ELECTRONIC SUPPLEMENTARY INFORMATION (ESI) FOR:

Tuning the pH-triggered self-assembly of dendritic peptide amphiphiles using fluorinated side chains

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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.
Purification via preparative flash column chromatography was carried out using silica gel with an average grain size of 15-40 µm (MERCK). Technical grade solvents that were used as a mobile phase were distilled before use. Analysis of the collected fractions was performed via TLC on silica coated aluminum sheets (60 F254, MERCK). Size exclusion chromatography was carried out using Sephadex^{TM} LH-20 beads (GE Healthcare Bio-Sciences, Uppsala) as stationary phase and distilled methanol as mobile phase or Sephadex^{TM} G-25 beads (GE Healthcare Bio-Sciences, Uppsala) as stationary phase and demineralised water as mobile phase.

Instrumentation

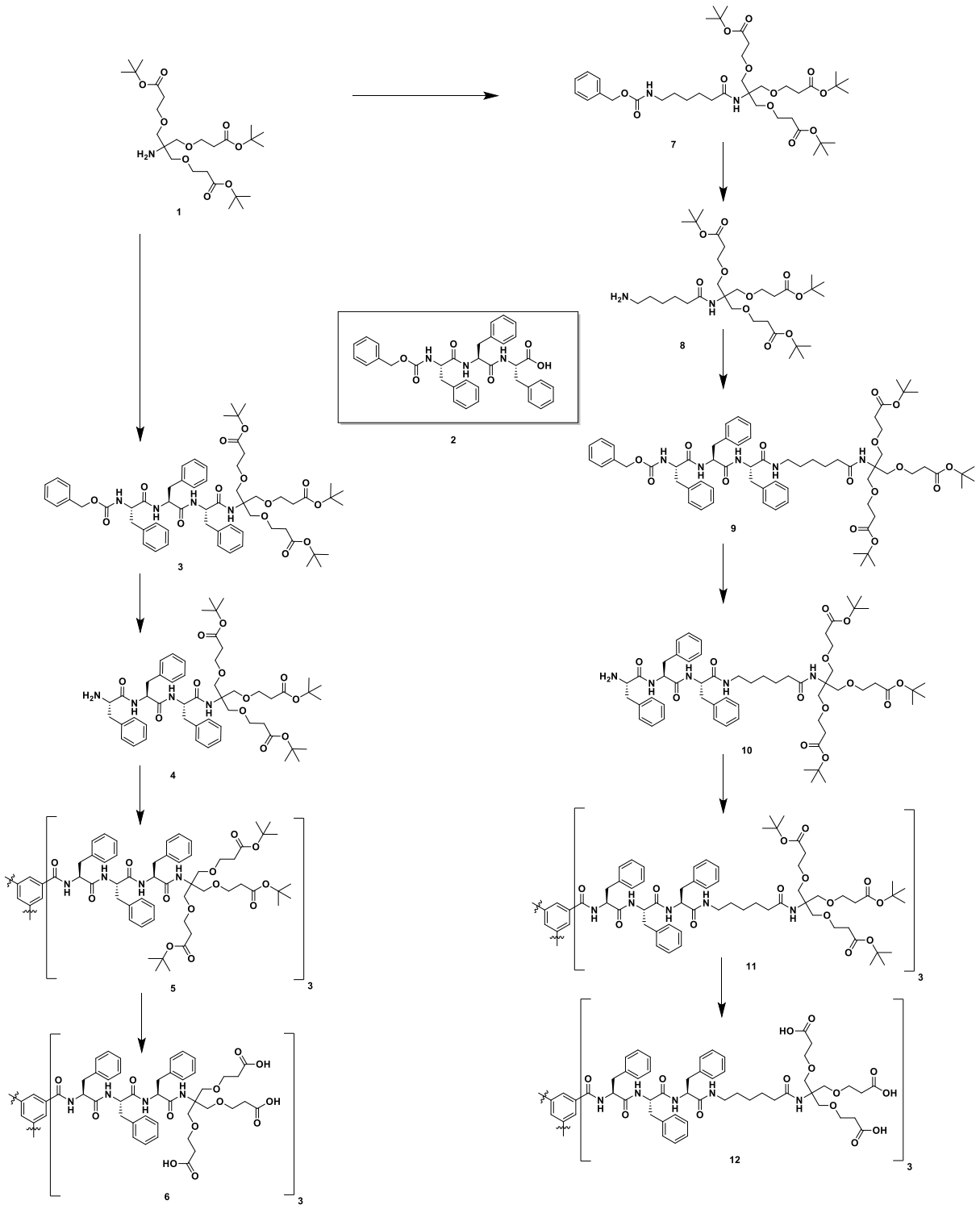
The NMR-spectra were recorded on the spectrometers AV 300 (BRUKER), ARX 300 (BRUKER) and AV 400 (BRUKER). All measurements were carried out in deuterated solvents. The chemical shift (δ) is recorded in parts per million (ppm) and relative to the residual solvent protons.[1] The measured coupling constants were calculated in Hertz (Hz). To analyse the spectra the software MESTRENOVA 9.0.1 was used. The description of the signals was done as follows: s = singlet, brs = broad singlet, d = doublet, t = triplet, q = quartet and m = multiplet.
Mass spectra were recorded on the electronspray ionisation spectrometers (ESI) Micro Tof (BRUKER) and Orbi-Trap LTQ-XL (THERMO SCIENTIFIC) using methanol, methanol/H_2O, CH_3CN, CH_2Cl_2, or $CHCl_3$ as solvents.
Molecules of a high molecular weight were detected using matrix assisted laser desorption ionization time of flight (MALDI-TOF) spectrometry using an Autoflex Speed (BRUKER).
CD spectra were recorded on a J-815 (JASCO) using the software Spectra Manager 2.08.04 and processed with Origin Pro 9.1G.

For transmission electron microscopy (TEM) investigations, standard 200 mesh EM grids, covered with a carbon coated plastic foil (Pioloform), were utilised. To render the support film more hydrophilic, the EM grids were plasma cleaned for 30-45 seconds. For sedimentation, 2-3 µL sample material was left 1 min and afterwards the residual material was blotted. Thereafter, the grids were washed once with 2-3 µL uranyl acetate (15 s). Followed by a second blotting step, the sample material was than stained by uranyl acetate for 45 s, blotted and left for air drying. The samples were examined on a Philips CM10 (FEI, Eindhoven, the Netherlands) operated at 80 kV. Electron micrographs were recorded by a side-mounted CCD camera (IDS, Obersulm, Germany) utilising acquisition software written in LabView.
For scanning transmission electron microscopy (STEM) investigations, EM high-resolution grids were utilized. Here, a holey carbon film is covered with a 2-4 nm thick amorphous carbon film, which acts as a support for the sample material. As in the case for TEM investigations, the grids were plasma cleaned prior to the sample preparation. Since no staining was performed for STEM investigations the preparation protocol was reduced to the following steps: 2-3 µL sample material were left for sedimentation (1 min). Hereafter, grids were either washed briefly with buffer or blotted directly and left for air drying. The samples were examined in a high-resolution in-lens scanning electron microscope (S-5000, Hitachi, Japan), equipped with a homemade annular dark-filed (ADF) detector. ADF images were taken at 30 kV acceleration voltage.

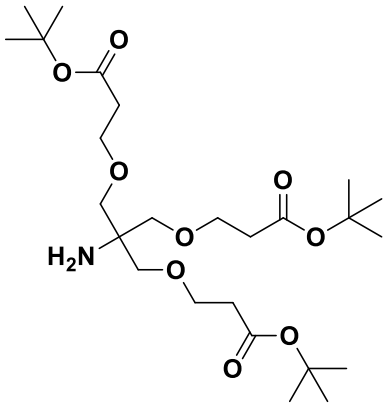
Synthesis of the 2-chlorotrityl resin bound peptide via SPPS

The loading of the resin was performed according to literature known procedure.[2-4] The appropriate Fmoc-protected amino acid (9 mmol; 3.0 eq.) was dissolved in 20 mL DCM/DMF (4:1) and added to the 2-chlorotrityl-chloride resin (2.0 g; 3 mmol) followed by the addition of 1.20 mL DIPEA. After stirring for 5 min at room temperature an additional 6.0 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.[5] 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 colour 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 SPPS grade 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 protected amino acid for every coupling process, starting with the Fmoc deprotection on the resin. In case of the triphenylalanine, Cbz-protected phenylalanine was coupled as the last amino acid.

Synthesis route for the dendritic peptide amphiphiles 6 and 12



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



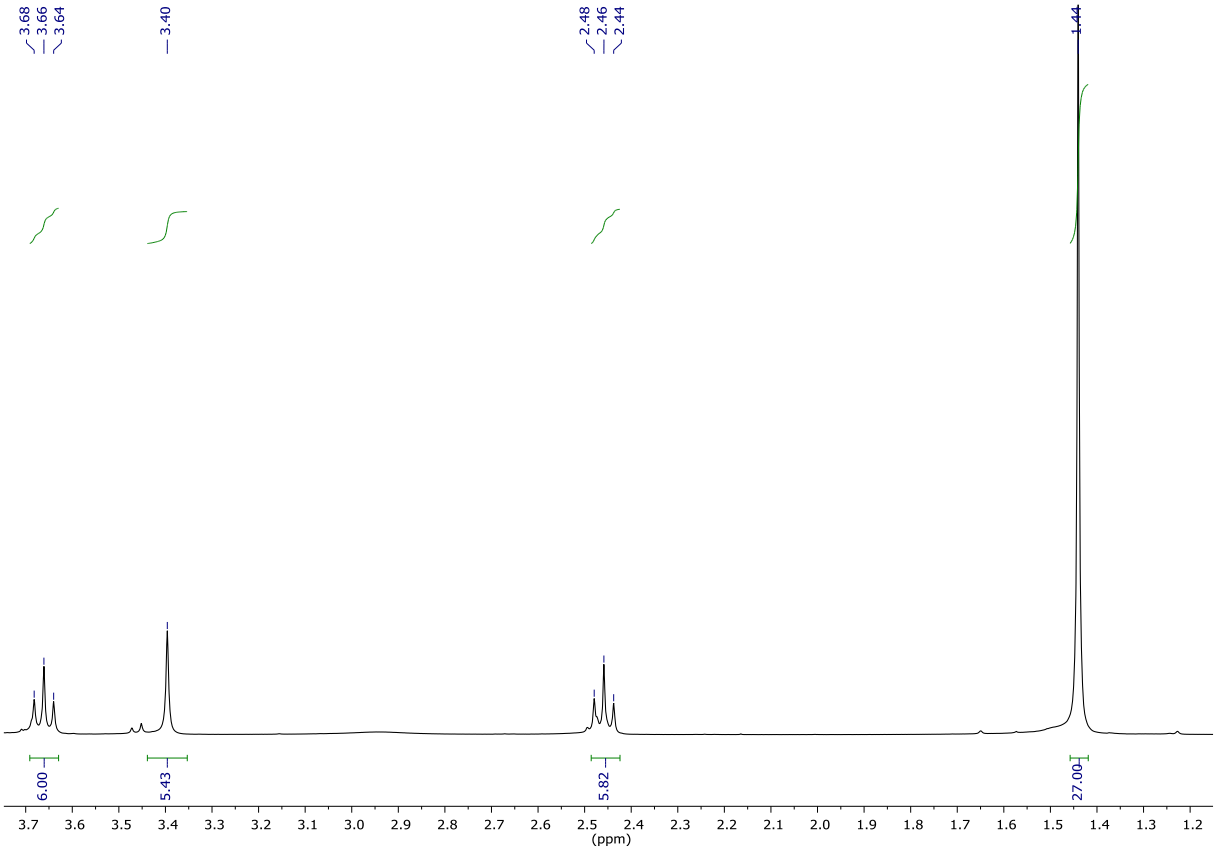
1

Tris(hydroxymethyl)aminomethane (2.40 g; 20 mmol; 1.0 eq.) was dissolved in DMSO (4 mL) under argon atmosphere. Under stirring 5 M NaOH (0.4 mL) was added first, followed by the dropwise addition of *tert*-butyl acrylate (6.0 mL; 68 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, SiO_2) = 0.43.

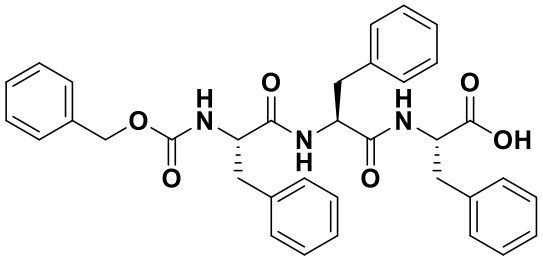
Yield: 3.39 g (6.71 mmol, 34%), $C_{25}H_{47}NO_9$, colourless oil.

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm]= 3.66 (t, *J* = 6.3 Hz, 6H, OCH_2CH_2), 3.40 (s, 6H, C^qCH_2O), 2.46 (t, *J* = 6.3 Hz, 6H, OCH_2CH_2), 1.44 (s, 27H, CH_3^{tBu}).

ESI-MS (positive mode): *m/z* calcd. for $[M+H]^+$ 506.3324; found: 506.3328 $[M+H]^+$ and *m/z* calcd. for $[M+Na]^+$ 528.3151; found: 528.3145 $[M+Na]^+$.



Cbz-Triphenylalanine



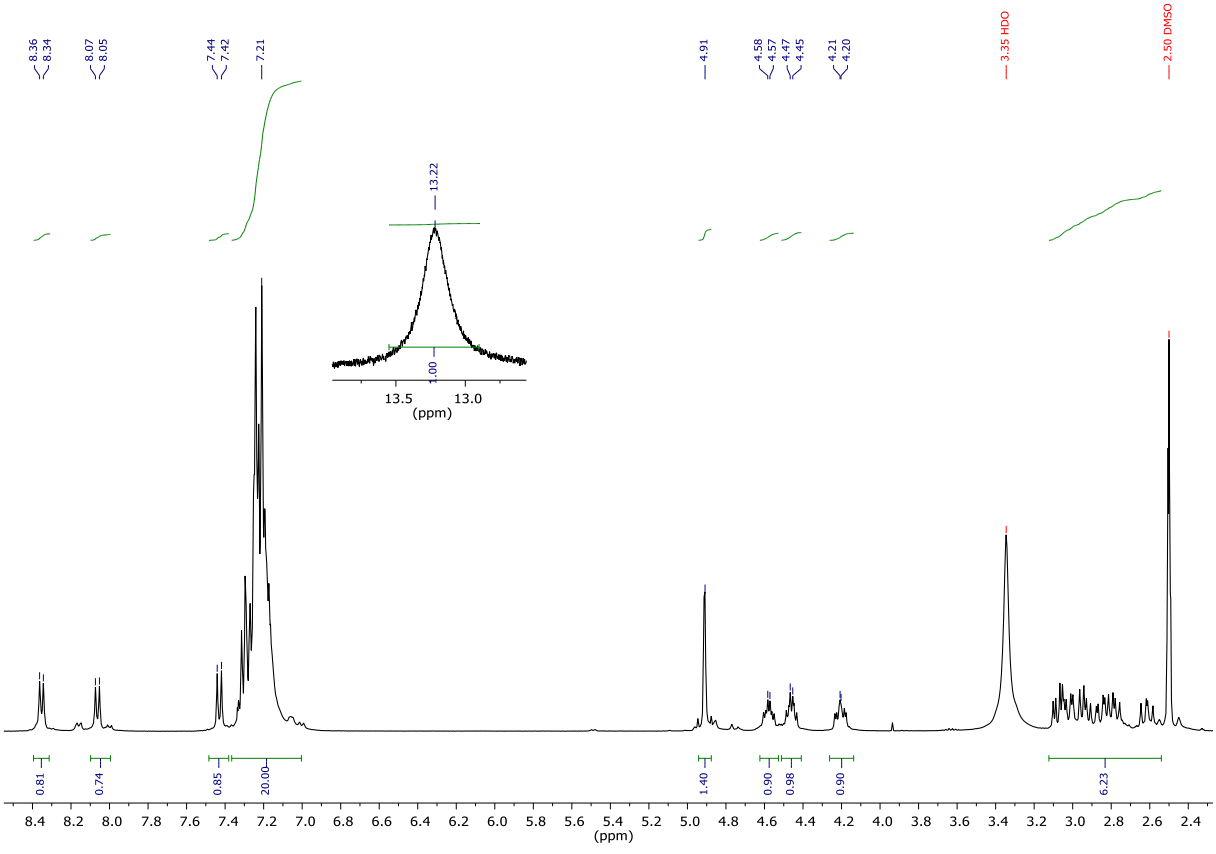
2

Cbz-protected triphenylalanine bound to the 2-chlorotrityl resin (from SPPS) was stirred twice in a 10 mL of a mixture of trifluoroethanol and DCM (8:1) for 45 min each time. After draining the resin was washed three times with DCM. The organic solvent was removed under reduced pressure. The residue was purified via precipitation from THF into a mixture of cyclohexane and diethylether (1:2) and isolated via centrifugation.

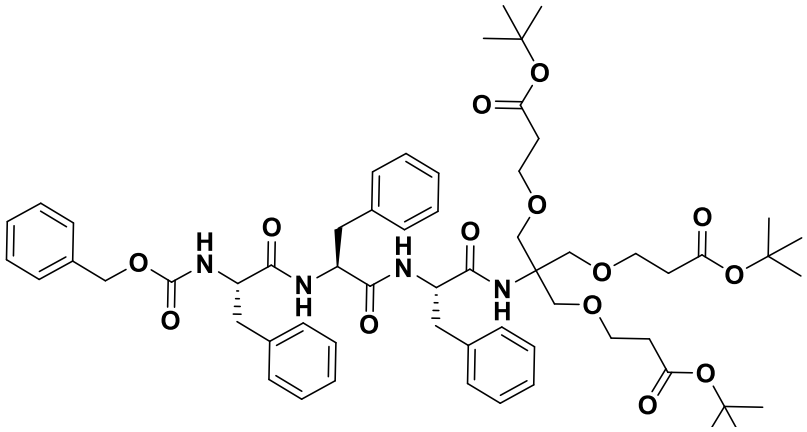
Yield: 599 mg (1.01 mmol) $C_{35}H_{35}N_3O_6$, white solid.

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm]= 13.22 (brs, 1H, COO*H*), 8.35 (d, *J* = 7.8 Hz, 1H, N*H*), 8.06 (d, *J* = 8.3 Hz, 1H, N*H*), 7.43 (d, *J* = 8.9 Hz, 1H, N*H*), 7.36 – 6.86 (m, 20H, $CH_{aro}^{Phe\ and\ Cbz}$), 4.91 (s, 2H, CH_2^{Cbz}), 4.62 - 4.54 (m, 1H, CH^{α}), 4.50 – 4.42 (m, 1H, CH^{α}), 4.24 - 4.16 (m, 1H, CH^{α}), 3.12 – 2.54 (m, 6H, CH_2^{Phe}).

ESI-MS (positive mode): *m/z* calcd. for $[M+H]^+$ 594.2599; found: 594.2592 $[M+H]^+$, *m/z* calcd. for $[M+Na]^+$ 616.2418; found: 616.2407 $[M+Na]^+$ and *m/z* calcd. for $[2M+Na]^+$ 1209.4944; found: 1209.4899 $[2M+Na]^+$.
ESI-MS (negative mode): *m/z* calcd. for $[M]^-$ 592.2442 found: 592.2464 $[M]^-$ and *m/z* calcd. for $[2M]^-$ 1185.4968; found: 1185.4985 $[2M]^-$.



Cbz-triphenylalanine-tris{[2-(*tert*-butoxycarbonyl)-ethoxy]methyl}methylcarboxamide



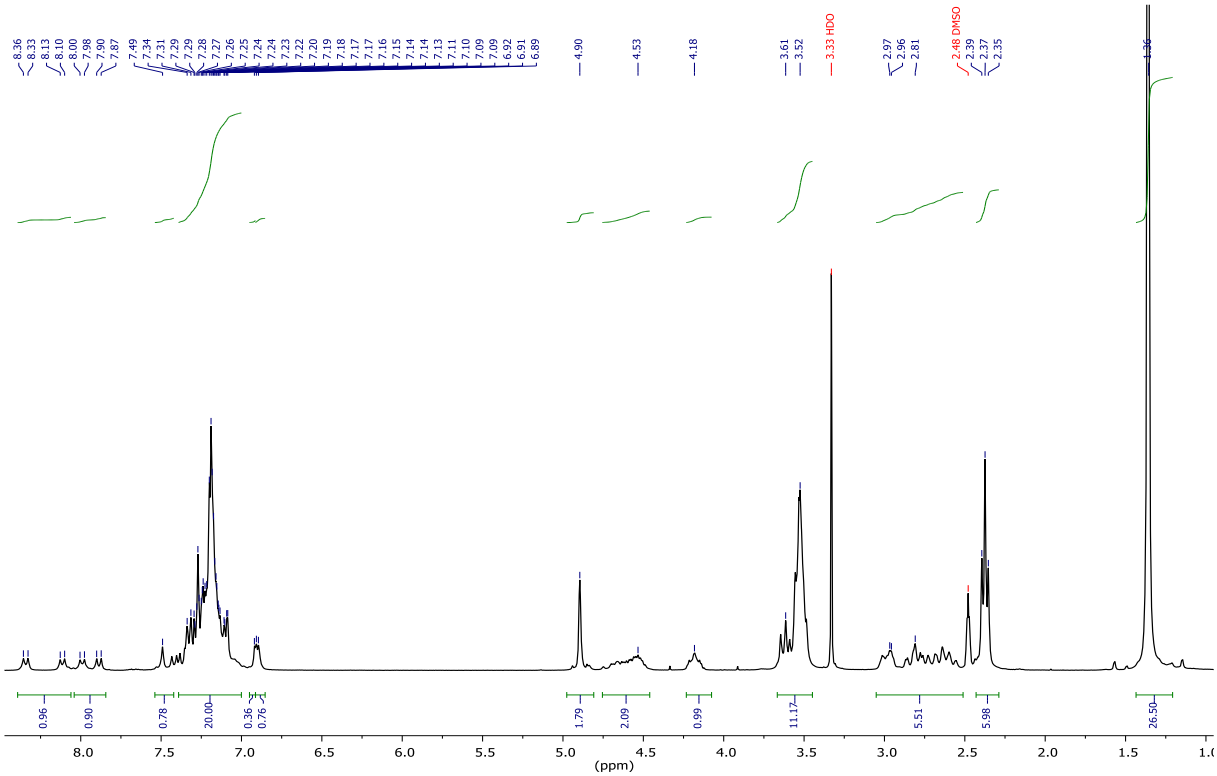
3

1 (254 mg; 0.51 mmol; 1.5 eq.), PyBOP (1052 mg; 2.02 mmol; 6.0 eq.) and **2** (200 mg; 0.34 mmol; 1.0 eq.) were dissolved under argon in dry DMF (5 mL) and DIPEA (622 mg; 1.08 mmol; 12.0 eq.) was subsequently then added. The reaction mixture was stirred at room temperature for 12 h. The solvent was removed under reduced pressure and the residue was purified via flash chromatography over SiO_2 (EtOAc:DCM 1:1), R_f(EtOAc:DCM 1:1, SiO_2) = 0.73.

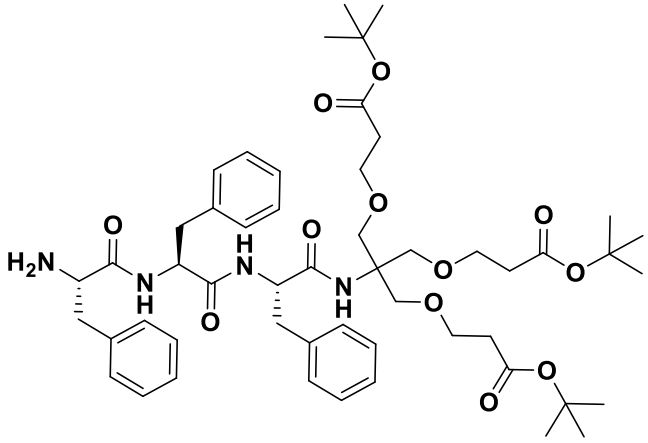
Yield: 238 mg (0.22 mmol, 64%), $C_{66}H_{91}N_5NaO_{15}$, white solid.

^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm]= 8.39 – 8.07 (m, 1H, N*H*), 8.01 – 7.84 (m, 1H, N*H*), 7.61 – 7.41 (m, 1H, NH), 7.32 – 7.10 (m, 20H, $CH_{aro}^{Phe\ and\ Cbz}$), 6.97 – 6.77 (m, 1H, N*H*), 4.90 (s, 2H, CH_2^{Cbz}), 4.74 – 4.47(m, 2H, CH^{α}), 4.29 – 4.07 (m, 1H, CH^{α}), 3.69 – 3.42 (m, 12H, CH_2OCH_2), 3.03 – 2.52 (m, 6H, CH_2^{Phe}), 2.37 (t, *J* = 6.1 Hz, 6H, OCH_2CH_2), 1.36 (s, 27H, CH_3^{tBu}).

ESI-MS (positive mode): *m/z* calcd. for $[M+Na]^+$ 1103.5569; found: 1103.5506 $[M+Na]^+$.



Triphenylalanine-tris{[2-(*tert*-butoxycarbonyl)-ethoxy]methyl}methylcarboxamide



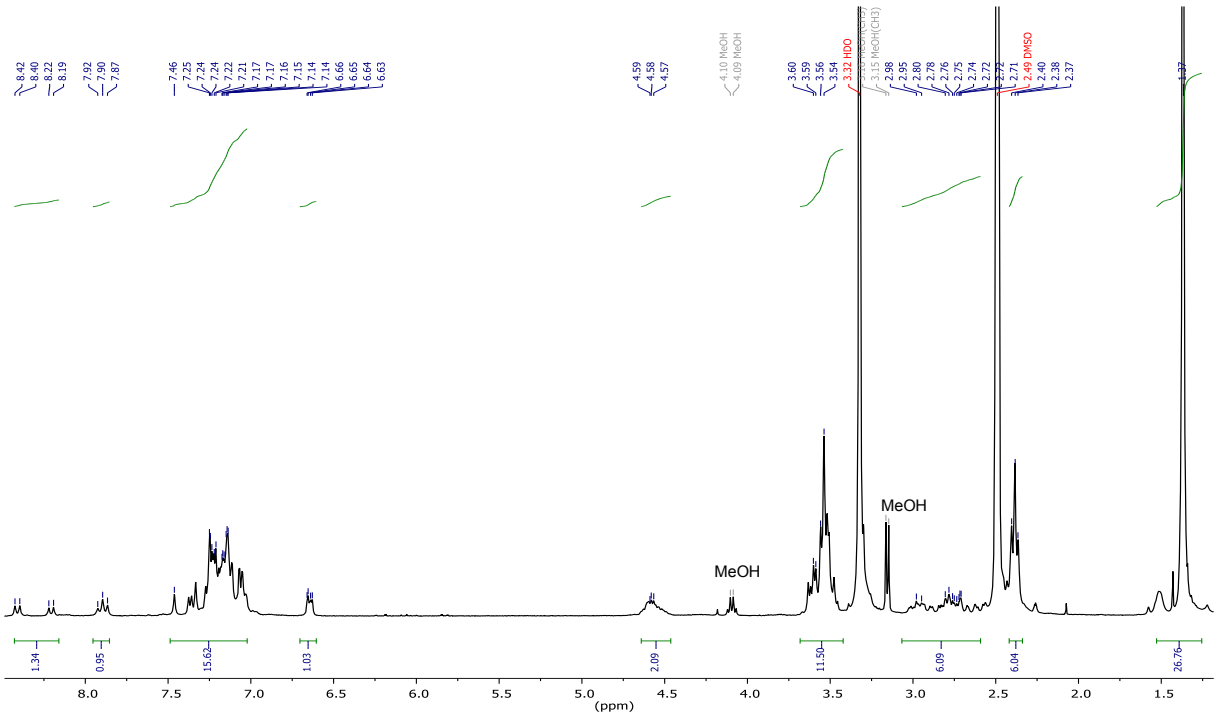
4

3 (200 mg, 0.185 mmol, 1.0 eq.) was dissolved in methanol (5 mL) and Pd/C (10 wt%, 20 mg) was added. The heterogeneous mixture was stirred under an atmosphere of hydrogen for 18 h. The catalyst was removed by filtration over Celite and afterwards the solvent was removed under reduced pressure.

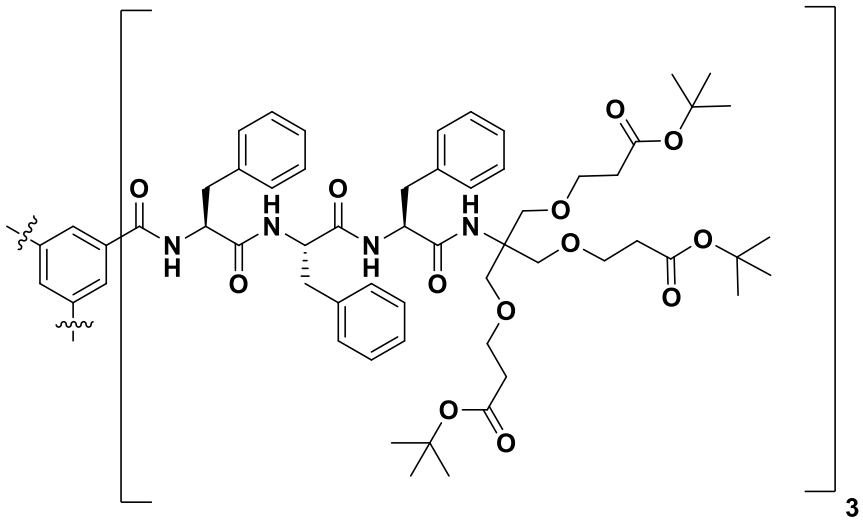
Yield: 160 mg (0.169 mmol, 91%), $C_{52}H_{74}N_4O_{12}$, colourless oil.

^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm]= 8.45 – 8. 12 (m, 1H, N*H*), 8.97 - 7.82 (m, 1H, N*H*), 7.49 – 6.99 (m, 15H, CH_{aro}^{Phe}), 6.71 – 6.58 (m, 1H, N*H*), 4.69 – 4.47 (m, 2H, CH^{α}), 3.69 – 3.43 (m, 12H, CH_2OCH_2), 3.07 – 2.56 (m, 6H, CH_2^{Phe}), 2.39 (t, *J* = 6.1 Hz, 6H, OCH_2CH_2), 1.37 (s, 27H, CH_3^{tBu}).

ESI-MS (positive mode): *m/z* calcd. for $[M+H]^+$ 947.5376; found: 947.5367 $[M+H]^+$ and *m/z* calcd. for $[M+Na]^+$ 969.5195; found: 969.5184 $[M+Na]^+$.



BTA-(triphenylalanine-tris{[2-(*tert*-butoxycarbonyl)-ethoxy]methyl}methylcarboxamide)$_3$



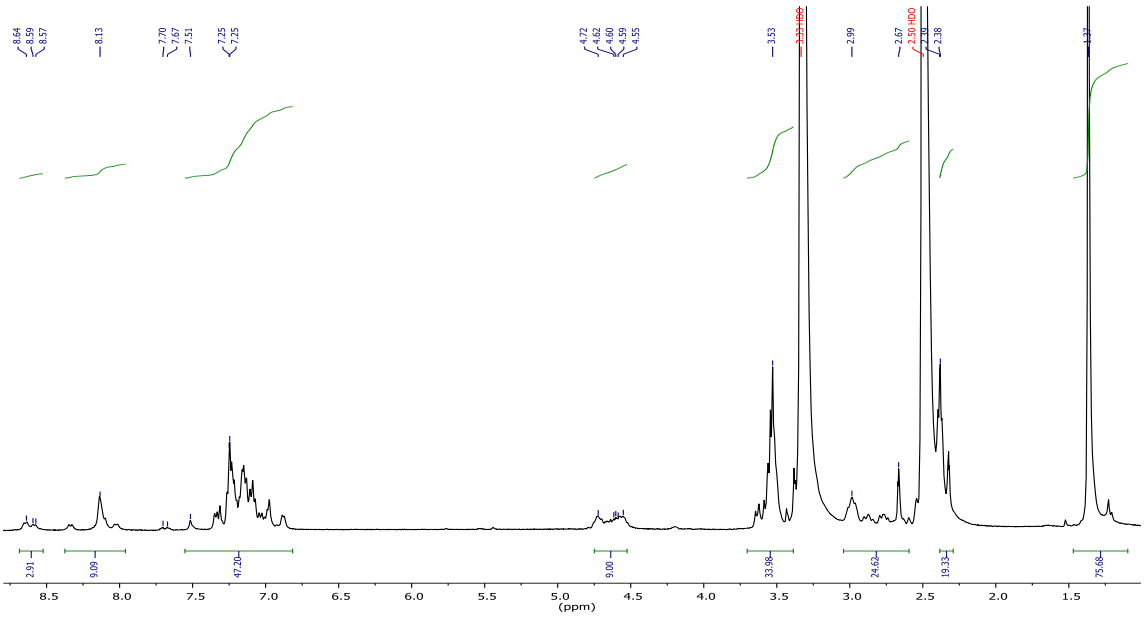
5

4 (159 mg; 0.169 mmol; 3.0 eq.) and 1,3,5-benzenetricarbonyl trichloride (15 mg; 0.056 mmol; 1.0 eq.) were dissolved under argon in dry DMF (5 mL) and DIPEA (109 mg; 0.845 mmol; 15.0 eq.) were then added. The reaction mixture was stirred at room temperature for 20 h, after 2 h PyBOP (179 mg; 0.344 mmol; 7.0 eq.) was added to react hydrolysed acid chloride with remaining amine **10**. The organic solvent was removed under reduced pressure. The residue was purified via precipitation in water and isolated via centrifugation. The residue was purified again via SEC in methanol. R_f(DCM:EtOAc 1:1 + 20 vol% MeOH, SiO_2) = 0.90.

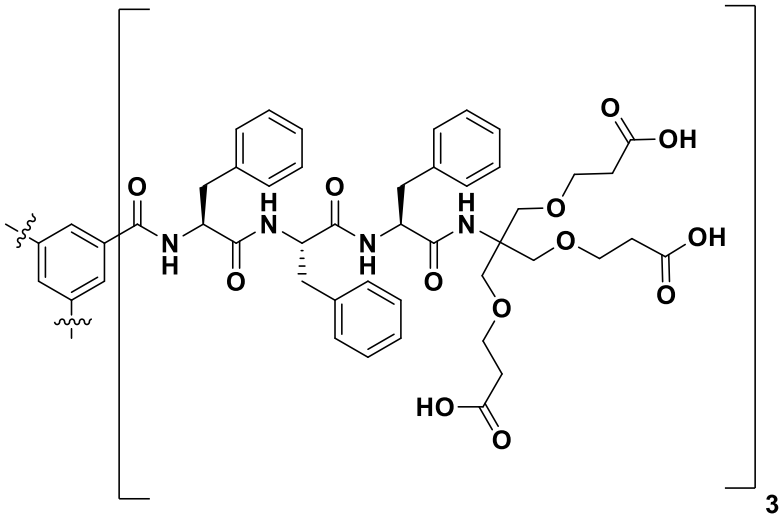
Yield: 83 mg (0.028 mmol, 50%) $C_{165}H_{222}N_{12}O_{39}$, light brownish solid.

^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm]= 8.70 – 8.54 (m, 3H, N*H*), 8.43 – 7.97 (m, 9H, N*H* and CH_{aro}^{BTA}), 7.60 – 6.82 (m, 48H, CH_{aro}^{Phe} and N*H*), 4.80 – 4.49 (m, 9H, CH^{a}), 3.67 – 3.44 (m, 36H, CH_2OCH_2), 2.90 – 2.58 (m, 18H, CH_2^{Phe}), 2.47 – 2.33 (m, 18H, OCH_2CH_2), 1.37 (s, 81H, CH_3^{tBu}).

MALDI-MS (positive mode) (DHB [EtOAc]): *m/z* calcd. for $[M+Na]^+$ 3020.57; found: 3020.71 $[M+Na]^+$.



BTA-(triphenylalanine-tris{[2-carboxyethoxy]methyl}methylcarboxamide)$_3$



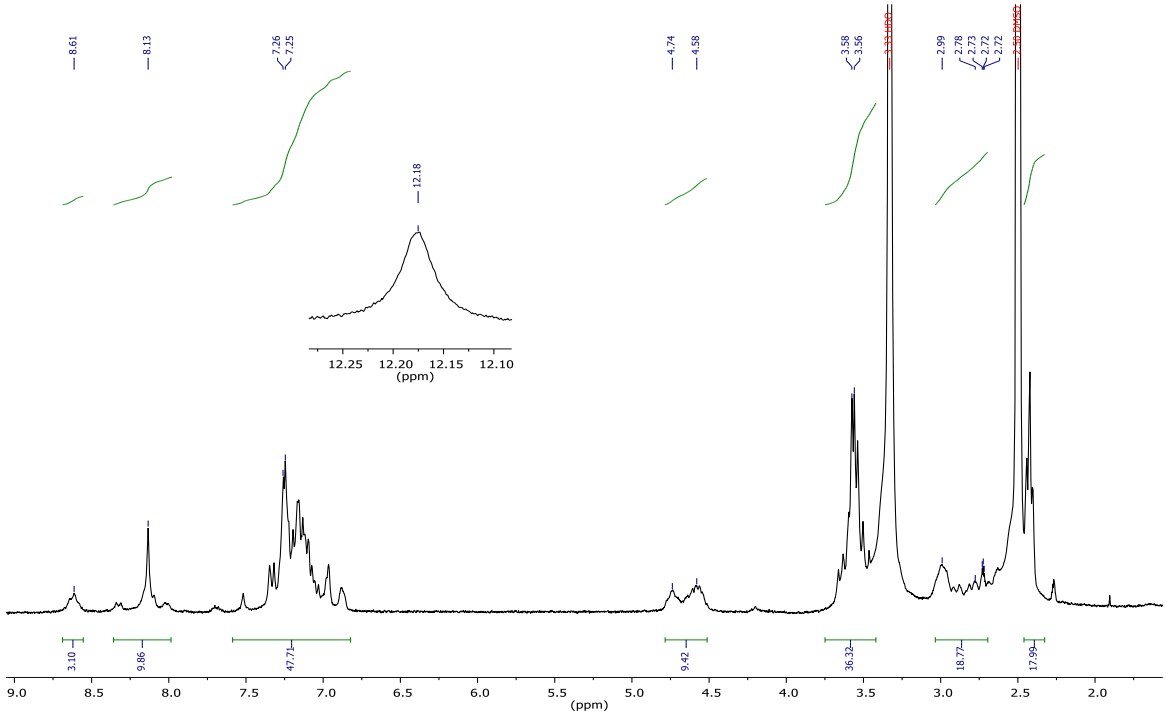
6

5 (80 mg; 0.027 mol) was stirred three times in 3.0 mL of a mixture of TFA and DCM (1:1) for 1 h. The organic solvent was removed in each step under reduced pressure. Finally the residue was taken up in water and freeze-dried over night.

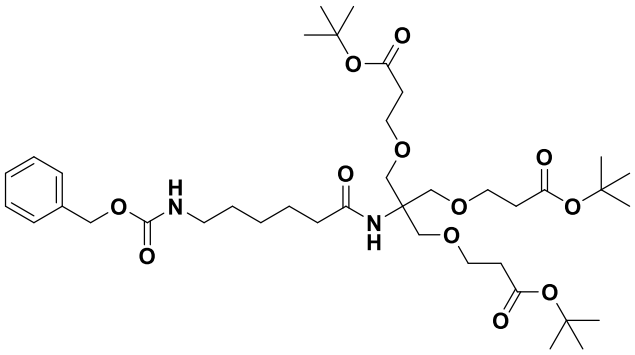
Yield: 29 mg (0.012 mmol, 44%) $C_{129}H_{150}N_{12}O_{39}$, white solid.

^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm]= 12.18 (brs, 9H, COO*H*), 8.69 – 8.52 (m, 3H, N*H*), 8.39 – 7.97 (m, 9H, N*H* and CH_{aro}^{BTA}), 7.59 – 6.82 (m, 48H, CH_{aro}^{Phe} and N*H*), 4.78 - 4.51 (m, 9H, CH^{α}), 3.75 – 3.41 (m, 36H, CH_2OCH_2), 3.05 – 2.64 (m, 18H, CH_2^{Phe}), 2.47 – 2.33 (m, 18H, OCH_2CH_2).

MALDI-MS (positive mode) (DHB [H_2O/ACN]): *m/z* calcd. for $[M+Na]^+$ 2515.00; found: 2515.00 $[M+Na]^+$.



6-Cbz-aminohexane-tris{[2-(*tert*-butoxycarbonyl)-ethoxy]methyl}methylcarboxamide



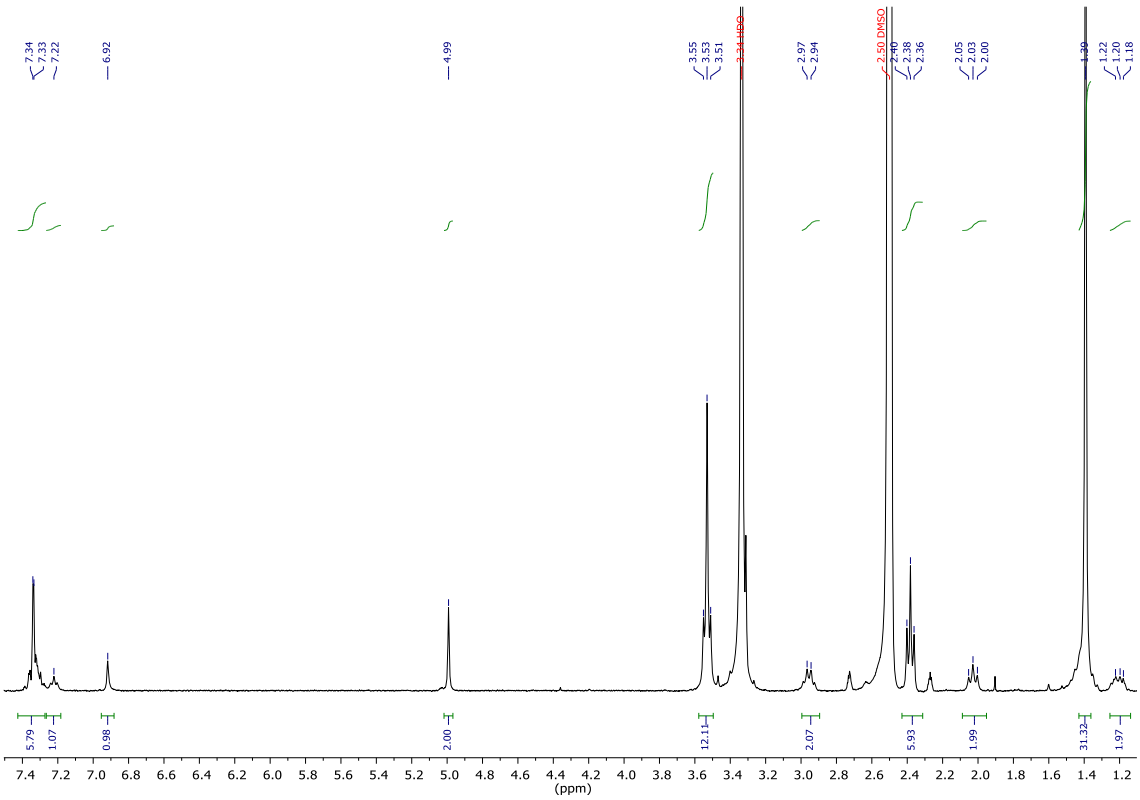
7

Cbz-6-aminohexanoic acid (976 mg; 3.68 mmol; 1.2 eq.) and **1** (1.55 mg; 3.07 mmol; 1.0 eq.) were dissolved in dry DCM (10 mL) and treated with DIPEA (1.59 mg; 12.28 mmol; 4.0 eq.) and PyBOP (2.40 g; 1.8 mmol; 1.5 eq.). The solution was stirred at room temperature over night and purified after concentration under reduced pressure via column chromatography over SiO_2 (EtOAc:DCM 1:1), R_f(EtOAc:DCM 1:1, SiO_2) = 0.78.

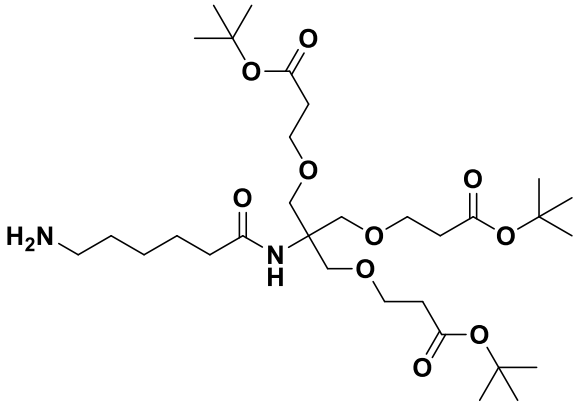
Yield: 2.29 g (3.04 mmol, 99%), $C_{39}H_{64}N_2O_{12}$, colourless oil.

^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm]= 7.41-7.27 (m, 5H, C*H*^{Cbz}),7.22 (t, *J* = 5.9 Hz, 1H, N*H*), 6.92 (s, 1H, N*H*), 4.99 (s, 2H, CH_2^{Cbz}), 3.58 – 3.47 (m, 12H, CH_2OCH_2), 3.04 – 2.87 (m, 2H, NCH_2^{Ahx}), 2.38 (t, *J* = 6.1 Hz, 6H, OCH_2CH_2,), 2.03 (t, *J* = 7.7 Hz, 2H, CH_2CON^{Ahx}), 1.46 - 1.33 (m, 4H, CH_2^{Ahx}), 1.39 (s, 27H, CH_3^{tBu}), 1.27 – 1.15 (m, 2H, CH_2^{Ahx}).

ESI-MS (positive mode): *m/z* calcd. for $[M+H]^+$ 753.4538; found: 753.4511 $[M+H]^+$and *m/z* calcd. for $[M+Na]^+$ 775.4362; found: 775.4346 $[M+Na]^+$.



6-Aminohexane-tris{[2-(*tert*-butoxycarbonyl)-ethoxy]methyl}methylcarboxamide



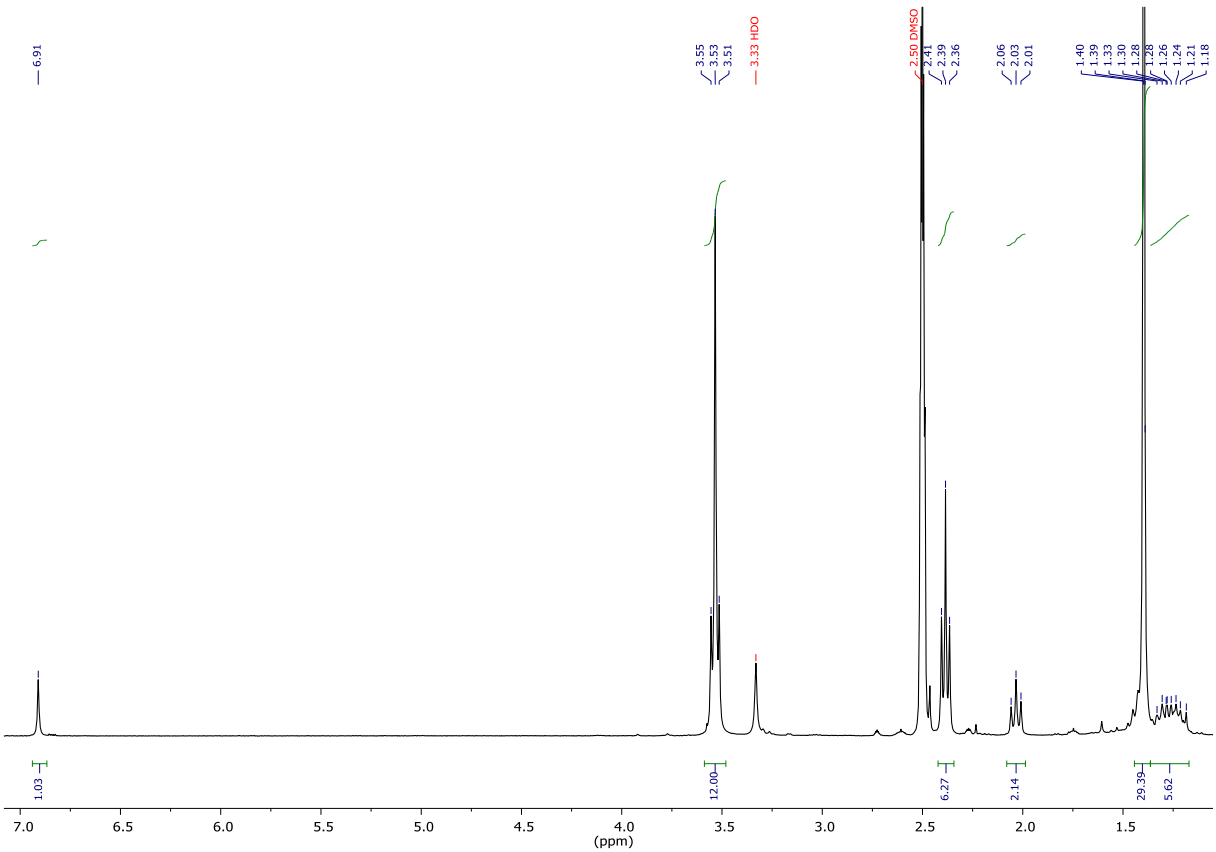
8

7 (889 mg; 1.18 mmol; 1.0 eq.) was dissolved in methanol (5 mL) and Pd/C (10 wt%; 89 mg) was added. The heterogeneous mixture was stirred under an atmosphere of hydrogen over night (12 h). After the catalyst was removed by filtration over Celite the solvent was removed under reduced pressure.

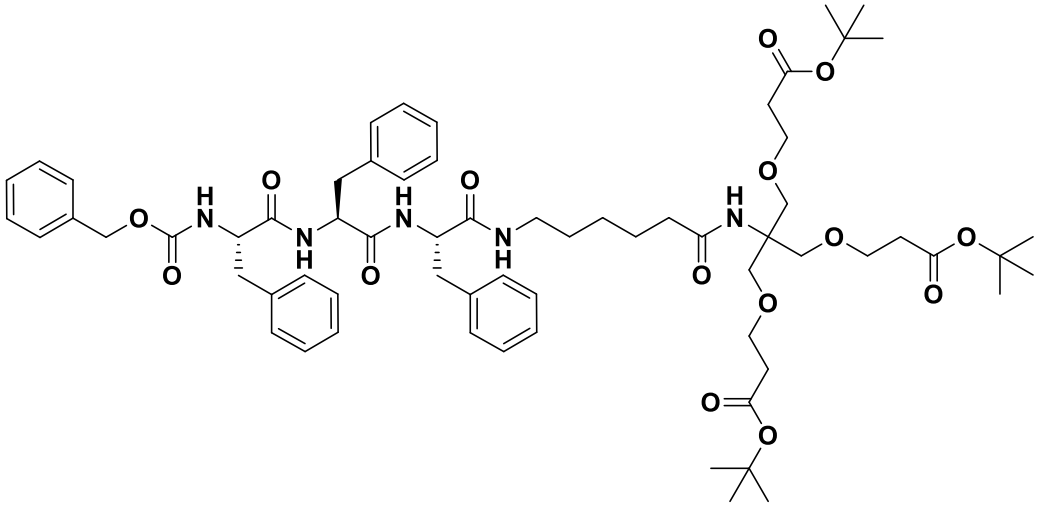
Yield: 636 mg (1.03 mmol, 87%) $C_{31}H_{58}N_2O_{10}$, colourless oil.

^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm]= 6.91 (s, 1H, N*H*), 3.61 – 3.46 (m, 12H, CH_2OCH_2), 2.39 (t, *J* = 6.1 Hz, 6H, 0CH$_2$CH_2), 2.03 (t, *J* = 7.4 Hz, 2H, CH_2CON^{Ahx}), 1.46 – 1.35 (m, 2H, CH_2^{Ahx}), 1.40 (s, 27H, CH_3^{tBu}), 1.34 – 1.17 (m, 6H, CH_2^{Ahx}).

ESI-MS (positive mode): *m/z* calcd. for $[M+H]^+$ 619.4175; found: 619.4151 $[M+H]^+$ and *m/z* calcd. for $[M+Na]^+$ 641.3995; found: 641.3967 $[M+Na]^+$.



Cbz-Triphenylalanine-6-aminohexane-tris{[2-(*tert*-butoxycarbonyl)-ethoxy]methyl}methylcarboxamide



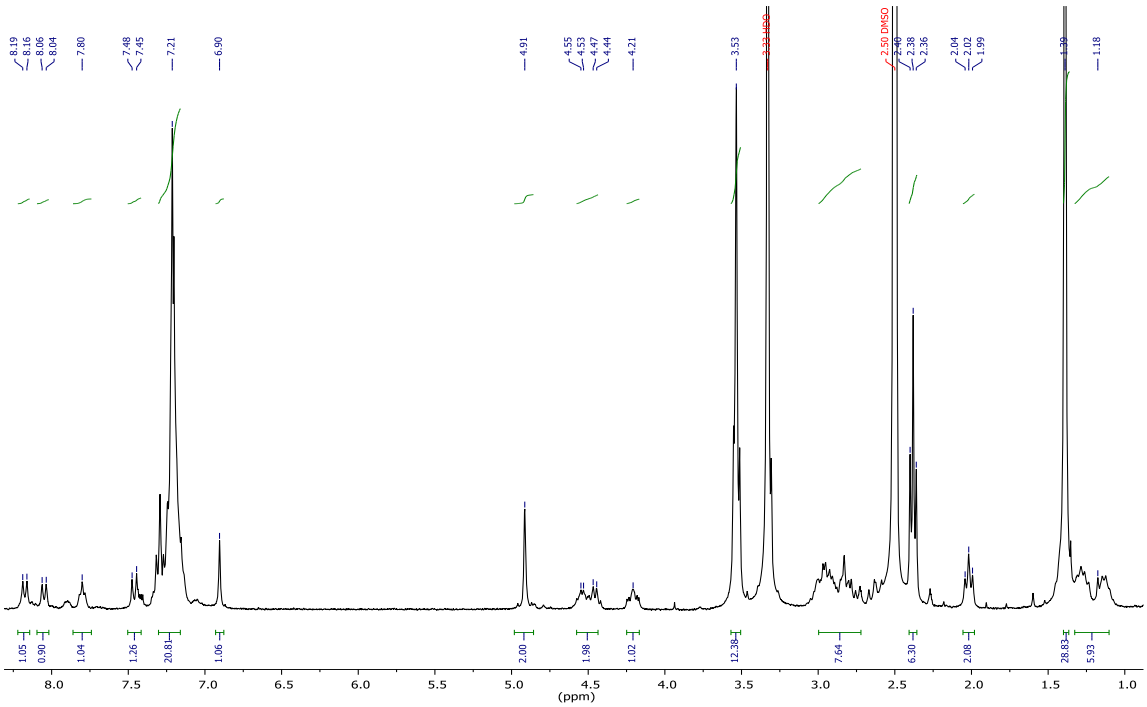
9

2 (127 mg; 0.21 mmol; 1.3 eq.), PyBOP (123 mg; 0.24 mmol; 1.5 eq.) and **8** (94 mg; 0.16 mmol; 1.0 eq.) were dissolved under argon in dry THF (5 mL) and DIPEA (82 mg; 0.64 mmol; 4.0 eq.) were then added. The reaction mixture was stirred at room temperature for 12 h. The solvent was removed under reduced pressure and the residue was purified via flash chromatography over SiO_2 (EtOAc), R_f(EtOAc, SiO_2) = 0.63.

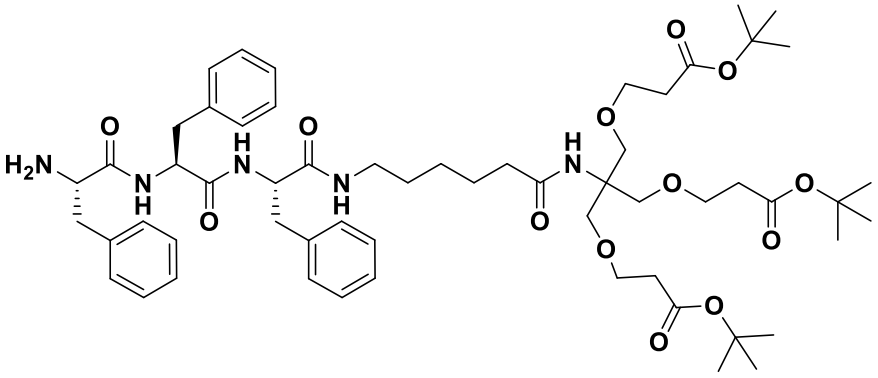
Yield: 150 mg (0.13 mmol, 81%) $C_{66}H_{91}N_5O_{15}$, colourless oil.

^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm]= 8.18 (d, *J* = 8.0 Hz, 1H, N*H*), 8.05 (d, 1H, N*H*, *J* = 8.3 Hz), 7.80 (t, *J* = 5.4 Hz, 1H, N*H*), 7.48 (d, *J* = 8.4 Hz, 1H, N*H*), 7.36 - 7.03 (m, 20H, $CH_{aro}^{Phe\ and\ Cbz}$), 6.90 (s, 1H, N*H*), 4.91 (s, 2H, CH_2^{Cbz}), 4.58 - 4.43 (m, 2H, CH^{α}), 4.25 - 4.27 (m, 1H, CH^{α}), 3.61 – 3.44 (m, 12H, CH_2OCH_2), 3.12 - 2.54 (m, 8H, CH_2^{Phe} and $NHCH_2^{Ahx}$), 2.38 (t, *J* = 6.1 Hz, 6H, OCH_2CH_2,), 2.02 (t, *J* = 7.2 Hz, 2H, CH_2CON^{Ahx}), 1.39 (s, 27H, CH_3^{tBu}), 1.32 – 1.07 (m, 6H, CH_2^{Ahx}).

ESI-MS (positive mode): *m/z* calcd. for $[M+Na]^+$ 1216.6404; found: 1216.6385 $[M+Na]^+$.



Triphenylalanine-6-aminohexane-tris{[2-(*tert*-butoxycarbonyl)-ethoxy]methyl}methylcarboxamide



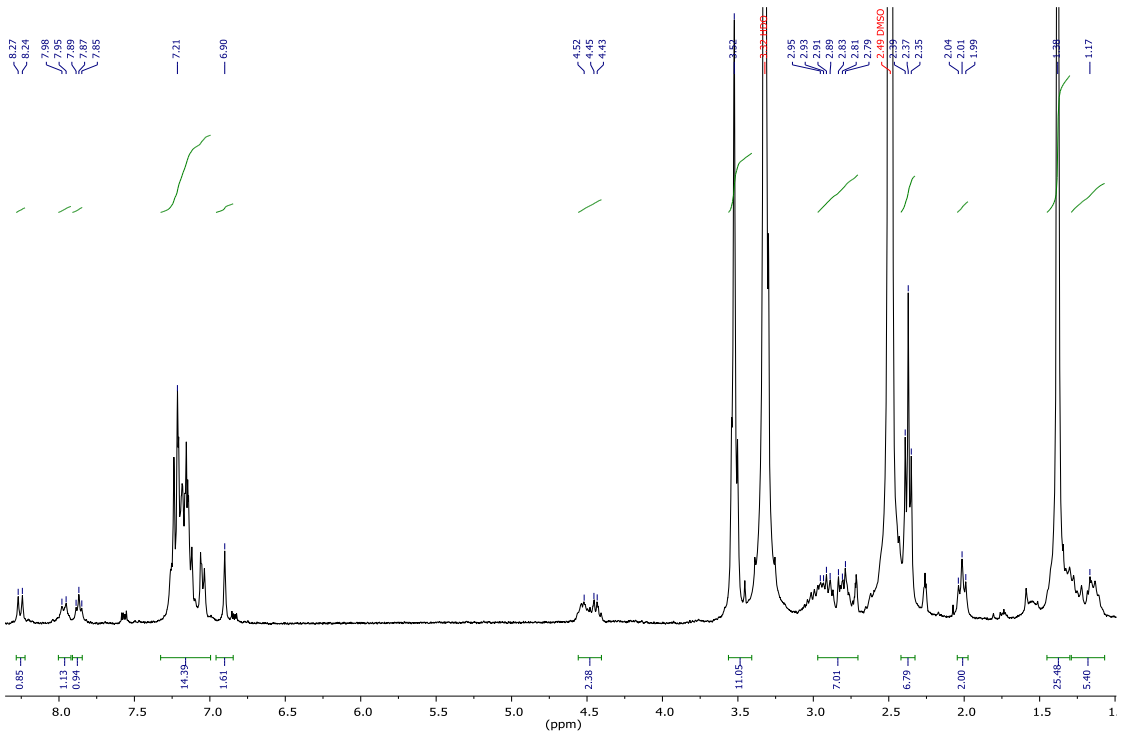
10

9 (150 mg; 0.13 mmol; 1.0 eq.) was dissolved in methanol (3 mL) and Pd/C (10 wt%, 15 mg) was added. The heterogeneous mixture was stirred under an atmosphere of hydrogen over night. After the catalyst was removed via filtration over Celite and the solvent was removed under reduced pressure.

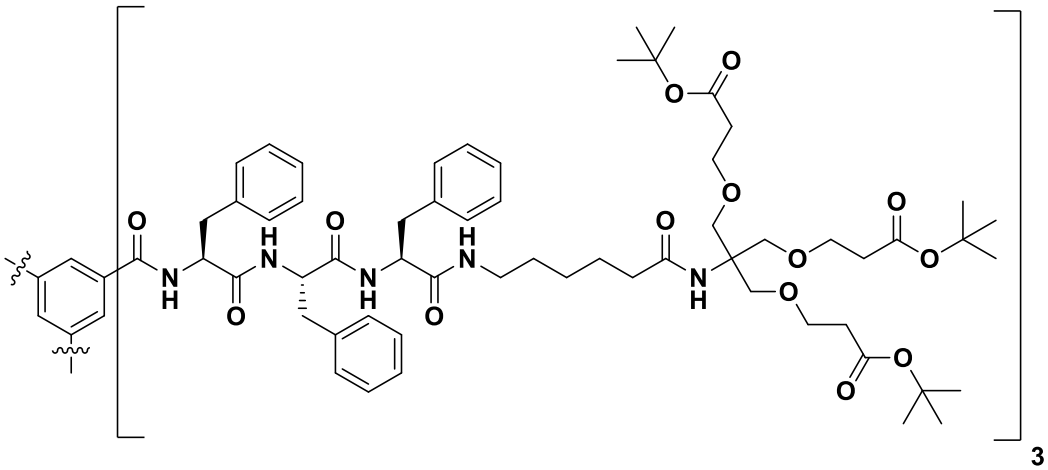
Yield: 118 mg (0.11 mmol, 89%) $C_{58}H_{85}N_5O_{13}$, colourless oil.

^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm]= 8.26 (d, *J* = 8.3 Hz, 1H, N*H*), 7.96 (d, *J* = 8.4 Hz, 1H, N*H*), 7.87 (t, *J* = 5.8 Hz, 1H, N*H*), 7.32 - 7.00 (m, 15H, CH_{aro}^{Phe}), 6.90 (s, 1H, N*H*), 4.56 - 4.38 (m, 2H, CH^{α}), 3.52 - 2.47 (m, 12H, CH_2OCH_2), 2.96 - 2.70 (m, 8H, CH_2^{Phe} and NHCH_2^{Ahx}), 2.37 (t, *J* = 6.0 Hz 6H, OCH_2CH_2), 2.01 (t, *J* = 7.3 Hz, 2H, CH_2CON^{Ahx}), 1.38 (s, 27H, CH_3^{tBu}), 1.18 - 1.09 (m, 6H, CH_2^{Ahx}).

ESI-MS (positive mode): *m/z* calcd. for $[M+H]^+$ 1060.6217, found: 1060.6170 $[M+H]^+$and *m/z* calcd. for $[M+Na]^+$ 1082.6036, found: 1082.5989 $[M+Na]^+$.



BTA-(triphenylalanine-6-aminohexane-tris{[2-(*tert*-butoxycarbonyl)-ethoxy]methyl}methylcarboxamide)$_3$



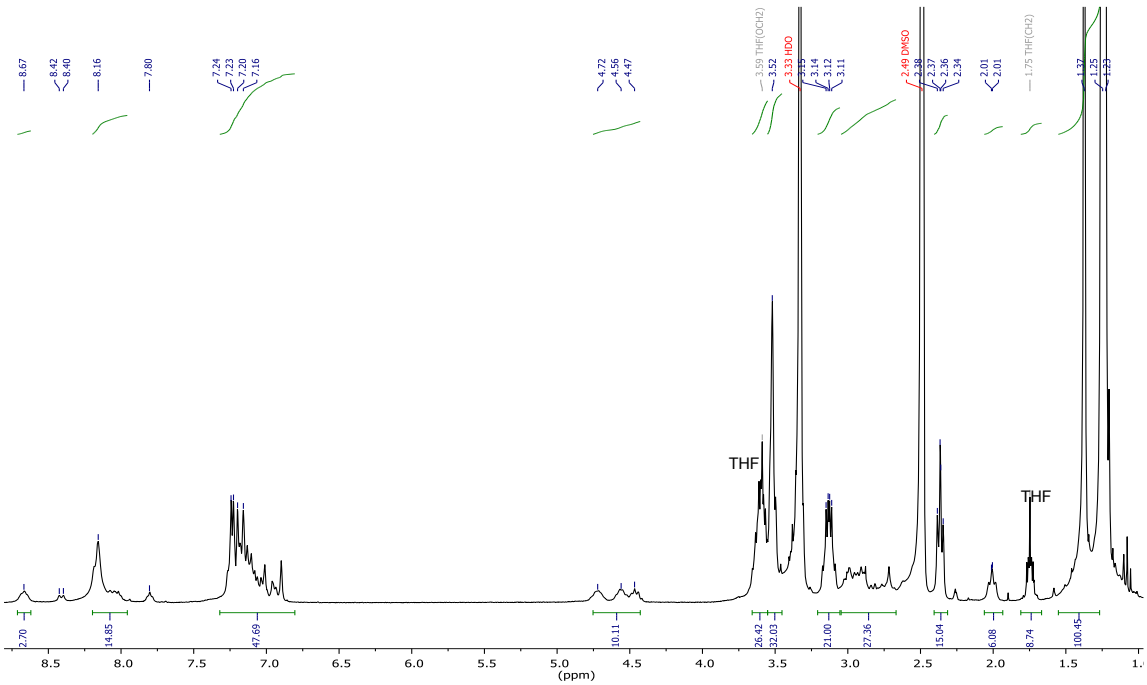
11

10 (171.5 mg; 0.162 mmol; 3.3 eq.) and 1,3,5-benzenetricarbonyl trichloride (13.0 mg; 0.049 mmol; 1.0 eq.) were dissolved under argon in dry DMF (5 mL) and DIPEA (82 mg; 0.64 mmol; 4.0 eq.) were then added. The reaction mixture was stirred at room temperature for 18 h. The organic solvent was removed under reduced pressure. The residue was purified via precipitation from THF into a mixture of *n*-pentan and diethylether (1:1) and then isolated via centrifugation. The residue was purified via flash chromatography over SiO_2 (DCM:EtOAc:MeOH 1:1:1), R_f(DCM:EtOAc:MeOH 1:1:1, SiO_2) = 0.50.

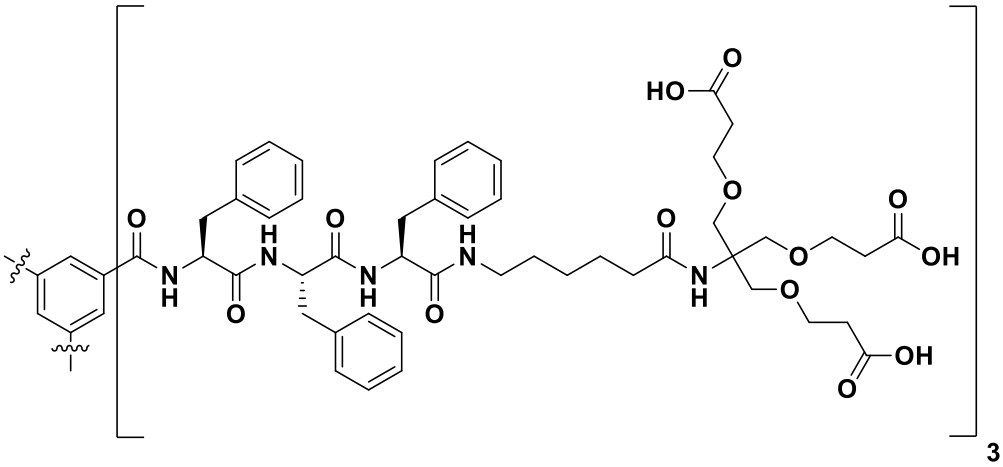
Yield: 105 mg (0.031 mmol, 63%) $C_{183}H_{255}N_{15}O_{42}$, light brownish solid.

^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm]= 8.72 – 8.61 (m, 3H, N*H*), 8.41 (d, *J* = 8.5 Hz, 3H, N*H*), 8.25 - 7.99 (m, 9H, CH_{aro}^{BTA} and N*H*), 7.80 (t, J = 5.6 Hz, 3H, N*H*), 7.33 – 6.87 (m, 48H, CH_{aro}^{Phe} and N*H*), 4.82- 4.38 (m, 9H, CH^{α}), 3.59 (m, 36H, CH_2OCH_2), 3.08 - 2.67 (m, 24H, CH_2^{Phe} and NHCH_2^{Ahx}), 2.36 (t, *J* = 6.3 Hz, 18H, OCH_2CH_2), 2.01 (t, *J* = 7.0 Hz, 6H,CH_2CON^{Ahx}), 1.50 – 1.28 (m, 99H, CH_3^{tBu} and CH_2^{Ahx}).

MALDI-MS (positive mode) (DHB [EtOAc]): *m/z* calcd. for $[M+Na]^+$ 3357.82; found: 3357.86 $[M+Na]^+$.



BTA-(triphenylalanine-6-aminohexane-tris{[2-carboxyethoxy]methyl}methylcarboxamide)$_3$



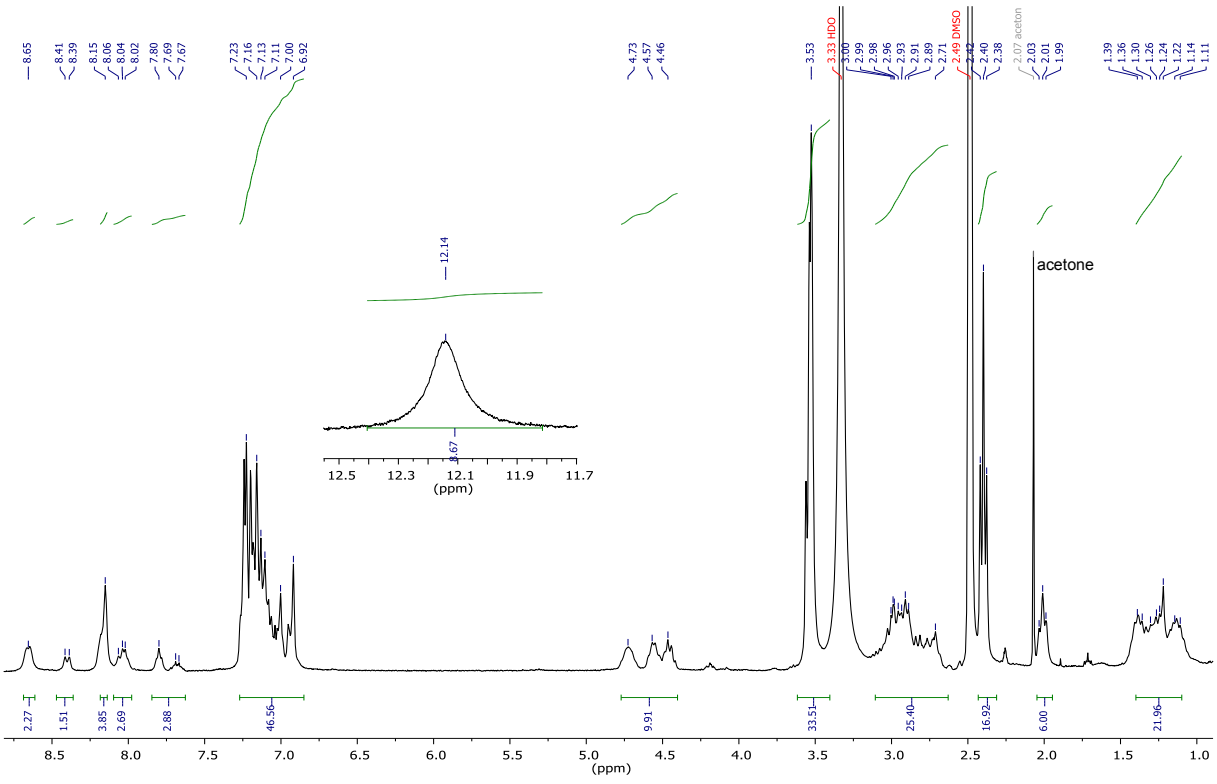
12

11 (105 mg; 0.031 mol) was stirred three times in 2.5 mL of a mixture of TFA and DCM (1:1) for 1 h. The organic solvent was removed after each step under reduced pressure. Finally the residue was taken up in water and freeze-dried over night.

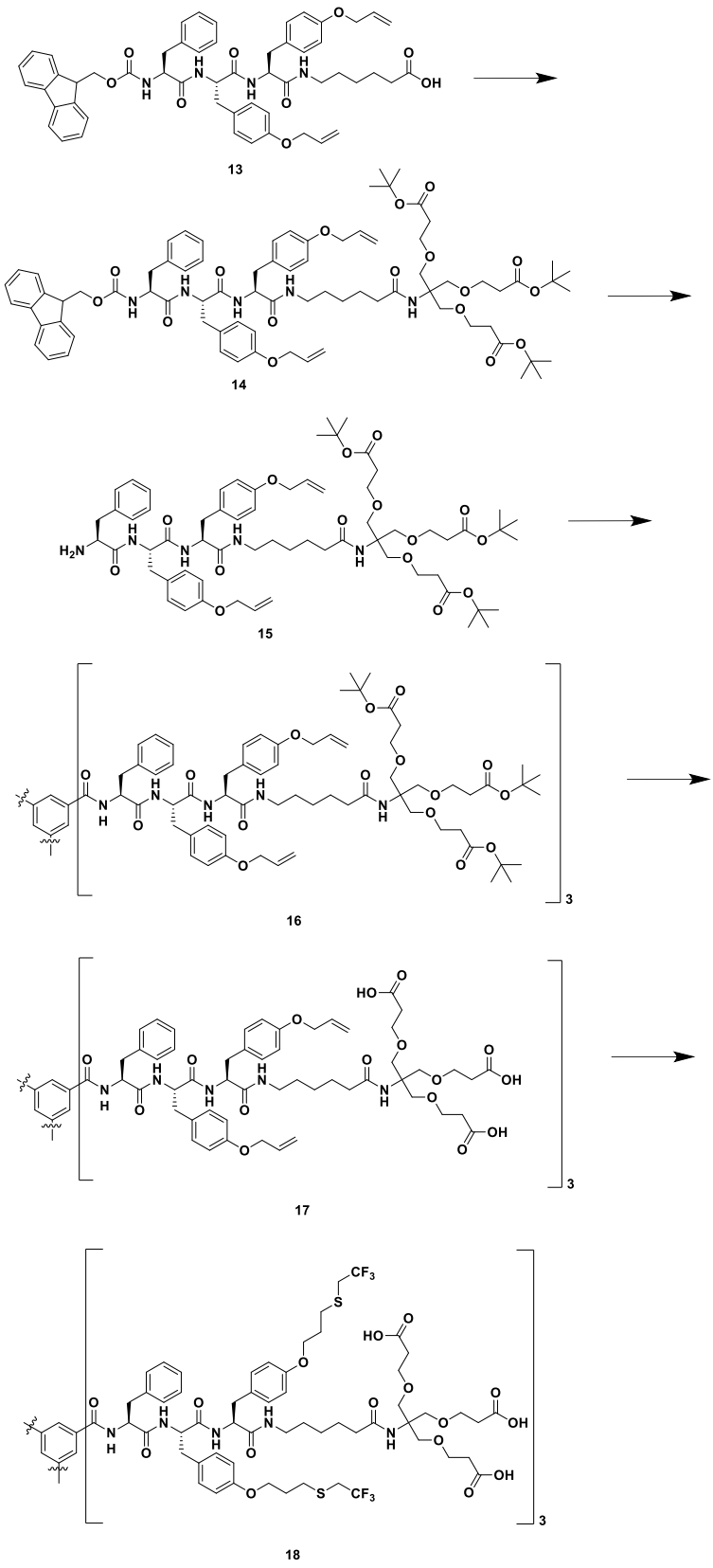
Yield: 42 mg (0.015 mmol, 48%) $C_{147}H_{183}N_{15}O_{42}$, white solid.

^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm]= 12.14 (brs, 9H, COO*H*), 8.73 – 8.58 (m, 3H, N*H*), 8.49 – 8.32 (m, 3H, N*H*), 8.15 (s, 3H, CH_{aro}^{BTA}), 8.10 – 7.96 (m, 3H, N*H*), 7.84 – 7.62 (m, 3H, N*H*), 7.33 – 6.87 (m, 48H, H_{aro}^{Phe} and N*H*), 4.83- 4.37 (m, 9H, CH^{α}), 3.53 (m, 36H, CH_2OCH_2), 3.08 -2.67 (m, 24H, CH_2^{Phe} and $NHCH_2^{Ahx}$), 2.40 (t, *J* = 6.3 Hz, 18H, OCH_2CH_2), 2.01 (t, *J* = 7.0 Hz, 6H, CH_2CON^{Ahx}), 1.46 - 1.11 (m, 18H, CH_2^{Ahx}).

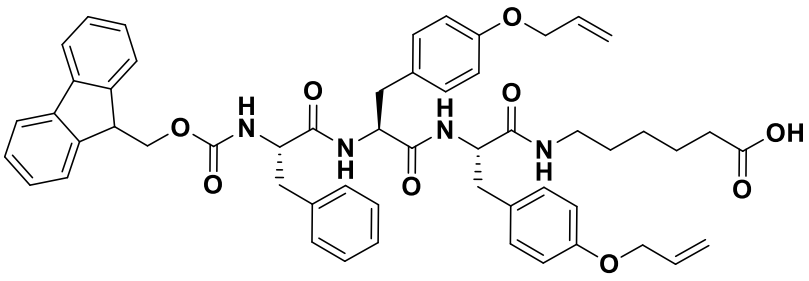
ESI-MS (positive mode): *m/z* calcd. for $[M+2Na]^{2+}$ 1438.6231; found: 1438.6249 $[M+2Na]^{2+}$.



Synthesis route for the dendritic fluorinated peptide amphiphiles 18



***N*-(Fluorenylmethyloxycarbonyl)-L-phenylalanine-di-(*O*-allyl-L-tyrosine)-6-aminohexanoic acid**



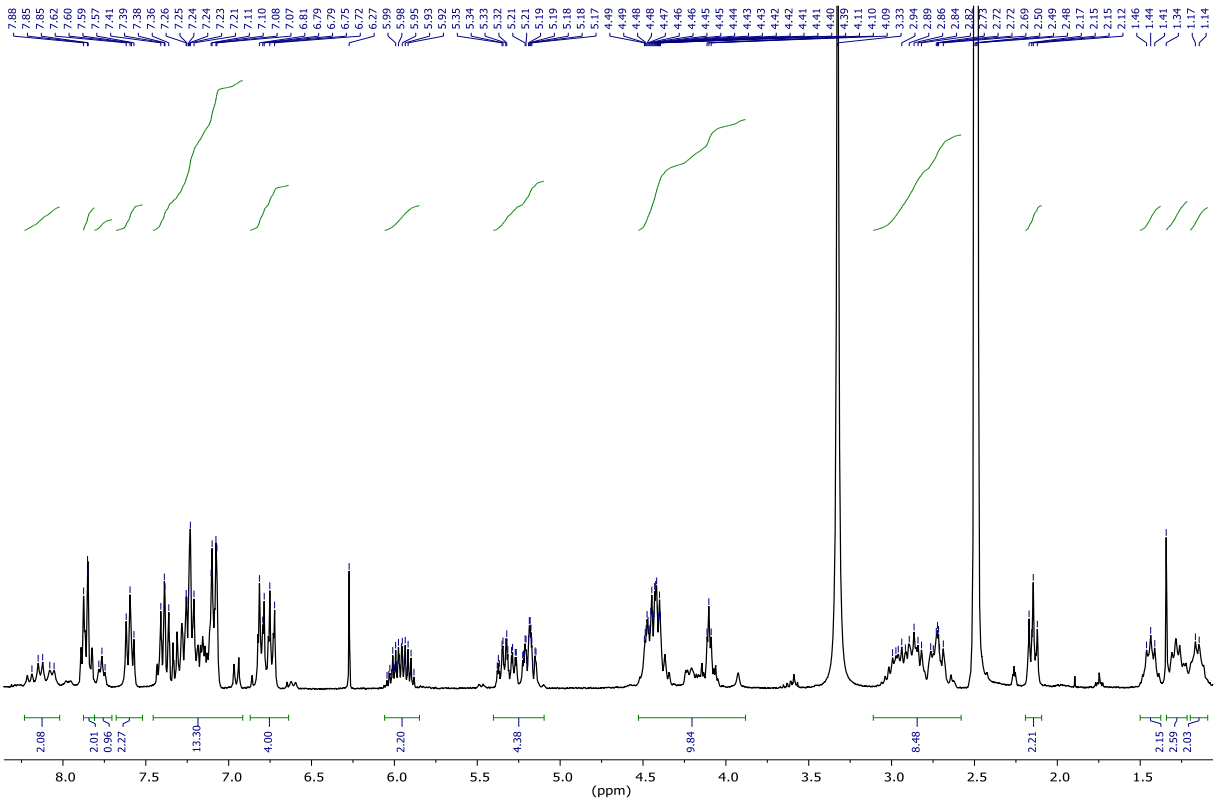
13

The Fmoc-protected peptide bound to the 2-chlorotrityl resin (from SPPS) was stirred three times in 10 mL of a mixture of HFIP and DCM (1:4) for 45 min each and washed subsequently with 10 mL of DCM. The collected organic phase was concentrate. The residue was purified via precipitation from a mixture THF, DMF and DCM (1:1:1) into a mixture of *n*-pentane and diethylether (1:1) and isolated via centrifugation.

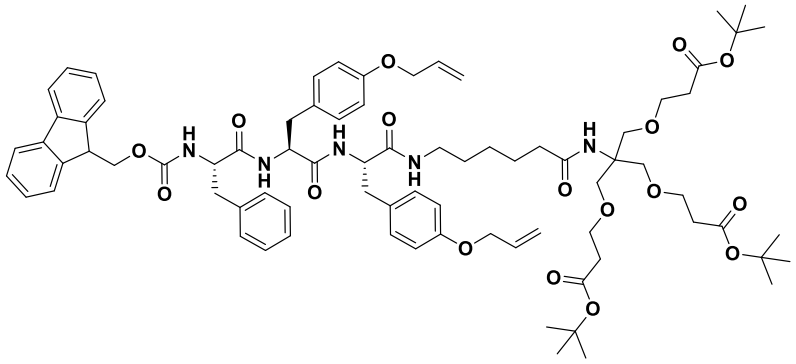
Yield: 858 mg (0.97 mmol) $C_{54}H_{58}N_4O_9$, white solid.

^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm]= 8.26 – 8.01 (m, 2H, N*H*), 7.94 – 7.79 (m, 2H, CH_{aro}^{Fmoc}), 7.81 – 7.72 (m, 1H, N*H*), 7.65 – 7.52 (m, 3H, N*H*, CH_{aro}^{Fmoc}), 7.47 – 6.90 (m, 13H, C$H_{aro}^{Fmoc, Phe and Tyr}$), 6.85 – 6.67 (m, 4H, C$H_{aro}^{Tyr}$), 5.96 (ddt, *J* = 21.9, 10.6, 5.3 Hz, 2H, OCH_2CH=CH_2), 5.39 – 5.11 (m, 4H, OCH_2CH=CH_2), 4.53 – 3.88 (m, 10H, OCH_2CH=CH_2, CH^{α}, CH^{Fmoc} and CH_2^{Fmoc}), 3.06 – 2.60 (m, 8H, C$H_2^{Phe\ an\ Tyr}$ and NHCH_2^{Ahx}), 2.15 (t, *J* = 7.4 Hz, 2H,CH_2CON^{Ahx}), 1.48 - 1.38 (m, 2H, CH_2^{Ahx}), 1.33 - 1.23 (m, 2H, CH_2^{Ahx}), 1.20 - 1.11 (m, 2H,CH_2^{Ahx}).

ESI-MS (positive mode): *m/z* calcd. for $[M+Na]^+$ 929.4096; found: 929.4095 $[M+Na]^+$



N-(Fluorenylmethyloxycarbonyl)-L-phenylalanine-di-(*O*-allyl-L-tyrosine)-6-aminohexane)-tris{[2-(*tert*-butoxycarbonyl)-ethoxy]methyl}methylcarboxamide



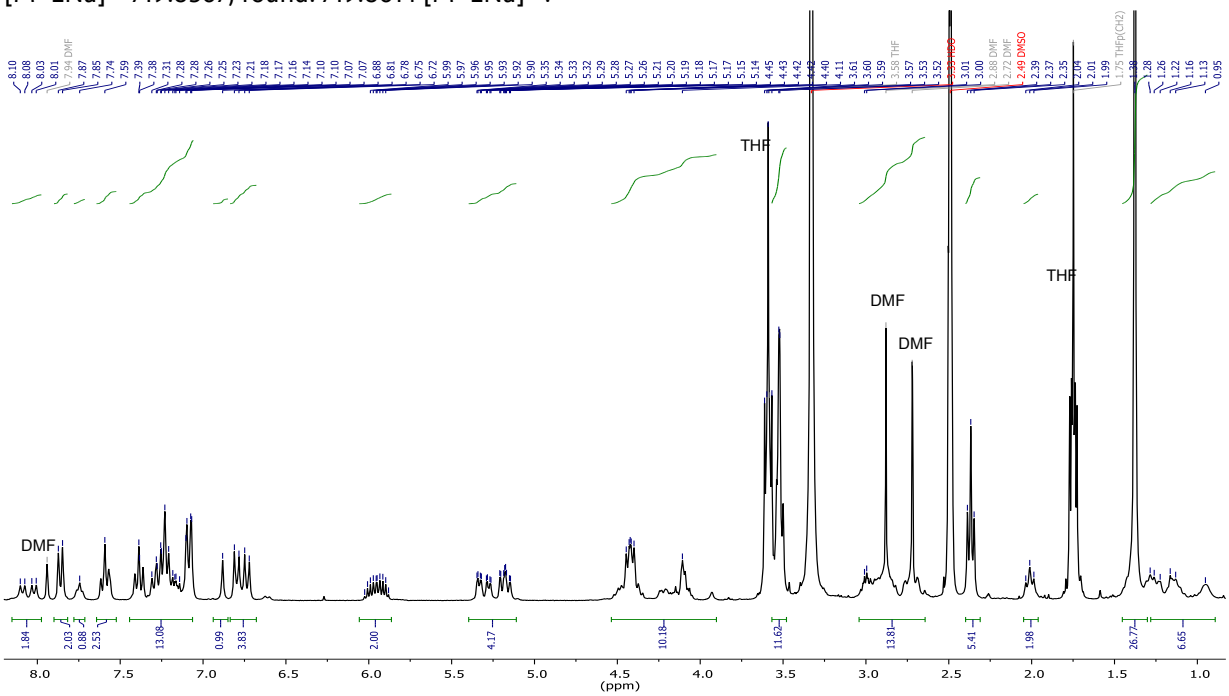
14

13 (740 mg; 0.84 mmol; 1.0 eq.) and **1** (460 mg; 0.92 mmol; 1.1 eq.) were dissolved in dry DCM (5 mL) and treated with DIPEA (433 mg; 3.35 mmol; 4.0 eq.) and PyBOP (654 mg; 1.26 mmol; 1.5 eq.). The solution was stirred at room temperature for 19 h. After evaporation of the organic solvent under reduced pressure the residue was purified via flash chromatography over SiO_2 (EtOAc:DCM 2:1), R_f(EtOAc:DCM 2:1) = 0.64.

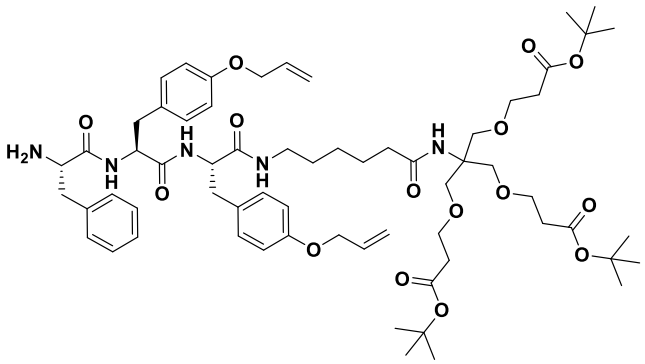
Yield: 833 mg (0.60 mmol, 71%), $C_{79}H_{103}N_5O_{17}$, white solid.

^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm]= 8.17 – 7.99 (m, 2H, N*H*), 7.91 – 7.82 (m, 2H, CH_{aro}^{Fmoc}), 7.78 – 7.70 (m, 1H, N*H*), 7.65 – 7.55 (m, 3H, N*H* and CH_{aro}^{Fmoc}), 7.46 – 7.01 (m, 13H, C$H_{aro}^{Fmoc,\ Phe\ and\ Tyr}$), 6.88 (s, 1H, N*H*), 6.77 (dd, *J* = 18.7, 8.3 Hz, 4H, CH_{aro}^{Tyr}), 5.94 (ddt, *J* = 22.4, 10.3, 5.1 Hz, 2H, OCH_2CH=CH_2), 5.40 – 5.10 (m, 4H, OCH_2CH=CH_2), 4.54 – 3.86 (m, 10H, OCH_2CH=CH_2, CH^{α}, CH^{Fmoc} and CH_2^{Fmoc}), 3.55 – 3.48 (m, 12H, CH_2OCH_2), 3.05 – 2.66 (m, 8H, C$H_2^{Phe\ and\ Tyr}$ and NHCH_2^{Ahx}), 2.37 (t, *J* = 6.1 Hz, 6H, OCH_2CH_2), 2.01 (t, *J* = 7.4 Hz, 2H, CH_2CON^{Ahx}), 1.38 (m, 27H, CH_3^{tBu}), 1.32 – 0.90 (m, 6H, CH_2^{Ahx}).

ESI-MS (positive mode): *m/z* calcd. for $[M+Na]^+$ 1416.7241; found: 1416.7238 $[M+Na]^+$ and *m/z* calcd. for $[M+2Na]^{2+}$ 719.8567; found: 719.8614 $[M+2Na]^{2+}$.



L-Phenylalanine-di-(*O*-allyl-L-tyrosine)-6-aminohexane)-tris{[2-(*tert*-butoxycarbonyl)-ethoxy]methyl}methylcarboxamide



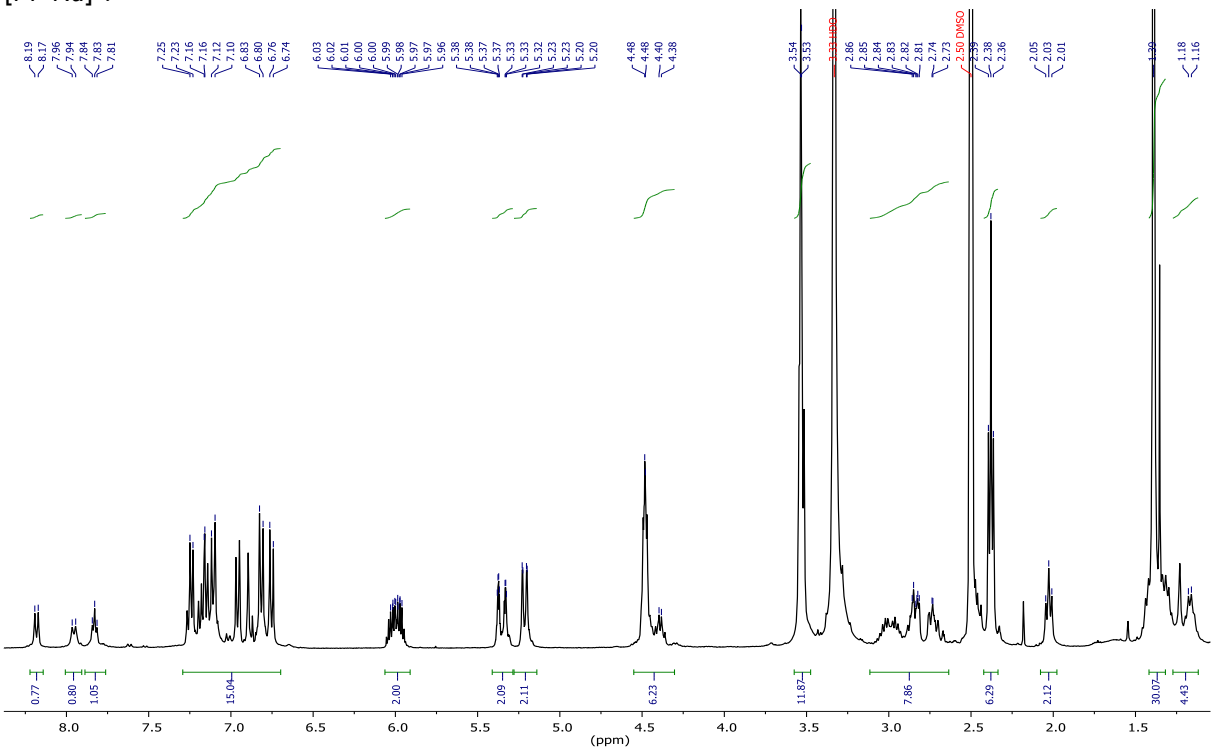
15

14 (241 mg; 0.173 mmol; 1.0 eq.) was dissolved in 5 mL acetonitrile and 1 mL piperidine was added. The mixture was stirred for 1 h at room temperature. The solvent was removed under reduced pressure. This procedure was repeated twice. The residue was purified via SEC in methanol, R_f(DCM:EtOAc 2:1, SiO_2) = 0.35.

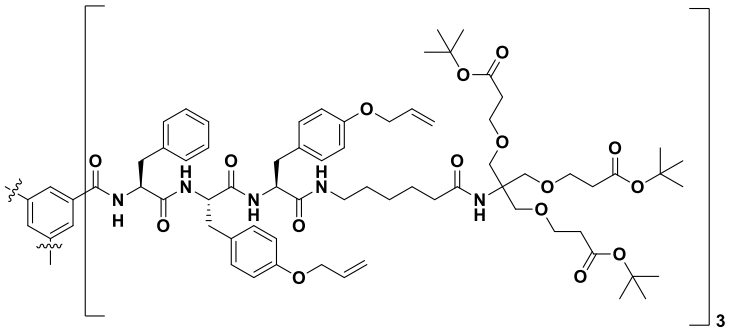
Yield: 90 mg (0.076 mmol, 44%), $C_{64}H_{93}N_5O_{15}$, colourless oil.

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm]= 8.18 (d, *J* = 8.3 Hz, 1H, N*H*), 7.98 (d, *J* = 8.4 Hz, 1H, N*H*), 7.83 (t, *J* = 5.6 Hz, 1H, N*H*), 7.34 – 6.70 (m, 14H, $CH_{aro}^{Phe\ and\ Tyr}$ and N*H*), 6.00 (ddt, *J* = 12.1, 10.5, 5.3 Hz, 2H, OCH_2CH=CH_2), 5.40 – 5.29 (m, 2H, OCH_2CH=CH_2), 5.21 (dd, *J* = 10.5, 1.6 Hz, 2H, OCH_2CH=CH_2), 4.56 – 4.40 (m, 6H, OCH_2CH=CH_2 and CH^{α}), 3.59 – 3.44 (m, 12H, CH_2OCH_2), 3.06 – 2.60 (m, 8H, $CH_2^{Phe\ and\ Tyr}$ and $NHCH_2^{Ahx}$), 2.38 (t, *J* = 6.1 Hz, 6H, OCH_2CH_2), 2.02 (t, *J* = 7.4 Hz, 2H, CH_2CON^{Ahx}), 1.43 – 1.28 (m, 29H, CH_3^{tBu} and CH_2^{Ahx}), 1.28 – 1.12 (m, 4H, CH_2^{Ahx}).

ESI-MS (positive mode): *m/z* calcd. for $[M+Na+H]^{2+}$ 597.8317; found: 597.8304 $[M+Na+H]^{2+}$, *m/z* calcd. for $[M+H]^+$ 1172.6741; found: 1172.6728 $[M+H]^+$ and *m/z* calcd. for $[M+Na]^+$ 1194.6560; found: 1194.6545 $[M+Na]^+$.



BTA-[L-phenylalanine-di-(*O*-allyl-L-tyrosine)-6-aminohexane-tris{[2-(*tert*-butoxycarbonyl)-ethoxy]methyl}methylcarboxamide]$_3$



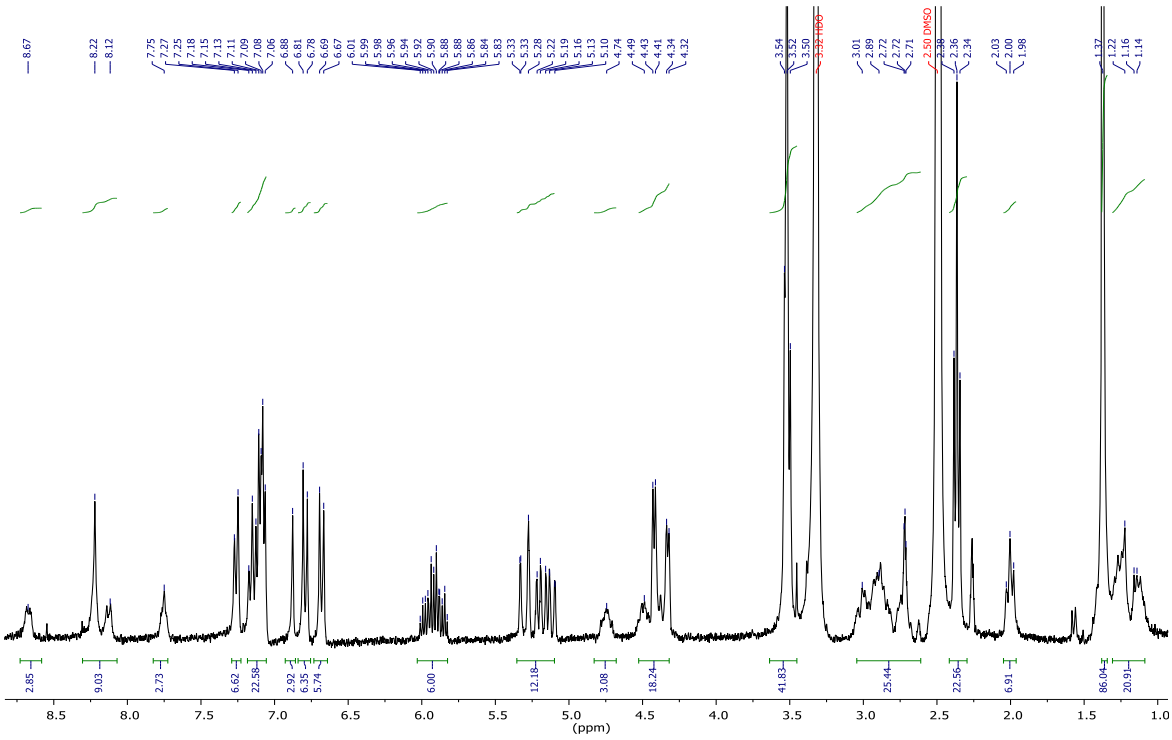
16

15 (89 mg; 0.07 mmol; 3.3 eq.) and 1,3,5-benzenetricarbonyl trichloride (6 mg; 0.02 mmol; 1.0 eq.) were dissolved in 3 mL DMF (SPPS grade) and treated with DIPEA (89 mg; 0.69 mmol; 30.0 eq.). The solution was stirred at room temperature for 45 h. The solvent was removed under reduced pressure. The residue was purification via precipitation from water and washing subsequently with methanol to remove excess **15**.

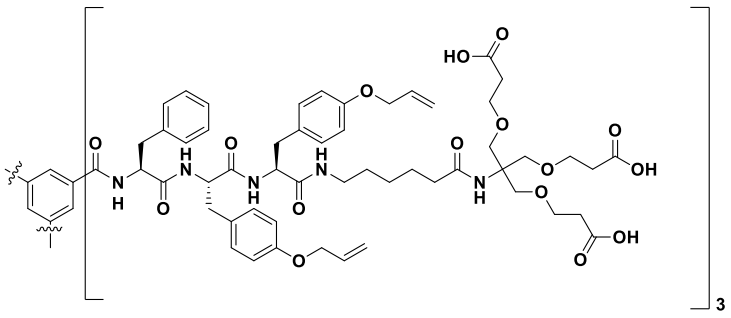
Yield: 38 mg (0.01 mmol, 50%), $C_{201}H_{279}N_{15}O_{48}$, white solid.

^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm]= 8.77 – 8.60 (m, 3H, N*H*), 8.26 – 8.08 (m, 9H, N*H* and CH_{aro}^{BTA}), 7.84 – 7.69 (m, 3H, N*H*), 7.26 (d, *J* = 7.4 Hz, 6H, CH_{aro}^{Tyr}), 7.18 – 7.05 (m, 21H, C$H_{aro}^{Phe\ and\ Tyr}$), 6.88 (s, 1H, s, 3H, N*H*), 6.79 (d, *J* = 8.5 Hz, 3H, CH_{aro}^{Tyr}), 6.68 (d, *J* = 8.4 Hz, 3H, CH_{aro}^{Tyr}), 5.92 (ddt, *J* = 17.1, 10.4, 5.1 Hz, 6H, OCH$_2$C*H*=CH$_2$), 5.39 – 5.04 (m, 12H, OCH$_2$CH=C*H*$_2$), 4.81 – 4.65 (m, 3H, C*H*$^{\alpha}$), 4.53 – 4.28 (m, 18H, C*H*$^{\alpha}$ and OC*H*$_2$CH=CH$_2$), 3.57 – 3.45 (m, 36H, C*H*$_2$OC*H*$_2$), 3.08 – 2.58 (m, 24H, C$H_2^{Phe\ and\ Tyr}$ and NHCH_2^{Ahx}), 2.36 (t, *J* = 6.1 Hz, 18H, OCH$_2$C*H*$_2$), 2.00 (t, *J* = 7.5 Hz, 6H, C*H*$_2$CON^{Ahx}), 1.37 (m, 81H, CH_3^{tBu}), 1.30 – 1.04 (m, 18H, CH_2^{Ahx}).

MALDI-MS (positive mode) (DHB [H$_2$O/ACN]): *m/z* calcd. for [M+Na]$^+$ 3695.98; found: 3696.16 [M+Na]$^+$.



BTA-[L-phenylalanine-di-(*O*-allyl-L-tyrosine)-6-aminohexane-tris{[2-carboxyethoxy]methyl} methylcarboxamide]$_3$



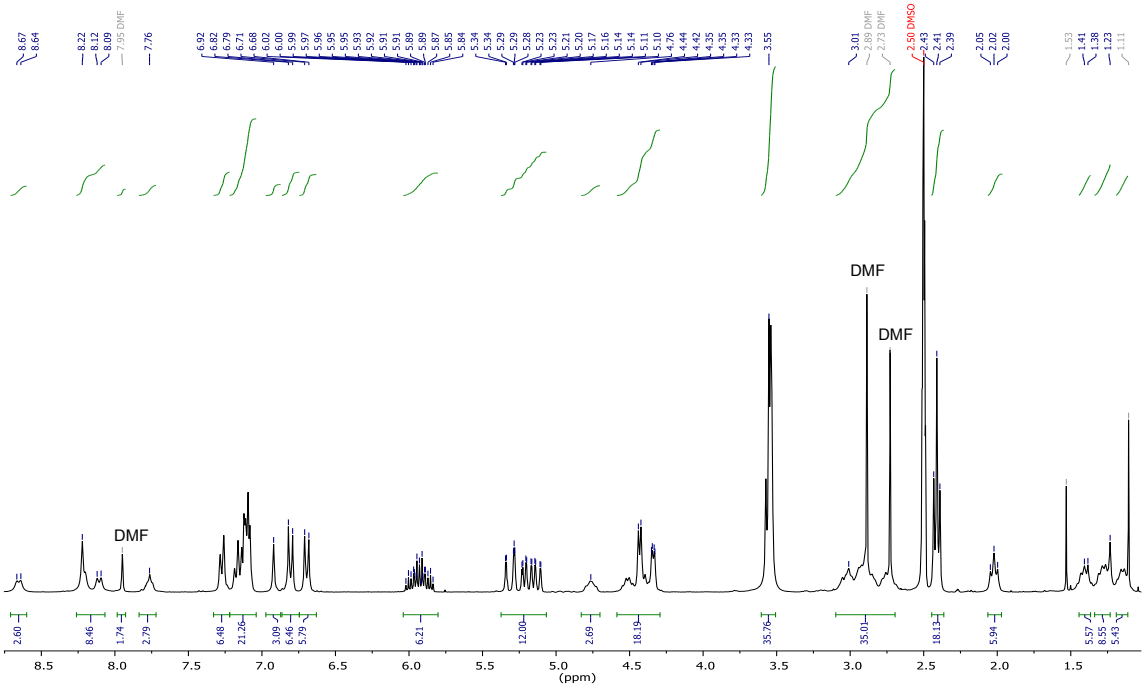
17

16 (38 mg; 0.01 mmol) was stirred twice in 2.0 mL of a mixture of TFA and DCM (1:1) for 45 min. The organic solvent was removed after each step under reduced pressure.

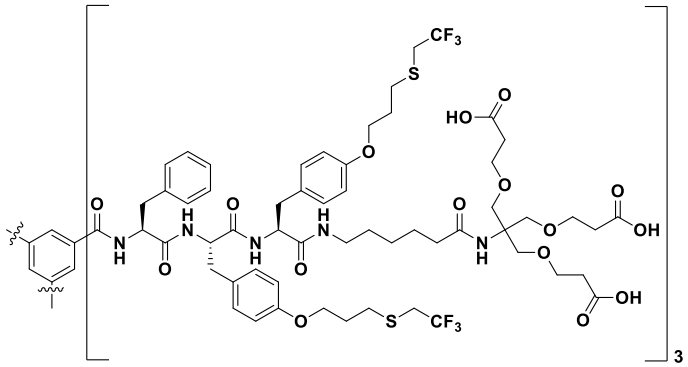
Yield: 30 mg (0.009 mmol, 93%), $C_{165}H_{207}N_{15}O_{48}$, white solid.

^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm]= 8.68 – 8.58 (m, 3H, N*H*), 8.27 – 8.08 (m, 9H, N*H* and CH_{aro}^{BTA}), 7.80 – 7.71 (m, 3H, N*H*), 7.27 (d, *J* = 6.9 Hz, 6H, CH_{aro}^{Tyr}), 7.21 – 7.06 (m, 21H, C$H_{aro}^{Tyr\ and\ Phe}$), 6.92 (s, 3H, N*H*), 6.81 (d, *J* = 8.7 Hz, 6H, CH_{aro}^{Tyr}), 6.70 (d, *J* = 8.6 Hz, 6H, CH_{aro}^{Tyr}), 5.93 (ddt, *J* = 17.2, 10.4, 5.2 Hz, 6H, CH_2CH=CH_2), 5.37 – 5.06 (m, 12H, OCH_2CH=CH_2), 4.81 – 4.71 (m, 3H, CH^{α}), 4.55 – 4.27 (m, 21H, CH^{α} and CH_2CH=CH_2), 3.59 – 3.49 (m, 36H, CH_2OCH_2), 3.09 – 2.68 (m, 24H, C$H_2^{Tyr\ and\ Phe}$ and NHCH_2^{Ahx}), 2.41 (t, *J* = 6.3 Hz, 18H, OCH_2CH_2), 2.02 (t, *J* = 7.4 Hz, 6H, CH_2CON^{Ahx}), 1.46 – 1.10 (m, 18H, CH_2^{Ahx}).

MALDI-MS (positive mode) (DHB [EtOAc]): *m/z* calcd. for $[M+Na]^+$ 3191.5172; found: 3191.534 $[M+Na]^+$.



BTA-[L-phenylalanine-di-(*O*-{3-[(2,2,2-trifluoroethyl)thio]propyl}-L-tyrosine)-6-aminohexane-tris{[2-carboxyethoxy]methyl}methylcarboxamide]$_3$



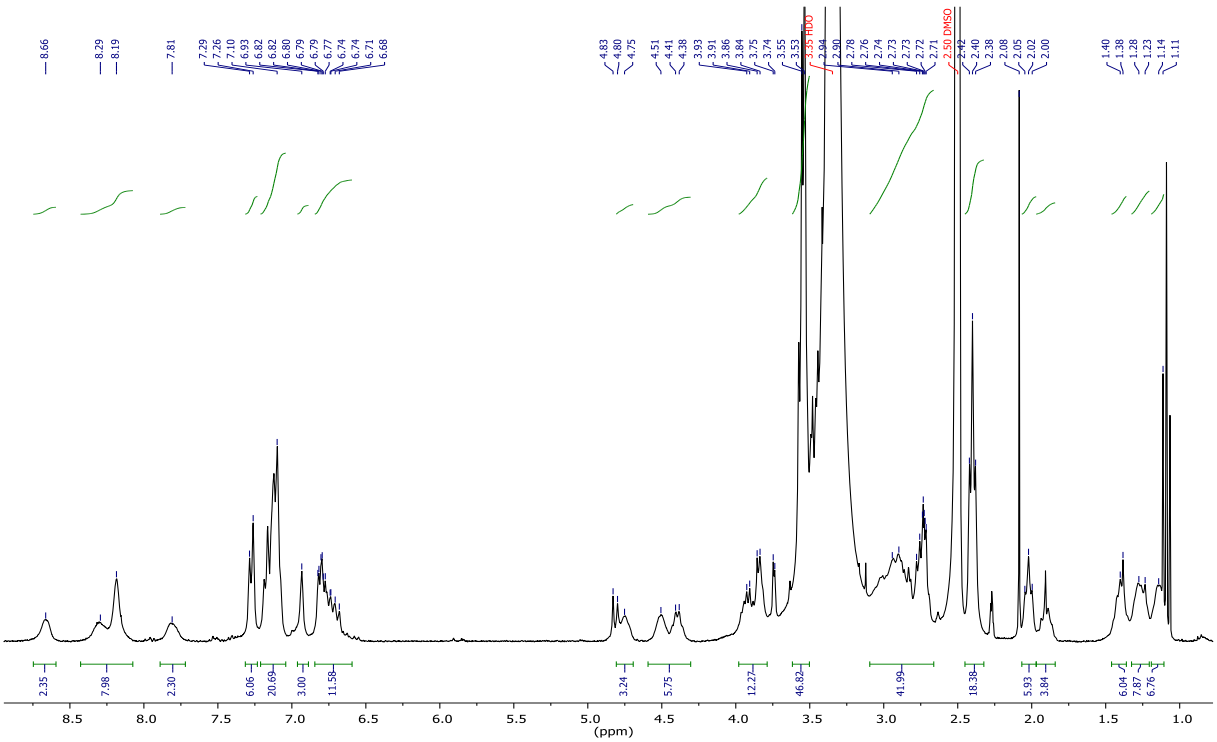
18

17 (29.5 mg; 0.0093 mmol; 1.0 eq.) was dissolved in a mixture of 6 mL THF and water (1:1). 1 mg 2,2-dimethoxy-2-phenylacetophenone (DMPA) and trifluorethanethiol (0.093 mmol, 10.8 mg 10.0 eq.) were added and the mixture was stirred for 18 h under irradiation with UV light (λ = 365 nm and 405 nm). The solvent was removed under reduced pressure and the residue was washed with diethylether. After dissolving the residue in basic water it was purified via SEC with water as a mobile phase.

Yield: 13.5 mg (0.0035 mmol, 38%), $C_{177}H_{225}F_{18}N_{15}O_{48}S_6$, white solid.

^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm]= 8.71 – 8.61 (m, 3H, N*H*), 8.41 – 8.06 (m, 9H, N*H* and CH_{aro}^{BTA}), 7.89 – 7.76 (m, 3H, N*H*), 7.27 (d, *J* = 7.4 Hz, 6H, CH_{aro}^{Tyr}), 7.21 – 7.06 (m, 21H, $CH_{aro}^{Phe\ and\ Tyr}$), 6.93 (s, 3H, N*H*), 6.84 – 6.64 (m, 12H, CH_{aro}^{Tyr}), 4.80 – 4.66 (m, 3H, CH^{α}), 4.56 - 4.32 (m, 6H, CH^{α}), 3.95-3.80 (m, 12H, ArOCH_2), 3.60 – 3.50 (m, 36H, CH_2OCH_2), 3.0 – 2.7 (m, 36H, $CH_2^{Tyr\ and\ Phe}$, NHCH_2^{Ahx} and CH_2CF_3), 2.40 (t, *J* = 6.3 Hz, 18H, OCH_2CH_2), 2.02 (t, *J* = 6.7 Hz, 6H, CH_2CON^{Ahx}), 1.48 – 1.34 (m, 6H, CH_2^{Ahx}), 1.30 – 1.23 (m, 6H, CH_2^{Ahx}), 1.18 – 1.09 (m, 6H, CH_2^{Ahx}).

^{19}F NMR (282 MHz, DMSO-d_6): δ [ppm]= -65.01 (t, *J* = 11.0 Hz, 9F), -65.20 (t, *J* = 10.8 Hz, 9F).



Circular Dichroism Spectroscopy

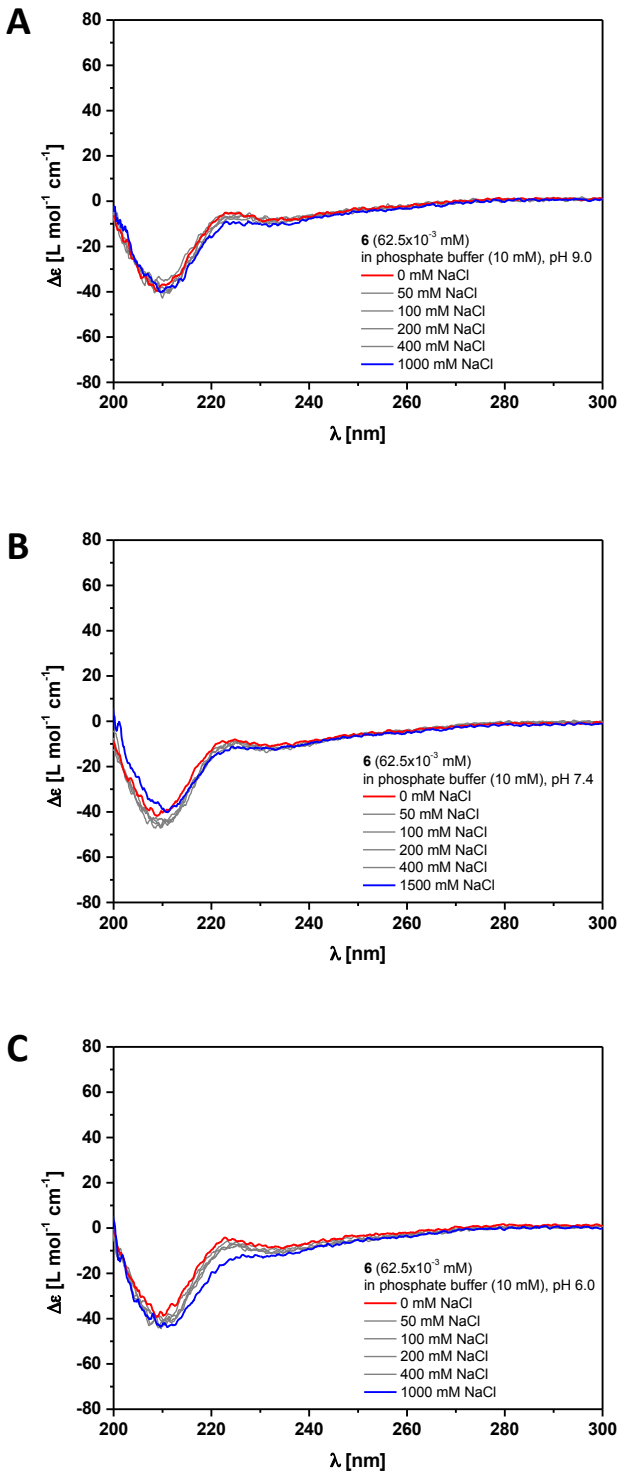


Figure S1 Ionic strength dependent CD spectra for dendritic peptide amphiphile **6** (62.5×10^{-3} mM, 293 K) in 10 mM phosphate buffer at pH 9.0 (**A**), pH 7.4 (**B**) and pH 6.0 (**C**).

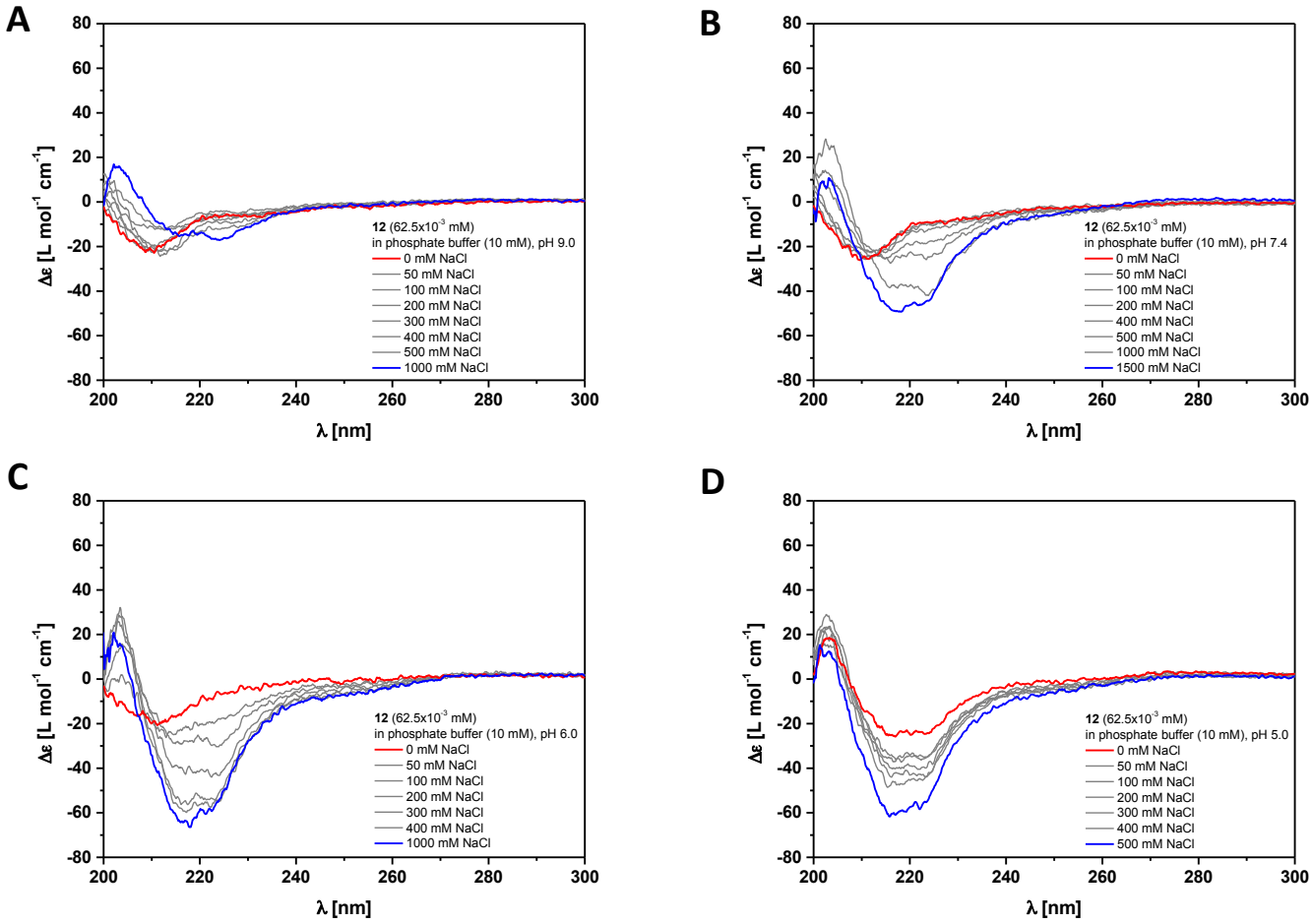


Figure S2 Ionic strength dependent CD spectra for dendritic peptide amphiphile **12** (62.5×10^{-3} mM, 293 K) in 10 mM phosphate buffer at pH 9.0 (**A**), pH 7.4 (**B**), pH 6.0 (**C**) and pH 5.0 (**D**).

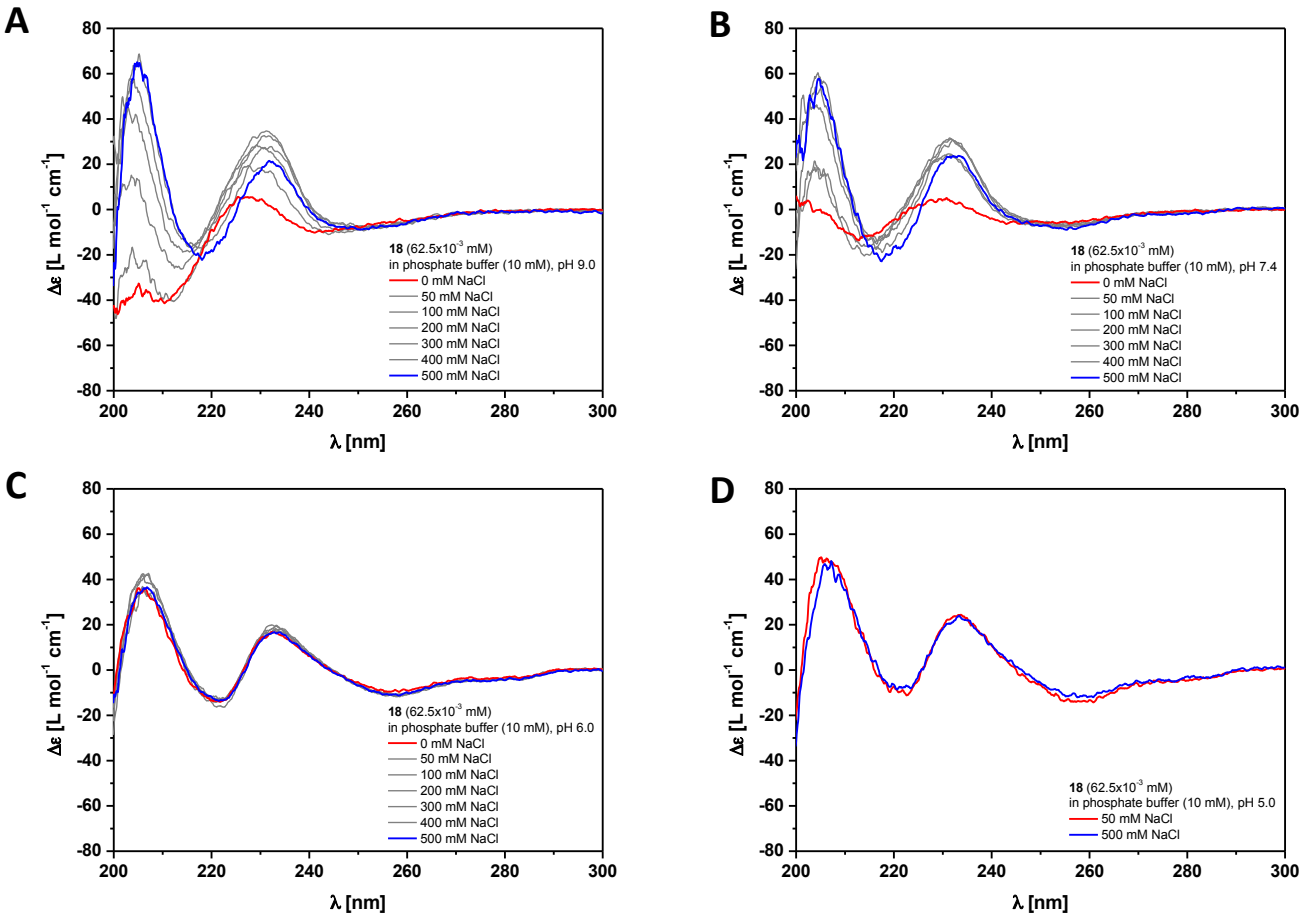


Figure S3 Ionic strength dependent CD spectra for dendritic peptide amphiphile **18** (62.5×10^{-3} mM, 293 K) in 10 mM phosphate buffer at pH 9.0 (**A**), pH 7.4 (**B**), pH 6.0 (**C**) and pH 5.0 (**D**).

Electron Microscopy

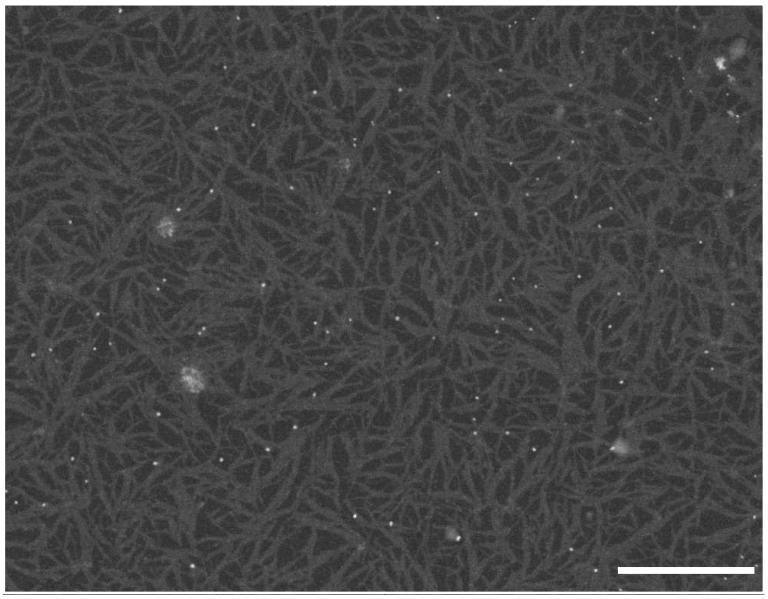


Figure S4 STEM image of the dendritic peptide amphiphile **12**, deposited on an EM grid covered with an amorphous carbon film from a 1 mg/mL clear solution of **12** in 10 mM phosphate buffer, pH 7.4 and 500 mM NaCl; the scale bar represents 500 nm.

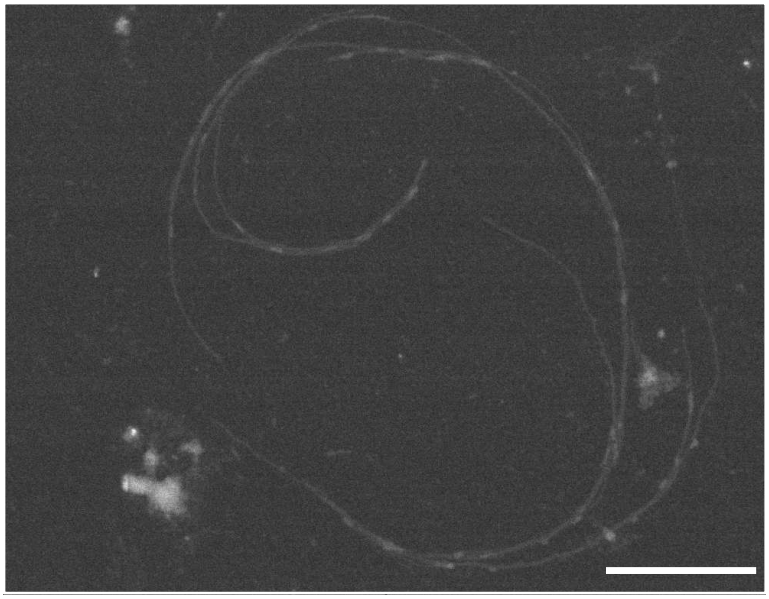


Figure S5 STEM image of the dendritic peptide amphiphile **18**, deposited on an EM grid covered with an amorphous carbon film from a 1 mg/mL clear solution of **18** in 10 mM phosphate buffer, pH 5.0 and 500 mM NaCl; the scale bar represents 400 nm.

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