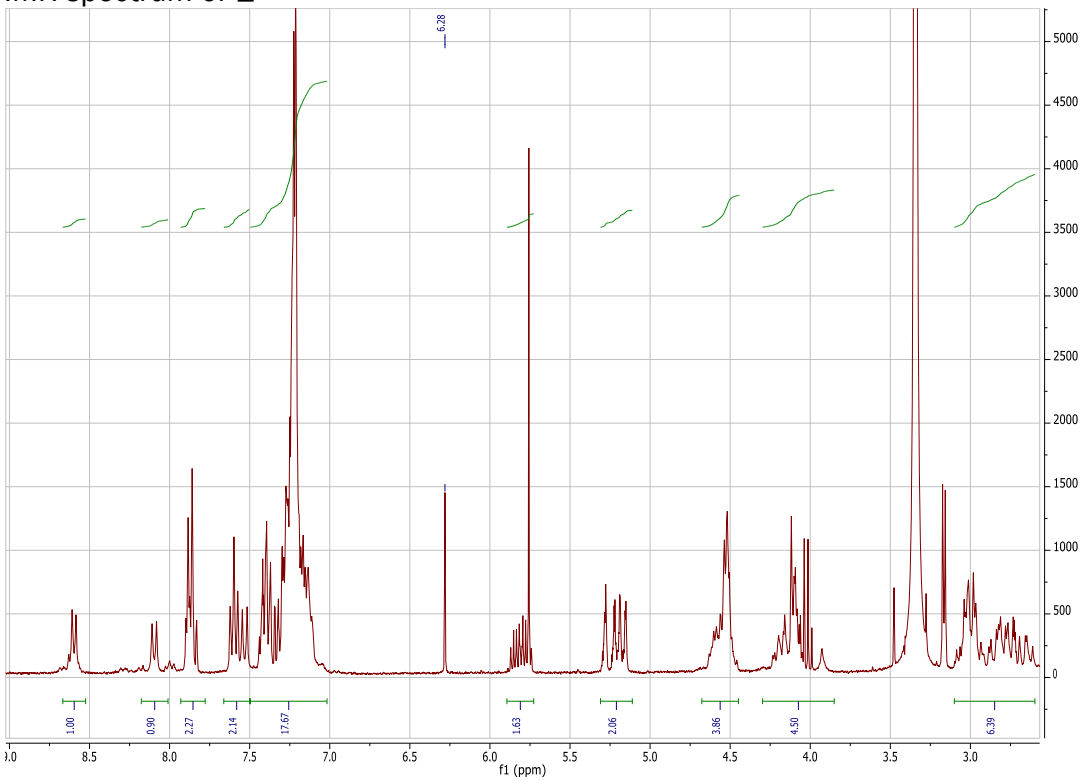


2 (52 mg; 0.03 mmol; 1 eq.), PyBOP (73 mg; 0.14 mmol; 5.1 eq.) and **4.4** (129 mg; 0.14 mmol; 5.1 eq.) were dissolved under argon in SPPS grade DMF (5 mL) and DIPEA (0.4 mL; 0.28 mmol; 9.4 eq.) was then added. The reaction mixture was stirred at room temperature for 18 h. The solvent was removed under reduced pressure and the residue purified via precipitation from THF into diethylether, isolated via centrifugation, followed by two washing steps with diethylether. The product could be purified further by flash chromatography over SiO_2 [DCM/MeOH (15%)], R_f [DCM/MeOH (15%)] = 0.78.

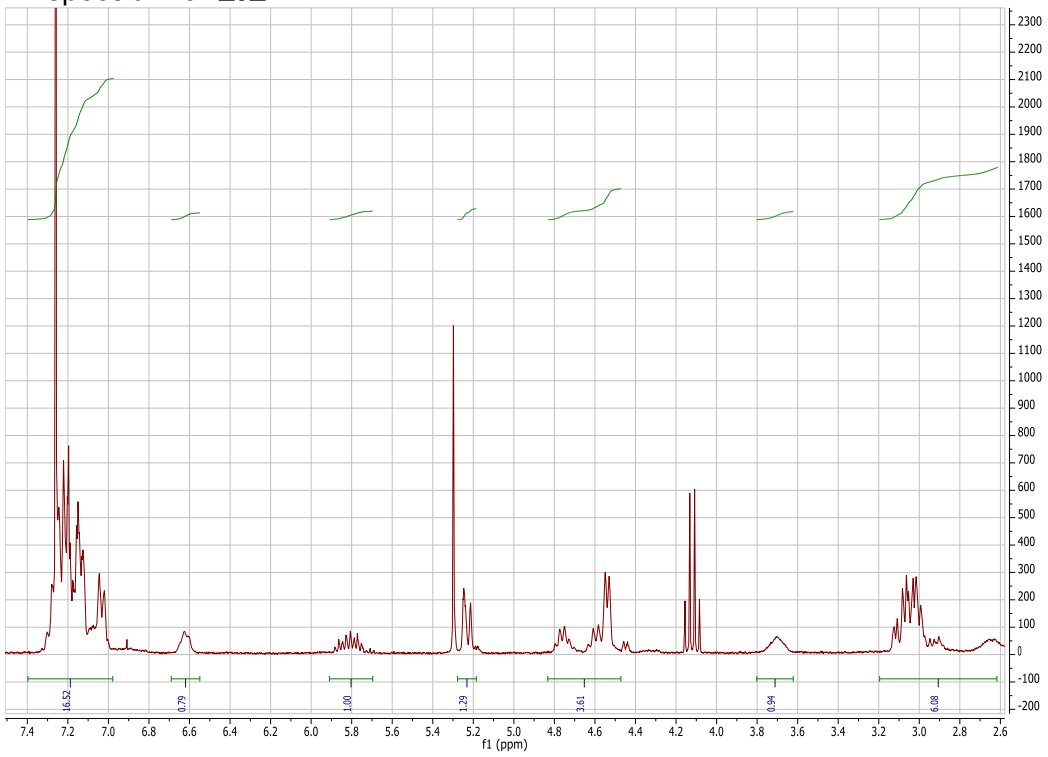
Yield: 47 mg; 0.01 mmol (34 %), $C_{228}H_{351}N_{21}O_{72}S_3$, white solid.
^{1}H-NMR (300 MHz, DMSO-d_6, 298 K): δ [ppm]= 1.55-1.75 (m, 6H $OCH_2CH_2CH_2S$), 2.29 (t, ^3J=6.2 Hz, 18H, OCH_2CH_2CON), 2.33-2.45 (m, 12 H, $OCH_2CH_2CH_2S$, SCH_2CH_2CON); 2.53-2.65 (t, ^3J=7.5 Hz, 6H, SCH_2CH_2CON); 2.95-3.06 (m, 18H, CH_2^{benzyl}); 3.12-3.19 (m, 18H, NCH_2CH_2O); 3.22 (s, 27H, CH_3); 3.28-3.62 (m, 144 CH_2OCH_2, 18H $C(CH_2)_3$ 4.03 (t, ^3J=6.2 Hz, 6H, $OCH_2CH_2CH_2S$); 4.43-4.56 (m, 3H, α-CH); 4.56-4.68 (m, 3H, α-CH); 4.68-4.81 (m, 3H, α-CH); 6.82-7.48 (m, 48, 45 $H^{aromatic}$, 3 NH); 7.92 (t, ^3J=5.6 Hz, 9H, NH); 8.14 (s, 3H, CH^{BTA}); 8.12-8.20 (m, 3H, NH); 8.56 (t, ^3J=7.4 Hz, 3H, NH); 8.61-8.73 (m, 3H, NH).
ESI-MS (positive mode): *m/z* calcd. for $[M+3Na]^{3+}$ 1567.4457; found: 1567.4425 $[M+3Na]^{3+}$; *m/z* calcd. for $[M+2Na]^{2+}$ 2339.6738; found: 2340.1693 $[M+2Na]^{2+}$.
ESI-MS (negative mode): *m/z* calcd. for $[M+2Cl]^{2-}$ 2351.65; found: 2351.09 $[M+2Cl]^{2-}$.

^{1}H-NMR spectra

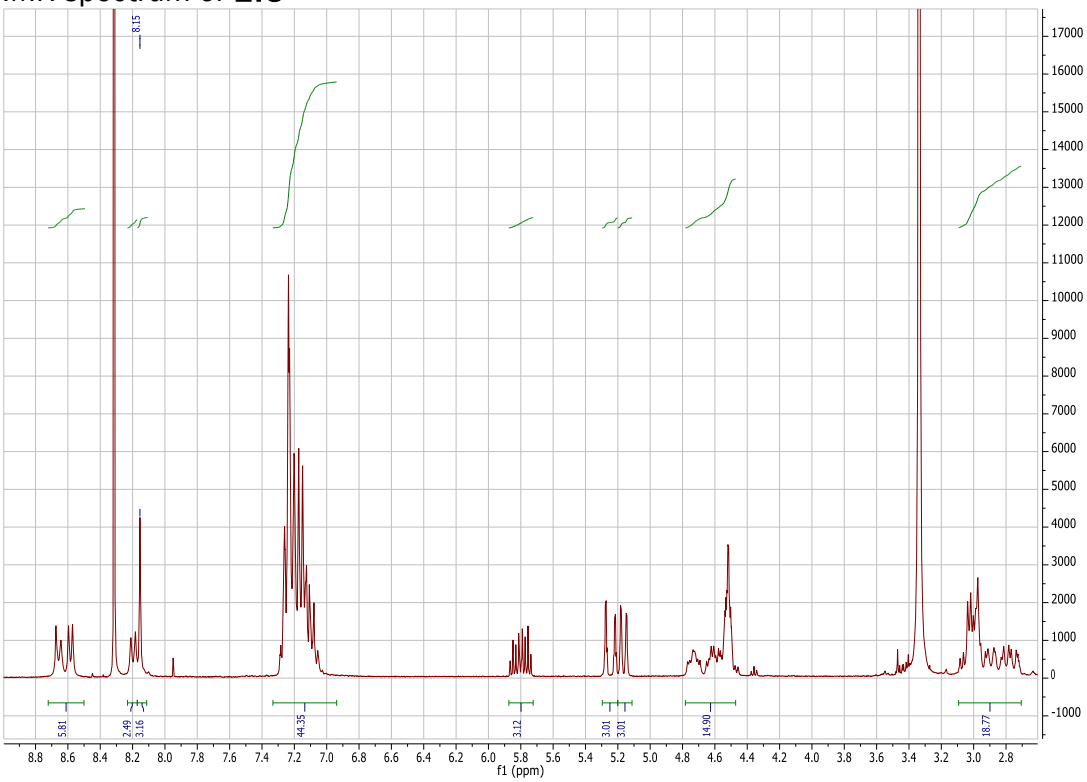
^{1}H-NMR spectrum of **1**



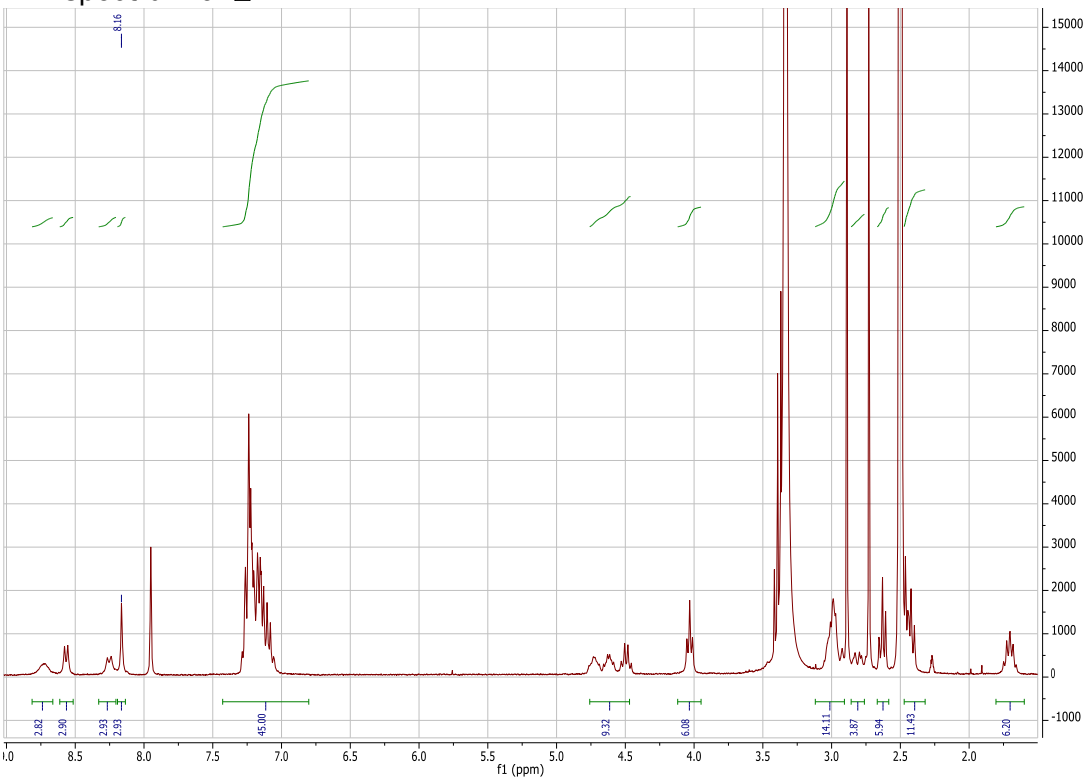
^{1}H-NMR spectrum of **1.2**



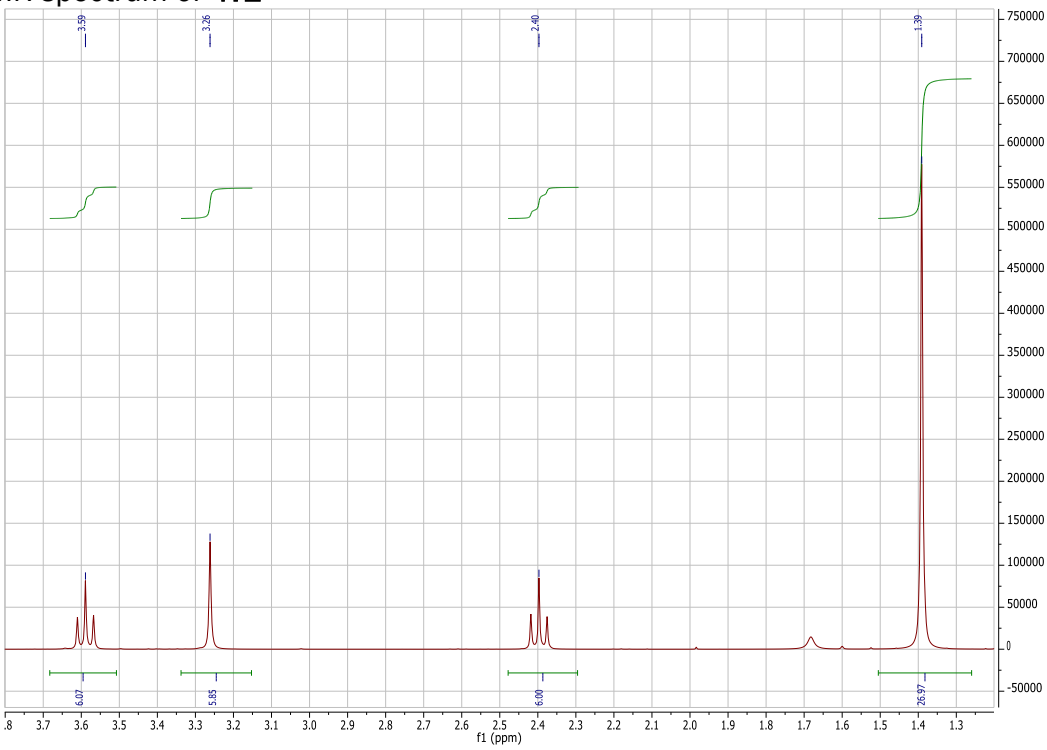
^{1}H-NMR spectrum of **1.3**



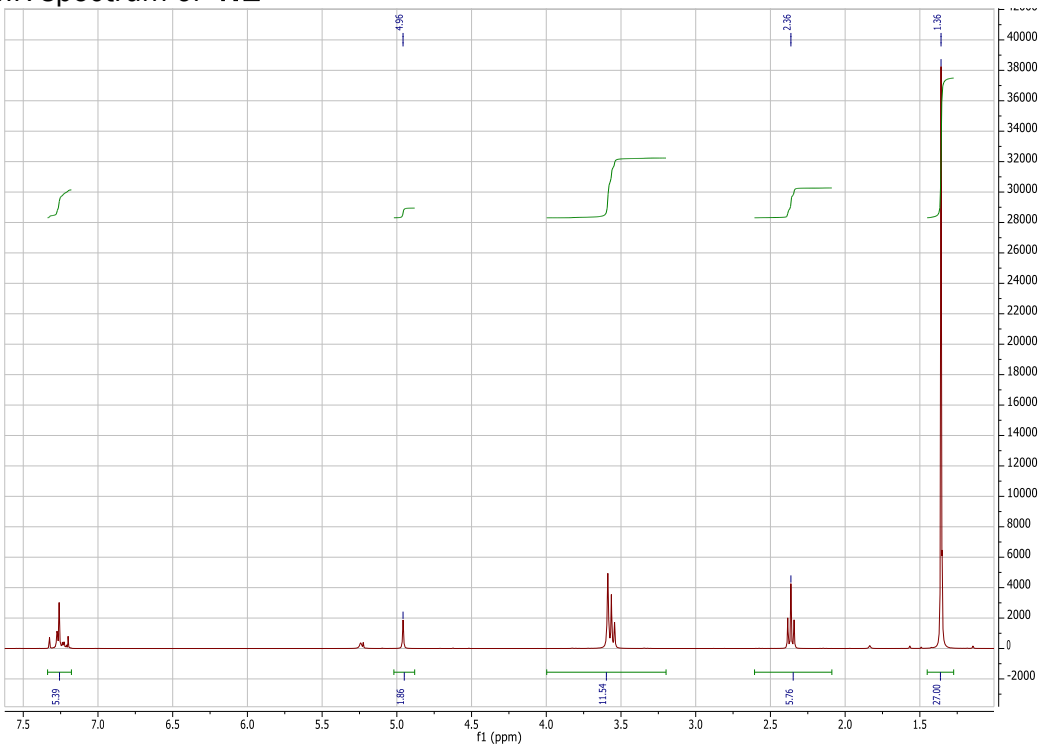
^{1}H-NMR spectrum of **2**



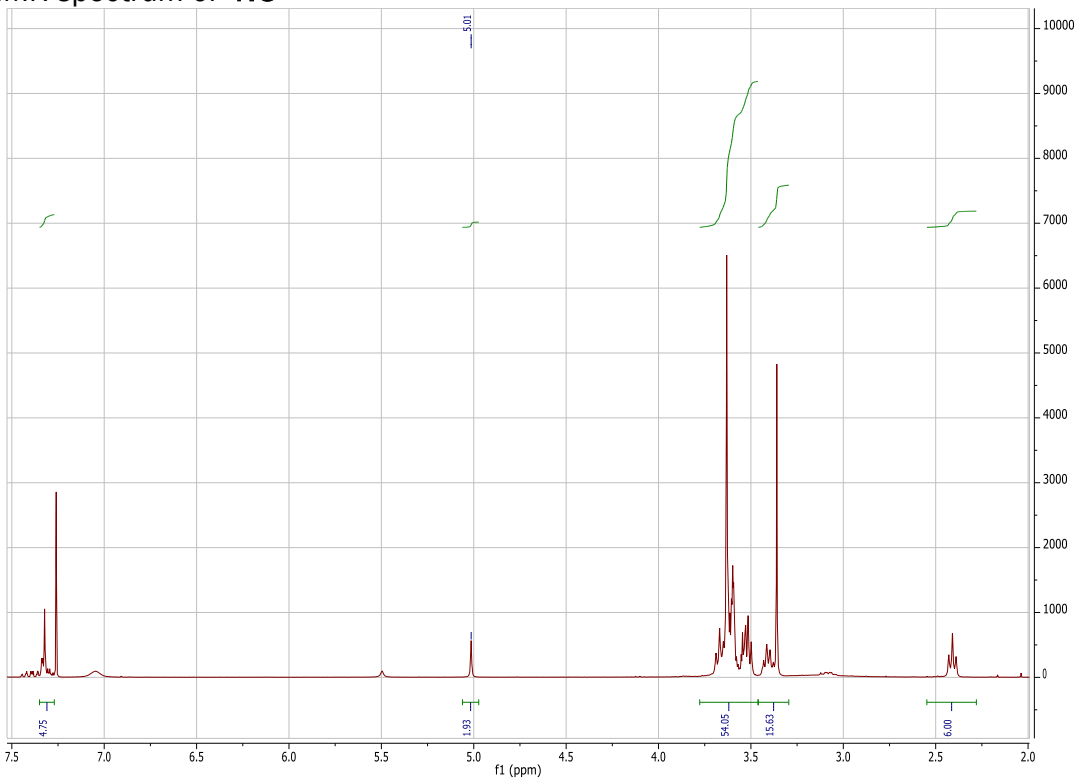
^{1}H-NMR spectrum of **4.1**



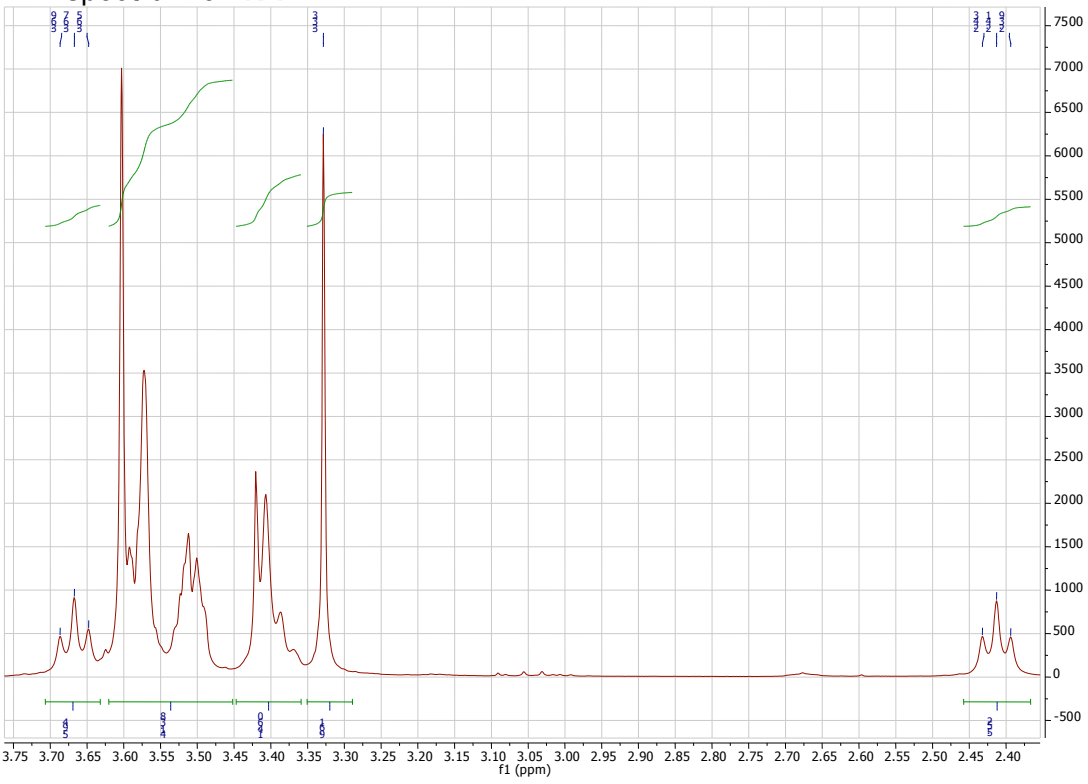
^{1}H-NMR spectrum of **4.2**



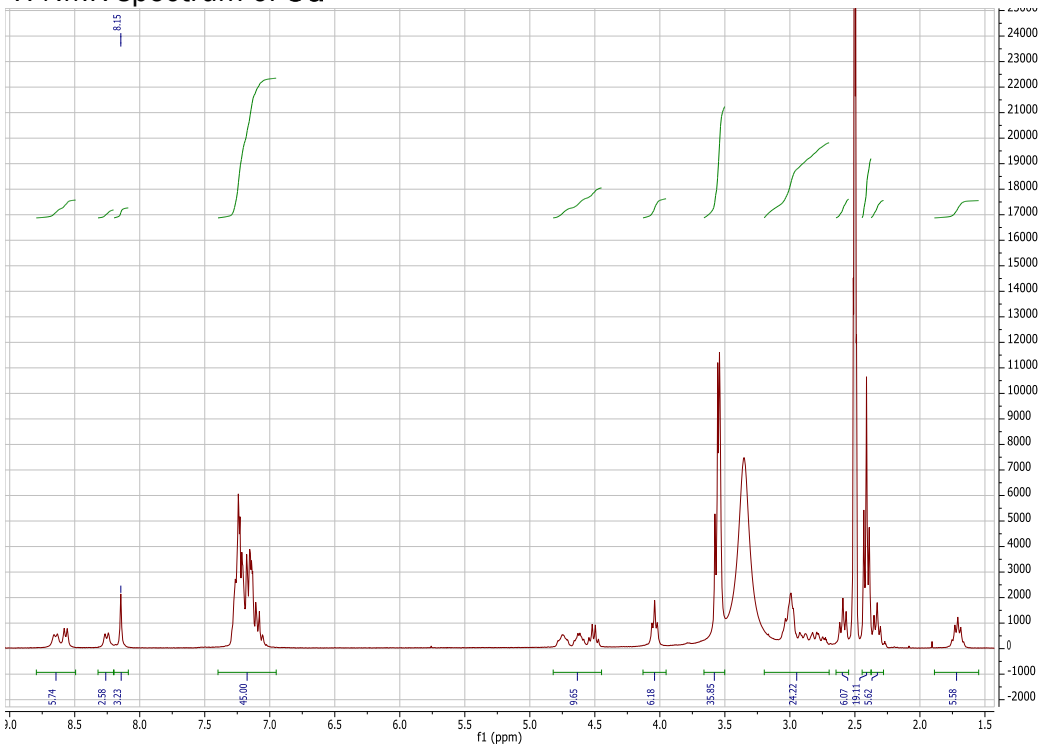
^{1}H-NMR spectrum of **4.3**



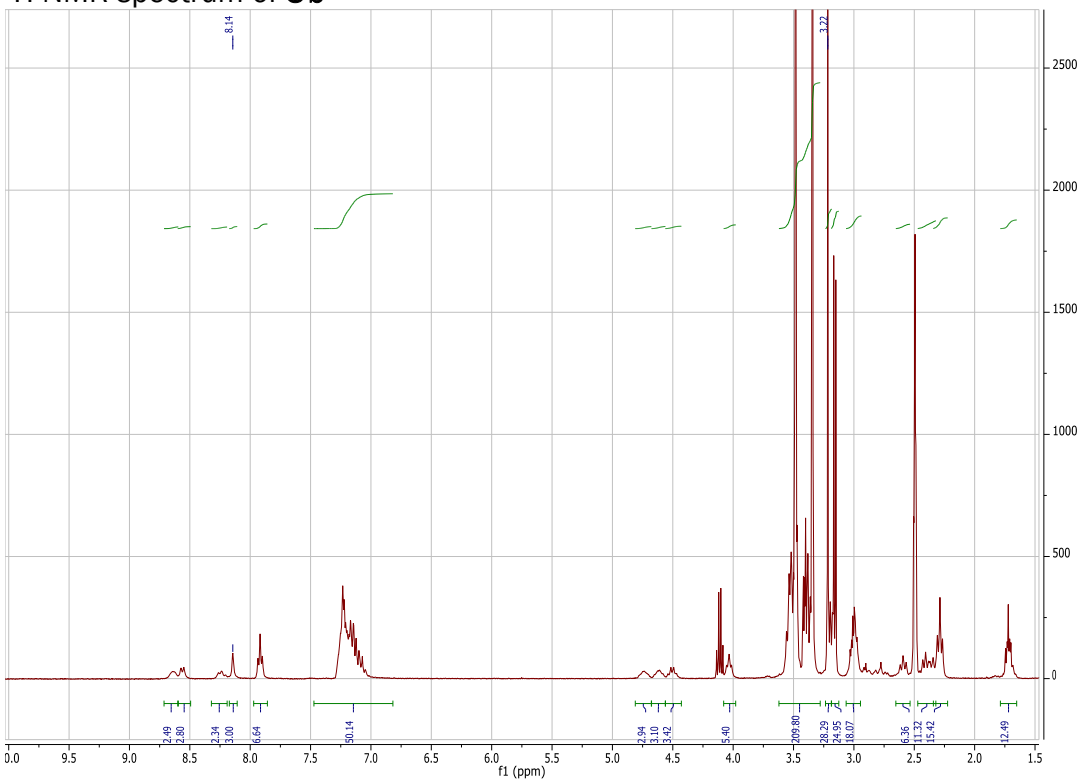
^{1}H-NMR spectrum of **4.4**



^{1}H-NMR spectrum of **3a**



^{1}H-NMR spectrum of **3b**



References

1. A. Kroeger, J. Belack, A. Larsen, G. Fytas and G. Wegner, *Macromolecules*, 2006, **39**, 7098-7106.
2. S. Bulut, J. Hamit, U. Olsson and T. Kato, *Eur. Phys. J. E*, 2008, **27**, 261-273.
3. K. Barlos, D. Gatos, J. Kallitsis, G. Papaphotiu, P. Sotiriu, Y. Wenqing and W. Schafer, *Tetrahedron Lett.*, 1989, **30**, 3943.
4. K. Barlos, O. Chatzi, D. Gatos and G. Stavropoulos, *Int. J. Peptide Protein Res.*, 1991, **37**, 513.
5. R. Bollhagen, M. Schmiedberger, K. Barlos and E. Grell, *Chem. Commun.*, 1994, 2559.
6. L. A. Carpino and A. El-Faham, *Tetrahedron*, 1999, **55**, 6813.
7. R. A. Turner, R. J. Weber and R. S. Lokey, *Org. Lett.*, 2010, **12**, 1852-1855.
8. C. M. Cardona and R. E. Gawley, *J. Org. Chem.*, 2002, **67**, 1411-1413.