Electronic Supplementary Information

Structure and Luminescence Properties of Supramolecular Polymers of Amphiphilic Aromatic Thioether-Peptide Conjugates in Water

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1. Materials and Instrumentation

All solvents and reagents were obtained from commercial sources at the highest purity available and used without further purification. The list of suppliers includes SIGMAALDRICH (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), ACROS ORGANICS (Thermo Fisher Scientific GmbH, Geel, Belgium), MERCK KGaA (Merck KGaA, Darmstadt, Germany). ALFA AESAR (Thermo Fisher (Kandel) GmbH, Karlsruhe, Germany), CARBOLUTION CHEMICALS (Carbolution Chemicals GmbH, Saarbrücken, Germany), IRIS BIOTECH (Iris Biotech GmbH, Marktredwitz, Germany), TOKYO CHEMICAL INDUSTRY (TCI Deutschland GmbH, Eschborn, Germany) and ABCR (abcr GmbH, Karlsruhe, Germany). Water was demineralized prior to use. Solvents used for air or moisture sensitive reactions were purchased anhydrous or dried prior to use according to common drying techniques. Purification *via* preparative flash column chromatography (FC) 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 Å F254, MACHEREY-NAGEL). Solvents which were needed for flash chromatography (FC) were purchased in technical quality and used without further purification. The solid phase peptide synthesis (SPPS) was carried out on a Peptide Synthesizer CS 136XT (CS Bio) using 2-chloro-tritylchloride resin (1.6 mmol/ g loading) and SPPS-grade reagents and solvents.

NMR-spectroscopy

NMR-spectra were recorded on a BRUKER ARX 300 spectrometer, BRUKER Avance II 400 and BRUKER Avance III 600 spectrometer. 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.[c] The measured coupling constants were calculated in Hertz (Hz). To analyse the spectra the software MESTRENOVA 10.0.1 was used. The signals were quoted as follows: s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet and m = multiplet.

Mass-spectrometry

High resolution electrospray ionization mass spectra (ESI-HRMS) were recorded on a *Micromass QTof Ultima 3* (WATERS) performed by the mass-spectrometric department of the *Institute of Organic Chemistry, Johannes Gutenberg-University Mainz*. Molecules of a high molecular mass were detected using matrix assisted laser desorption ionization-time of flight (MALDITOF) spectrometry using a Schimadzu Axima CFR.

Transmission electron microscopy (TEM)

Negative stain EM: grid preparation and image recording in brief: 5 µL sample droplets were adsorbed for 2 min on freshly glow-discharged copper grids (Electron Microscopy Sciences; CF300-CU) covered by a thin, continuous carbon film. The grids were then negatively stained with 2.0% uranyl acetate (Polysciences) for 1 min before blotting with filter papers (Whatman no. 4).[d] All images were recorded with a FEI Tecnai T12 electron microscope equipped with a LaB_6-cathode and operated at 120 kV. Digital electron micrographs were recorded with a 4k x 4k CMOS camera (TVIPS) under minimal dose conditions.

Photoluminescence lifetime measurements

Time-resolved photoluminescence measurements were performed with a Fluorolog3 spectrofluorometer equipped with a FluoroHub TCSPC (time-correlated single photon counting) unit (Horiba Jobin Yvon). As excitation source a pulsed NanoLED-370 with an emission wavelength of 370 nm, a repetition rate of 1 MHz and a pulse width of 1.2 ns was used (Horiba Jobin Yvon). The concentration of each sample was adjusted to keep the absorbance at the excitation wavelength λ_{exc} = 370 nm below values of 0.1, in order to avoid artefacts from the inner-filter effect. The photon arrival times with respect to the excitation pulse were collected in fluorescence decay histograms with a channel width of 13.6 ps, 119.4 ps and 5.9 ns. The overall timing resolution of the setup was quantified by the FWHM (full width at half maximum) IRF (instrumental response function) to 1.5 ns, measured at 370 nm with a scattering solution. Data analysis was performed with home-written software in Python utilizing a reconvolution fit according to the following equation:

$$I_{fl}(t) = \left[\sum_n a_n \, exp\left(-\frac{t}{\tau_n}\right)\right] \otimes irf(t) + b \tag{S1}$$

Using amplitudes a_n and lifetimes τ_n of a given decay, the intensity weighted average fluorescence lifetime $\langle\tau\rangle$ can be calculated by the following equation:

$$\langle\tau\rangle = \frac{\sum_n a_n \tau_n^2}{\sum_m a_m \tau_m} \tag{S2}$$

2. Additional Data

UV/Vis Absorption spectra

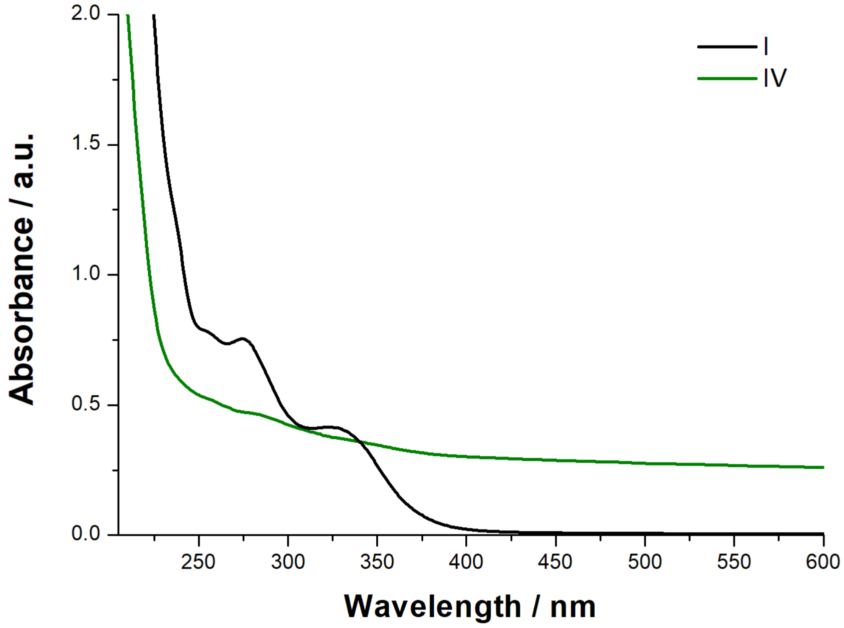


Figure S1: Absorption spectra of amphiphiles **I** (black line) and **IV** (green line) in 10 mM phosphate buffer (pH 7.4); monomer concentrations: 37.5 μM.

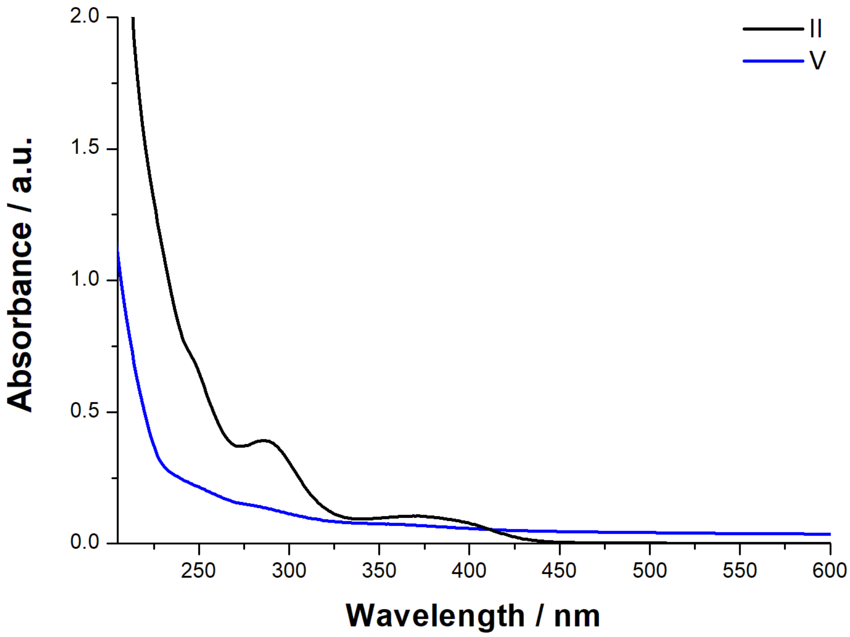


Figure S2: Absorption spectra of amphiphiles **II** (black line) and **V** (blue line) in 10 mM phosphate buffer (pH 7.4); monomer concentrations: 12.5 μM.

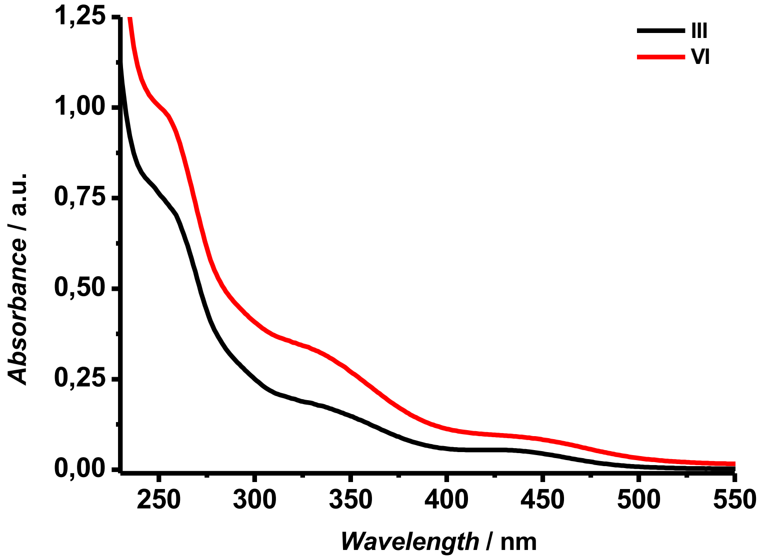


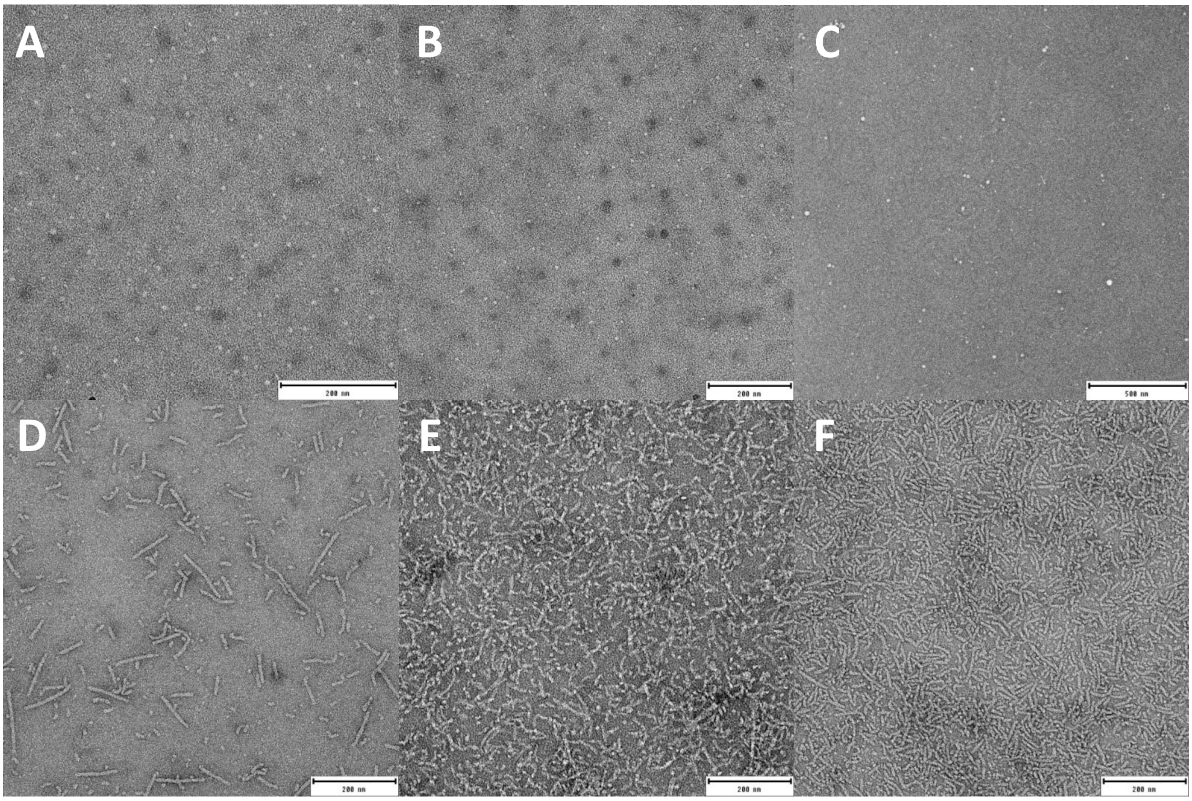
Figure S3: Absorption spectra of amphiphiles **III** (black line) and **VI** (red line) in 10 mM phosphate buffer (pH 7.4); monomer concentrations: 9.375 μM.

Photoluminescence lifetime data

The results for the time-resolved photoluminescence measurements are summarized in table T1. The decay curves for **I-V** were fitted with a sum of two exponential functions. The decay curve for **VI** was fitted by a three-exponential function, whereby the lifetimes from the fit of **III** were used and kept constant.

Table T1: Result of the fits of fluorescence decay curves and calculated intensity weighted average lifetimes ⟨τ⟩/ ns for peptide amphiphiles **I-VI.**

Sample	**λ_{em}/ nm**	**Fit**	**a_1**	**τ_1/ ns**	**a_2**	**τ_2/ ns**	**a_3**	**τ_3/ ns**	**⟨τ⟩/ ns**	**x^2_{red}**
I	461	biexp	0.92	0.85	0.08	5.41	-	-	2.46	1.07
II	461	biexp	0.75	1.65	0.25	3.30	-	-	2.30	1.07
III	458	biexp	0.84	0.86	0.16	5.77	-	-	3.64	1.09
IV	460	biexp	0.88	1.04	0.12	6.00	-	-	3.24	1.63
V	440	biexp	0.94	0.75	0.06	6.26	-	-	2.72	1.50
VI	615	triexp	0.94	0.86	0.04	5.77	0.02	337	-	-



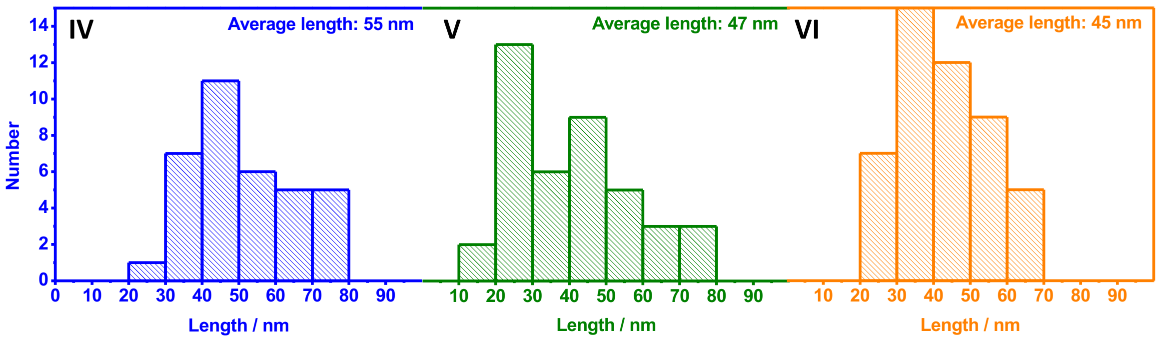


Figure S4: Top: Negative stained TEM imaged of peptide amphiphiles **I**-**VI** (A-F) in 10 mM TRIS buffer at pH 7.4. Scale bars A-E and F: 200 nm. G: 500 nm. Bottom: Histograms of polymeric structures **IV**-**VI**.

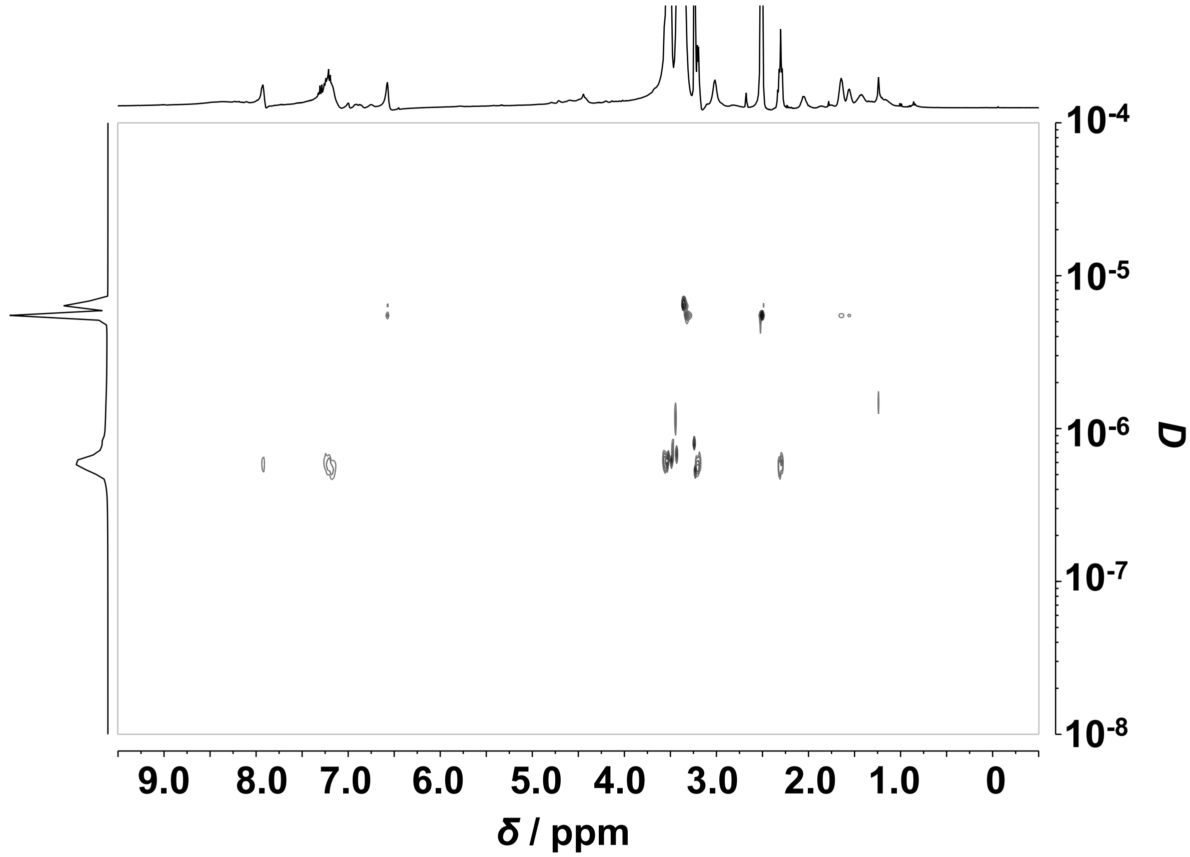


Figure S5: ^{1}H DOSY NMR of **III** in DMSO-d_6, 400 MHz (with diffusion coefficient D in $cm^2 \cdot s^{-1}$).

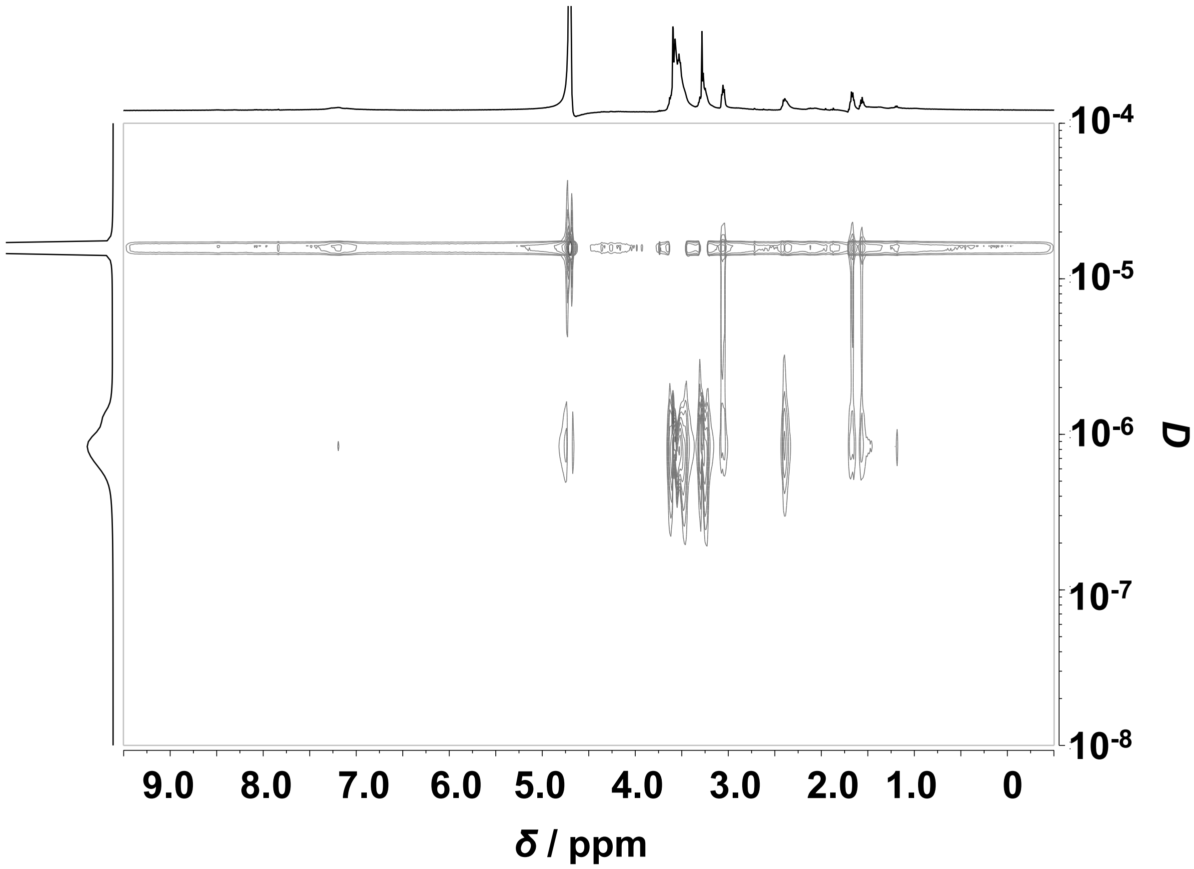


Figure S6: ^{1}H DOSY NMR of **III** in D_2O, 400 MHz (with diffusion coefficient D in $cm^2 \cdot s^{-1}$).

Table T2: Diffusion coefficients D, hydrodynamic radii R_H of luminophore-peptide conjugate **III** in DMSO-d_6 and D_2O, at 295 K.

	D (uncorrected) [10^{-11} $m^2 \cdot s^{-1}$]	D (corrected) [10^{-11} $m^2 \cdot s^{-1}$]	η[1, 2] [10^{-3} $kg \cdot m^{-1} \cdot s^{-1}$]	R_H [10^{-9} m]
III in DMSO-d_6	6.0	7.2	2.18	1.4
III in D_2O	8.4	10.2	1.1	1.9

The self-diffusion[2, 3] of HDO in D_2O (1.9 10^{-9} $m^2 \cdot s^{-1}$), and DMSO-d_5 in DMSO-d_6 (6.6 10^{-10} $m^2 \cdot s^{-1}$) were used to calibrate the measurements.

The model used to calculate the hydrodynamic radii of the molecules is the Stokes–Einstein relation[4, 5] for the diffusion of a spherical particle:

$$D = \frac{k\,T}{6\,\pi\,\eta\,R_H} \quad / \, m^2 \cdot s^{-1}$$

k = Boltzmann constant = 1.38065 10^{-23} $m^2 \cdot kg \cdot s^{-2} \cdot K^{-1}$
T = absolute temperature / K
η = dynamic viscosity / $kg \cdot m^{-1} \cdot s^{-1}$
R_H = hydrodynamic radius / m

3. Synthetic Procedures

Standard operating procedure for the synthesis of the protected peptides *via* SPPS (SOP 1)

The loading of the resin was performed according to a procedure described in literature.[6] The appropriate Fmoc-protected amino acid (2.0 eq. relative to resin loading capacity) was dissolved in DCM/ DMF (1:1, 10 mL/ g resin) and added to the 2-chlorotrityl-chloride resin. This is followed by the addition of DIPEA (2.0 eq. relative to the resin capacity). After shaking for 5 min at room temperature additional DIPEA (3.0 eq. relative to the resin capacity) was added. The reaction mixture was shaken for 1 h at room temperature and afterwards treated with MeOH (1 mL/g resin) and shaken for 15 min. 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 *in vac* overnight.

The following step-wise chain elongation was performed using the CS 136XT peptide synthesizer, which is an automated batch peptide synthesizer. The procedure is described in the following. The dried beads were swollen in DCM p.a. for 10 min while shaking the reaction vessel. After sucking off 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. The resin was treated with a solution of the corresponding Fmoc-protected amino acid (4.0 eq.), HBTU (4.0 eq.), HOBt (4.0 eq.) and DIPEA (6.0 eq.) in DMF. After shaking for 1 h the solution was removed and the resin was washed five times with DMF. This procedure was repeated with the corresponding amino acid for every coupling process, starting with the Fmoc deprotection on the resin. Finally the resin was washed with DCM.

The cleavage of resin-bound peptides was carried out according to a procedure described in literature.[7] The beads were shaken for 45 min in a solution of trifluoroacetic acid (TFA) and DCM (1:1). Afterwards the solution was drained from the reaction vessel and the beads were washed at least two times with a small amount of DCM. The collected solutions were concentrated under reduced pressure and the product precipitated out of a 5°C solution of Et_2O. After centrifugation, and decanting the liquid phase the precipitate was washed with H_2O and lyophilized. The whole procedure was repeated three times.

Standard operation procedure for the cleavage of the ^{t}Bu/ Trt-protection groups (SOP 2)

The ^{t}-Bu protected compound was treated with 8 mL TFA (50%) in DCM; for Trt-cleavage 5 vol% TIS was also added. The solution was stirred 1 h at room temperature, concentrated under reduced pressure and another 8 mL of the 1:1 TFA/ DCM solution were added. After removal of the solvent under reduced pressure and after lyophilization the desired product was obtained without further purification.

Standard operation procedure for the cleavage of Cbz-protection groups (SOP 3)

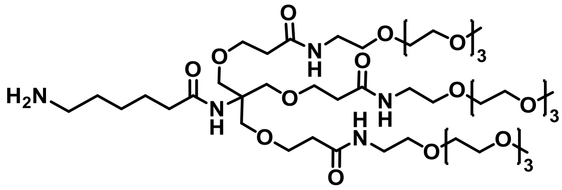
The Cbz-protected compound was dissolved in 10 mL MeOH. 10 wt% Pd/C were added and the suspension was stirred under an atmosphere of hydrogen for 12 h at room temperature. The catalyst was afterwards removed *via* filtration over Kieselguhr. After removal of the solvent under reduced pressure, the desired product was obtained without further purification.

Standard operation procedure for the cleavage of Fmoc-protection groups (SOP 4)

The Fmoc-protected compounds were dissolved in a 10 vol% solution of piperidine in $CHCl_3$ and stirred for 2 h at room temperature. The solvents were removed *in vac* and the residue purified *via* size exclusion chromatography (Sephadex® LH 20, MeOH). The obtained product was used after lyophilization

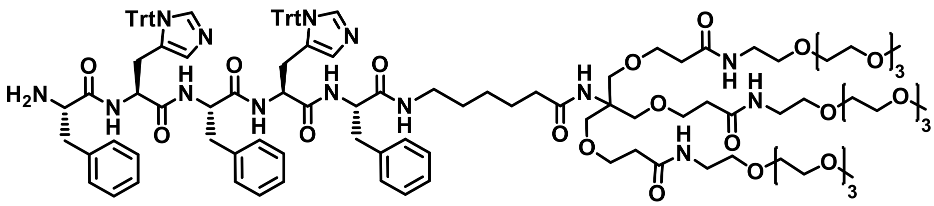
Synthesis

Compound 1



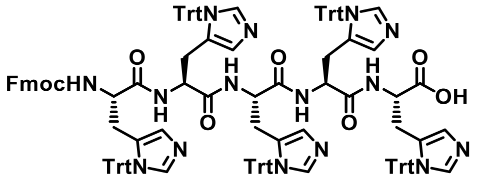
1 was synthesized according to literature.[8]
Yield: 580 mg (570 μmol, quant.); colorless oil.
Molecular formula: $C_{46}H_{91}N_5O_{19}$.
ESI-HRMS (MeOH) (*m/z*): Calculated for $[M+H]^+$: 1018.6387, found: 1018.6377; Calculated for $[M+Na]^+$: 1040.6206, found: 1040.6201.
^{1}H-NMR (400 MHz, DMSO-*d*$_6$, 296 K): δ/ ppm = 7.93 (t, J^3 = 5.6 Hz, 3H, N*H*CH$_2$), 6.97 (s, 1H, N*H*C$_q$), 3.64 - 3.37 (m, 54H, C*H*$_2$O), 3.28 - 3.13 (m, 15H, C*H*$_3$^{TEG}/ NCH$_2$C*H*$_2$O), 3.06 - 2.90 (m, 2H, NC*H*$_2$[CH$_2$]$_4$^{Ahx}), 2.29 (t, J^3 = 6.4 Hz, 6H, C*H*$_2$C=O), 2.05 (t, J^3 = 7.3 Hz, 2H, N[CH$_2$]$_4$C*H*$_2$^{Ahx}), 1.55 - 1.37 (m, 4H, NCH$_2$C*H*$_2$^{Ahx}/ N[CH$_2$]$_3$C*H*$_2$^{Ahx}), 1.35 - 1.16 (m, 2H, N[CH$_2$]$_2$C*H*$_2$^{Ahx}).

Compound 2



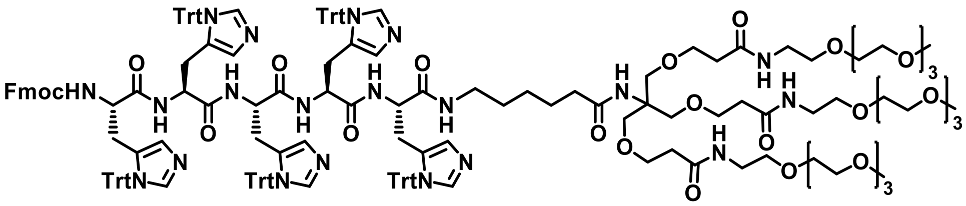
2 (153 mg, 62.7 μmol, 1.0 eq.) was deprotected according to literature.[8]
Yield: 129 mg (58 μmol, 93%); colorless, amorphous solid.
Molecular formula: $C_{123}H_{160}N_{14}O_{24}$.
ESI-HRMS (MeOH) (*m/z*): Calculated for $[M+Na]^+$: 2239.1670, found: 2240.1531.
^{1}H-NMR (400 MHz, DMSO-*d*$_6$, 298 K): δ/ ppm = 8.72 (d, J^3 = 7.6 Hz, 1H, α-N*H*), 8.24 (d, J^3 = 7.4 Hz, 1H, α-N*H*), 8.21 - 8.12 (m, 2H, α-N*H*), 8.05 - 7.98 (m, 1H, N*H*CH$_2$^{Ahx}), 7.91 (t, J^3= 5.6 Hz, 3H, N*H*CH$_2$), 7.84 (d, J^3= 8.1 Hz,1H, α-N*H*), 7.42 - 7.00 (m, 50H, C*H*^{Ar,Trt}/ C*H*^{Ar,Phe} / C*H*^{Ar,His}), 6.98 (s, 1H, N*H*C$_q$), 6.55 (s, 1H, C*H*^{Ar,His}), 6.53 (s, 1H, C*H*^{Ar,His}), 4.54 - 4.30 (m, 5H, α-C*H*), 3.57 - 3.35 (m, 54H, C*H*$_2$O), 3.25 - 3.15 (m, 15H, C*H*$_3$^{TEG}/ NCH$_2$C*H*$_2$O), 3.09 - 2.59 (m, 12H, β-C*H*$_2$^{Phe}/ β-C*H*$_2$^{His}/ NC*H*$_2$[CH$_2$]$_4$^{Ahx}), 2.29 (t, J^3 = 6.4 Hz, 6H, C*H*$_2$C=O), 2.01 (t, J^3 = 7.5 Hz, 2H, N[CH$_2$]4C*H*$_2$^{Ahx}), 1.41 - 1.31 (m, 2H, NCH$_2$C*H*$_2$^{Ahx}), 1.29 - 1.17 (m, 2H, N[CH$_2$]$_3$C*H*$_2$^{Ahx}), 1.15 - 1.02 (m, 2H, N[CH$_2$]$_2$C*H*$_2$^{Ahx}).

Compound 3



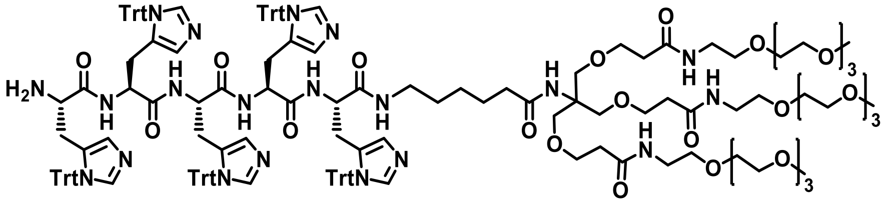
The synthesis was carried out according **SOP 1**.
Yield: 1.49 mg (698 μmol); colorless, amorphous solid.
Molecular formula: $C_{140}H_{117}N_{15}O_8$.
ESI-HRMS (MeOH) (*m/z*): Calculated for $[M+H]^+$: 2137.9316, found: 2137.8977.
^{1}H-NMR (400 MHz, DMSO-*d*$_6$, 296 K): δ/ ppm = 12.87 (bs, 1H, CO_2H), 8.51 (d, J^3 = 7.2 Hz, 1H, N*H*^{His}), 8.45 (d, J^3 = 3.4 Hz, 1H, N*H*^{His}), 8.11 (d, J^3 = 5.8 Hz, 1H, N*H*^{His}), 7.99 (d, J^3 = 6.9 Hz, 1H, N*H*^{His}), 7.87 (d, J^3 = 7.5 Hz, 2H, C*H*^{Ar,Fmoc}), 7.70 (d, J^3 = 8.0 Hz, 1H, N*H*^{Fmoc}), 7.57 (d, J^3 = 7.5 Hz, 2H, C*H*^{Ar,Fmoc}), 7.36 (t, J^3 = 8.1 Hz, 4H, C*H*^{Ar,Fmoc}), 7.32 - 6.94 (m, 80H, C*H*^{Trt}/ C*H*^{Ar,His}), 6.69 (s, 1H, C*H*^{Ar,His}), 6.67 (s, 1H, C*H*^{Ar,His}), 6.65 (s, 1H, C*H*^{Ar,His}), 6.60 (s, 1H, C*H*^{Ar,His}), 6.59 (s, 1H, C*H*^{Ar,His}), 4.46 - 4.38 (m, 1H, *α*-C*H*^{His}), 4.34 - 4.27 (m, 1H, *α*-C*H*^{His}), 4.24 - 4.14 (m, 3H, *α*-C*H*^{His}), 3.93 - 3.82 (m, 3H, C*H*^{Fmoc}/ C*H*$_2^{Fmoc}$), 2.89 - 2.53 (m, 10H, β-C*H*$_2^{His}$).

Compound 4



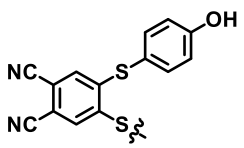
PyBOP (122 mg, 234 μmol, 1.5 eq.) was added to a stirring solution of **3** (401 mg, 187 μmol, 1.2 eq.), **1** (159 mg, 156 μmol, 1.0 eq.), HOAt (21 mg, 156 μmol, 1.0 eq.) and DIPEA (15 μL, 156 μmol, 1.0 eq.) in DMF (10 mL). The reaction mixture was stirred 18 h at room temperature. The solvent was removed under reduced pressure and the residue was purified *via* size exclusion chromatography (Sephadex® LH 20, MeOH).
Yield: 304 mg (97 μmol, 62%); colorless, amorphous solid.
Molecular formula: $C_{186}H_{206}N_{20}O_{26}$.
ESI-HRMS (MeOH) (*m/z*): Calculated for $[M+Na]^+$: 3160.5371, found: 3160.5491.
^{1}H-NMR (400 MHz, DMSO-*d*$_6$, 296 K): δ/ ppm = 8.51 (d, J^3 = 7.2 Hz, 1H, N*H*^{His}), 8.45 (d, J^3 = 3.4 Hz, 1H, N*H*^{His}), 8.11 (d, J^3 = 5.8 Hz, 1H, N*H*^{His}), 7.99 (d, J^3 = 6.9 Hz, 1H, N*H*^{His}), 7.93 (t, J^3 = 5.6 Hz, 3H, N*H*CH_2), 7.87 (d, J^3 = 7.5 Hz, 2H, C*H*^{Ar,Fmoc}), 7.82 (t, J^3 = 5.6 Hz, 1H, N*H*^{Ahx}), 7.70 (d, J^3 = 8.0 Hz, 1H, N*H*^{Fmoc}), 7.57 (d, *J* = 7.5 Hz, 2H, C*H*^{Ar,Fmoc}), 7.36 (t, J^3 = 8.1 Hz, 4H, C*H*^{Ar,Fmoc}), 7.32 - 6.94 (m, 81H, C*H*^{Trt}/ C*H*^{Ar,His}/N*H*C_q), 6.69 (s, 1H, C*H*^{Ar,His}), 6.67 (s, 1H, C*H*^{Ar,His}), 6.65 (s, 1H, C*H*^{Ar,His}), 6.60 (s, 1H, C*H*^{Ar,His}), 6.59 (s, 1H, C*H*^{Ar,His}), 4.46 - 4.38 (m, 1H, *α*-C*H*^{His}), 4.34 - 4.27 (m, 1H, *α*-C*H*^{His}), 4.24 - 4.14 (m, 3H, *α*-C*H*^{His}), 3.93 - 3.82 (m, 3H, C*H*^{Fmoc}/ C*H*$_2^{Fmoc}$), 3.64 - 3.37 (m, 54H, C*H*$_2$O), 3.28 - 3.13 (m, 15H, C*H*$_3^{TEG}$/ NCH_2CH_2O), 3.06 - 2.90 (m, 2H, N$CH_2[CH_2]_4^{Ahx}$), 2.89 - 2.53 (m, 10H, β-C*H*$_2^{His}$), 2.29 (t, *J* = 6.4 Hz, 6H, $CH_2C{=}O$), 2.05 (t, *J* = 7.3 Hz, 2H, $N[CH_2]_4CH_2^{Ahx}$), 1.55 - 1.37 (m, 4H, $NCH_2CH_2^{Ahx}$/ $N[CH_2]_3CH_2^{Ahx}$), 1.35 - 1.16 (m, 2H, $N[CH_2]_2CH_2^{Ahx}$).

Compound 5



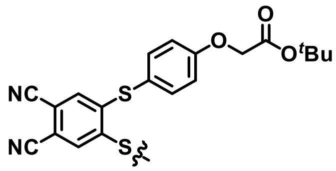
4 (294 mg, 93 μmol) was deprotected according to **SOP 4**.
Yield: 226 mg (80 μmol, 85%); colorless, amorphous solid.
Molecular formula: $C_{171}H_{196}N_{20}O_{24}$.
ESI-HRMS (MeOH) (*m/z*): Calculated for $[M+2Na]^{2+}$: 1458.2455, found: 1458.2153.
^{1}H-NMR (400 MHz, DMSO-*d*$_6$, 298 K): δ/ ppm = 8.51 (d, J^3 = 7.2 Hz, 1H, NH^{His}), 8.45 (d, J^3 = 3.4 Hz, 1H, NH^{His}), 8.11 (d, J^3 = 5.8 Hz, 1H, NH^{His}), 7.99 (d, J^3 = 6.9 Hz, 1H, NH^{His}), 7.93 (t, J^3 = 5.6 Hz, 3H, N*H*CH_2), 7.82 (t, J^3 = 5.6 Hz, 1H, NH^{Ahx}), 7.32 - 6.94 (m, 81H, CH^{Trt}/ C$H^{Ar,His}$/ N*H*C_q), 6.69 (s, 1H, C$H^{Ar,His}$), 6.67 (s, 1H, C$H^{Ar,His}$), 6.65 (s, 1H, C$H^{Ar,His}$), 6.60 (s, 1H, C$H^{Ar,His}$), 6.59 (s, 1H, C$H^{Ar,His}$), 4.46 - 4.38 (m, 1H, α-CH^{His}), 4.34 - 4.27 (m, 1H, α-CH^{His}), 4.24 - 4.14 (m, 3H, α-CH^{His}), 3.64 - 3.37 (m, 54H, CH_2O), 3.28 - 3.13 (m, 15H, CH_3^{TEG}/ NCH_2CH_2O), 3.06 - 2.90 (m, 2H, NCH_2[CH_2]$_4^{Ahx}$), 2.89 - 2.53 (m, 10H, β-CH_2^{His}), 2.29 (t, J^3 = 6.4 Hz, 6H, CH_2C=O), 2.05 (t, J^3 = 7.3 Hz, 2H, N[CH_2]$_4$CH_2^{Ahx}), 1.55 - 1.37 (m, 4H, NCH_2CH_2^{Ahx}/ N[CH_2]$_3$CH_2^{Ahx}), 1.35 - 1.16 (m, 2H, N[CH_2]$_2$CH_2^{Ahx}).

Compound 6[9]



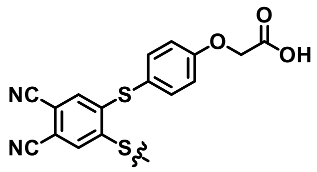
To a stirring solution of potassium carbonate (3.0 g, 15.3 mmol, 6.0 eq.) in dry DMF was added 4-hydroxy-thiophenol (1.0 g, 7.65 mmol, 3.0 eq.) and the remaining suspension was heated under stirring to 80°C for 30 minutes followed by the addition of 4,5-dichlorophthalonitrile (500 mg, 2.55 mmol, 1.0 eq.) and heating was continued for further 24 h. The slurry was poured carefully into 1N HCl (150 mL) and the precipitate was filtered. The residue was dissolved in EtOAc (50 mL) and extracted with distilled water until the aqueous layer became neutral. The organic layer was dried over $MgSO_4$ and evaporated to dryness.
Yield: 910 mg (2.43 mmol, 95%); yellow, amorphous solid.
Molecular Formula: $C_{20}H_{12}N_2O_2S_2$.
ESI-HRMS (MeOH) (*m/z*): Calculated for $[M+Na]^{+}$: 399.0238, found: 399.0332.
^{1}H-NMR (400 MHz, DMSO-*d*$_6$): δ/ ppm = 10.25 (s, 2H, O*H*), 7.45 (d, J^3 = 8.5 Hz, 4H, SCC*H*CH), 6.98 (s, 2H, CNCC*H*), 6.96 (d, *J*3 = 8.8 Hz, 4H, SCCHC*H*).
^{13}C{^{1}H}-NMR (101 MHz, DMSO-*d*$_6$): δ/ ppm = 160.06, 144.18, 137.58, 128.86, 117.91, 115.93, 115.47, 110.87.

Compound 7[9]



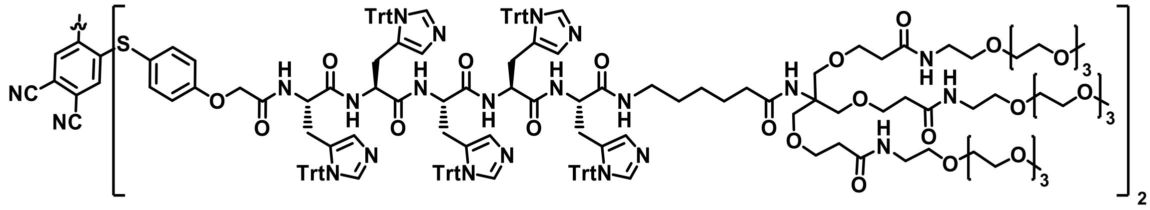
To a stirring solution of **6** (100 mg, 270 μmol 1.0 eq.) in dry DMF was added finely crushed potassium carbonate (1.00 g, 5.1 mmol, 18.9 eq.) and the slurry was heated to 60°C for 1 h, followed by the dropwise addition of bromo-*tert*-butyl acetic acid (300 mg, 1.54 mmol, 5.7 eq.) and a catalytic amount of postassium iodide (20 mg). The obtained solution was heated for further 48 h after which all solvents were removed *in vacuo*. The remaining oil was dissolved in 30 mL of DCM and extracted three times with 20 mL of distilled water. The organic layer was dried over $MgSO_4$ and the solvent was removed *in vacuo*. The residue was subjected to silica gel column chromatography using EtOAc as eluent.
Yield: 133 mg (220 μmol, 83%); yellow oil.
Molecular Formula: $C_{32}H_{32}N_2O_6S_2$.
ESI-HRMS (MeOH) (*m*/*z*): Calculated for $[M+Na]^+$: 627.1599, found: 627.1609.
^{1}H-NMR (400 MHz, $CDCl_3$): δ/ ppm = 7.49 (d, J^3 = 8.9 Hz, 4H, SCC*H*CH), 7.03 (d, J^3 = 8.9 Hz, 4H, SCCHC*H*), 6.89 (s, 2H, CNCC*H*), 4.60 (s, 4H, C*H*$_2$), 1.50 (s, 18H, C*H*$_3^{tBu}$).
^{13}C{^{1}H}-NMR (101 MHz, $CDCl_3$): δ/ ppm = 167.75, 160.49, 144.89, 137.73, 129.50, 119.86, 117.33, 115.91, 111.82, 83.33, 66.19, 28.69.

Compound 8[9]



7 (133 mg, 220 μmol) was deprotected according to **SOP 2**.
Yield: 88 mg (180 μmol, 81%); colorless, amorphous solid.
Molecular Formula: $C_{24}H_{16}N_2O_6S_2$.
ESI-HRMS (MeOH) (*m*/*z*): Calculated for $[M+Na]^+$: 515.0347, found: 515.0372.
^{1}H-NMR (300 MHz, DMSO-*d*$_6$): δ/ ppm = 13.13 (bs, 2H, CO$_2$*H*), 7.57 (d, J^3 = 8.9 Hz, 4H, SCC*H*CH), 7.12 (d, J^3 = 8.9 Hz, 4H, SCCHC*H*), 7.08 (s, 2H, CNCC*H*), 4.80 (s, 4H, C*H*$_2$).
^{13}C{^{1}H}-NMR (75 MHz, DMSO-*d*$_6$): δ/ ppm = 169.87, 159.68, 143.69, 136.94, 129.52, 118.45, 116.83, 115.70, 111.16.

Compound 9



PyBOP (23.5 mg, 45.2 µmol, 3.0 eq.) was added to a solution of **8** (7.4 mg, 15.1 µmol, 1.0 eq.), **5** (110 mg, 37.7 µmol, 2.5 eq.) and DIPEA (27 µL, 151 µmol, 10.0 eq.) in DMF (1 mL). The reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and the residue was purified *via* size exclusion chromatography (Sephadex® LH 20, MeOH).

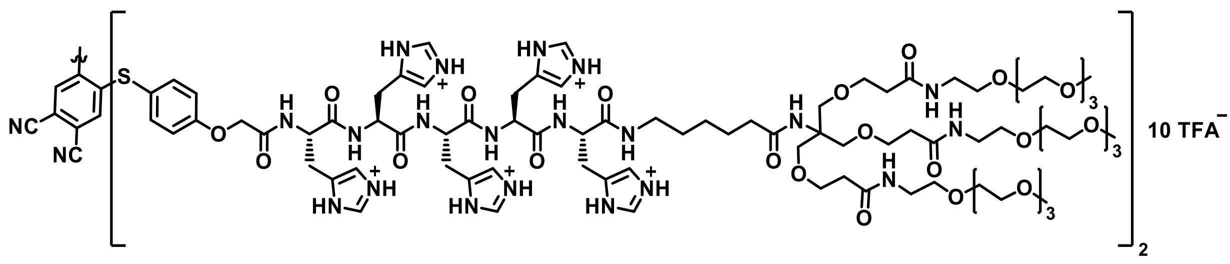
Yield: 111 mg (15.1 µmol, quant.); colorless, amorphous solid.

Molecular formula: $C_{366}H_{404}N_{42}O_{52}S_2$.

MALDI-MS (ACN/ H_2O) (*m*/*z*): Calculated for $[M+Na]^+$: 6310.6, found: 6310.2.

^{1}H-NMR (400 MHz, DMSO-*d*$_6$, 298 K): δ/ ppm = 8.52 (d, J^3 = 7.2 Hz, 2H, N*H*^{His}), 8.44 (d, J^3 = 3.4 Hz, 2H, N*H*^{His}), 8.24 (d, J^3 = 7.6 Hz, 2H, N*H*^{His}), 8.12 (d, J^3 = 5.8 Hz, 2H, N*H*^{His}), 7.99 (d, J^3 = 6.9 Hz, 2H, N*H*^{His}), 7.94 (t, J^3 = 5.6 Hz, 6H, N*H*CH$_2$), 7.81 (t, J^3 = 5.6 Hz, 2H, N*H*^{Ahx}), 7.53 (d, J^3 = 8.6 Hz, 4H, SCC*H*CH), 7.32 - 6.94 (m, 168H, CNCC*H*/ C*H*^{Trt}/ C*H*^{Ar,His}/N*H*C$_q$/ SCCHC*H*), 6.69 (s, 2H, C*H*^{Ar,His}), 6.67 (s, 2H, C*H*^{Ar,His}), 6.65 (s, 2H, C*H*^{Ar,His}), 6.60 (s, 2H, C*H*^{Ar,His}), 6.59 (s, 2H, C*H*^{Ar,His}), 4.52 (s, 4H, OC*H*$_2$CO), 4.46 - 4.38 (m, 2H, *α*-C*H*^{His}), 4.34 - 4.27 (m, 2H, *α*-C*H*^{His}), 4.24 - 4.14 (m, 6H, *α*-C*H*^{His}), 3.64 - 3.37 (m, 108H, C*H*$_2$O), 3.28 - 3.13 (m, 30H, C*H*$_3$^{TEG}/ NCH$_2$C*H*$_2$O), 3.06 - 2.90 (m, 4H, NC*H*$_2$[CH$_2$]$_4$^{Ahx}), 2.89 - 2.53 (m, 20H, β-C*H*$_2$^{His}), 2.29 (t, J^3 = 6.4 Hz, 12H, C*H*$_2$C=O), 2.05 (t, J^3 = 7.3 Hz, 4H, N[CH$_2$]$_4$C*H*$_2$^{Ahx}), 1.55 - 1.37 (m, 8H, NCH$_2$C*H*$_2$^{Ahx}/ N[CH$_2$]$_3$C*H*$_2$^{Ahx}), 1.35 - 1.16 (m, 4H, N[CH$_2$]$_2$C*H*$_2$^{Ahx}).

Compound I



9 (100 mg, 16 µmol) was deprotected according to **SOP 2**.

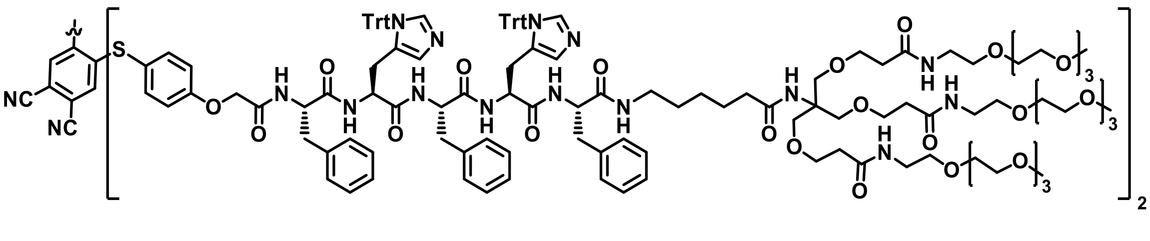
Yield: 65 mg (13 µmol, 79%); colorless, amorphous solid.

Molecular formula: $C_{195}H_{277}N_{42}O_{52}S_2$ * 10 TFA.

MALDI-MS (ACN/ H_2O) (*m*/*z*): Calculated for $[M+Na]^+$: 3886.87, found: 3887.08.

^{1}H-NMR (400 MHz, DMSO-*d*$_6$, 298 K): δ/ ppm = 14.35 (s, 20H, N*H*^{His}), 8.52 (d, J^3 = 7.2 Hz, 2H, N*H*^{His}), 8.44 (d, J^3 = 3.4 Hz, 2H, N*H*^{His}), 8.24 (d, J^3 = 7.6 Hz, 2H, N*H*^{His}), 8.12 (d, J^3 = 5.8 Hz, 2H, N*H*^{His}), 7.99 (d, J^3 = 6.9 Hz, 2H, N*H*^{His}), 7.94 (t, J^3 = 5.6 Hz, 6H, N*H*CH$_2$), 7.81 (t, J^3 = 5.6 Hz, 2H, N*H*^{Ahx}), 7.53 (d, J^3 = 8.6 Hz, 4H, SCC*H*CH), 7.34 (s, 5H, C*H*NH$_2$^{His}), 7.27 (s, 5H, C*H*NH$_2$^{His}), 7.05 (s, 2H, N*H*C$_q$), 7.03 (s, 2H, CNCC*H*), 6.99 (d, J^3 = 8.9 Hz, 4H, SCCHC*H*), 6.69 (s, 2H, C*H*^{Ar,His}), 6.67 (s, 2H, C*H*^{Ar,His}), 6.65 (s, 2H, C*H*^{Ar,His}), 6.60 (s, 2H, C*H*^{Ar,His}), 6.59 (s, 2H, C*H*^{Ar,His}), 4.52 (s, 4H, OC*H*$_2$CO), 4.46 - 4.38 (m, 2H, *α*-C*H*^{His}), 4.34 - 4.27 (m, 2H, *α*-C*H*^{His}), 4.24 - 4.14 (m, 6H, *α*-C*H*^{His}), 3.64 - 3.37 (m, 108H, CH_2O), 3.28 - 3.13 (m, 30H, CH_3^{TEG}/ NCH_2CH_2O), 3.06 - 2.90 (m, 4H, NCH_2[CH_2]$_4$^{Ahx}), 2.89 - 2.53 (m, 20H, β-CH_2^{His}), 2.29 (t, J^3 = 6.4 Hz, 12H, CH_2C=O), 2.05 (t, J^3 = 7.3 Hz, 4H, N[CH_2]$_4$CH_2^{Ahx}), 1.55 - 1.37 (m, 8H, NCH_2CH_2^{Ahx}/ N[CH_2]$_3$CH_2^{Ahx}), 1.35 - 1.16 (m, 4H, N[CH_2]$_2$CH_2^{Ahx}).

Compound 10



PyBOP (37 mg, 72 µmol, 3.0 eq.) was added to a solution of **7** (12 mg, 24 µmol, 1.0 eq.), **2** (175 mg, 60 µmol, 2.5 eq.) and DIPEA (42 µL, 240 µmol, 10.0 eq.) in DMF (1 mL). The reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and the residue was purified *via* size exclusion chromatography (Sephadex® LH 20, MeOH).

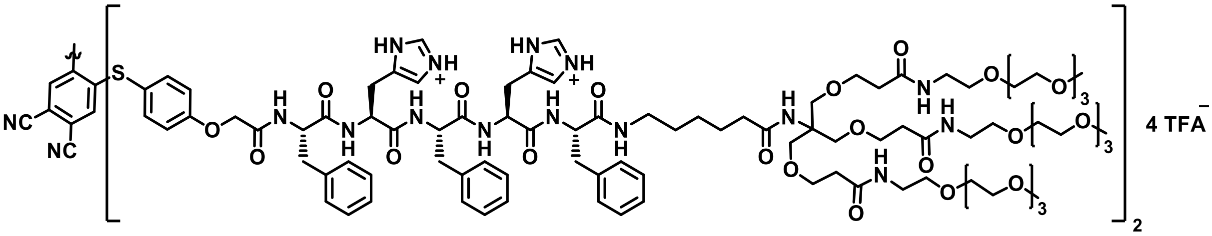
Yield: 105 mg (21 µmol, 89%); colorless, amorphous solid.

Molecular formula: $C_{270}H_{332}N_{30}O_{52}S_2$.

MALDI-MS (ACN/ H_2O) (*m/z*): Calculated for [M+Na]$^+$: 4915.37, found: 4916.90.

^{1}H-NMR (400 MHz, DMSO-*d*$_6$, 298 K): δ/ ppm = 8.72 (d, J^3 = 7.6 Hz, 2H, α-N*H*), 8.56 (d, J^3 = 7.8 Hz, 1H, α-N*H*^{Phe}), 8.24 (d, J^3 = 7.4 Hz, 2H, α-N*H*), 8.21 - 8.12 (m, 4H, α-N*H*), 8.05 - 7.98 (m, 2H, N*H*CH_2^{Ahx}), 7.91 (t, J^3 = 5.6 Hz, 6H, N*H*CH_2), 7.84 (d, J^3 = 8.1 Hz, 2H, α-N*H*), 7.53 (d, J^3 = 8.6 Hz, 4H, SCC*H*CH), 7.42 - 7.00 (m, 100H, C*H*^{Ar,Phe}/ CNCC*H*/ C*H*^{Trt}/ C*H*^{Ar,His}/ SCCHC*H*), 6.98 (s, 2H, N*H*C$_q$), 6.55 (s, 2H, C*H*^{Ar,His}), 6.53 (s, 2H, C*H*^{Ar,His}), 4.54 - 4.30 (m, 14H, OCH_2CO/ α-C*H*), 3.57 - 3.35 (m, 108H, CH_2O), 3.25 - 3.15 (m, 30H, CH_3^{TEG}/ NCH_2CH_2O), 3.09 - 2.59 (m, 24H, β-CH_2^{Phe}/ β-CH_2^{His}/ NCH_2[CH_2]$_4$^{Ahx}), 2.29 (t, J^3 = 6.4 Hz, 12H, CH_2C=O), 2.01 (t, J^3 = 7.5 Hz, 4H, N[CH_2]$_4$CH_2^{Ahx}), 1.41 - 1.31 (m, 4H, NCH_2CH_2^{Ahx}), 1.29 - 1.17 (m, 4H, N[CH_2]$_3$CH_2^{Ahx}), 1.15 - 1.02 (m, 4H, N[CH_2]$_2$CH_2^{Ahx}).

Compound IV



10 (50 mg, 10.0 µmol) was deprotected according to **SOP 2**.

Yield: 43 mg (9.8 µmol, 98%); colorless, fluorescent solid.

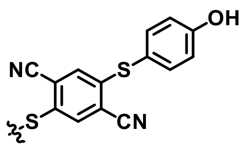
Molecular formula: $C_{194}H_{276}N_{30}O_{52}S_2$ * 4 TFA.

MALDI-MS (ACN/ H_2O) (*m/z*): Calculated for [M+Na]$^+$: 3947.61, found: 3948.49.

^{1}H-NMR (400 MHz, DMSO-*d*$_6$, 298 K): δ/ ppm = 14.35 (s, 8H, N*H*^{His}), 8.72 (d, J^3 = 7.6 Hz, 2H, α-N*H*), 8.56 (d, J^3 = 7.8 Hz, 1H, α-N*H*^{Phe}), 8.24 (d, J^3 = 7.4 Hz, 2H, α-N*H*), 8.21 - 8.12 (m, 4H,

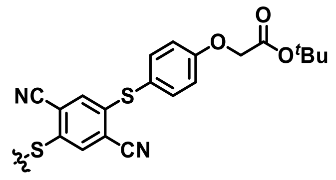
α-N*H*), 8.05 - 7.98 (m, 2H, N*H*CH_2^{Ahx}), 7.91 (t, J^3 = 5.6 Hz, 6H, N*H*CH_2), 7.84 (d, J^3 = 8.1 Hz, 2H, α-N*H*), 7.53 (d, J^3 = 8.6 Hz, 4H, SCC*H*CH), 7.34 (s, 2H, C*H*NH_2^{His}), 7.27 (s, 2H, C*H*NH_2^{His}), 7.32 - 7.11 (m, 30H, C$H^{Ar,Phe}$), 7.05 (s, 2H, N*H*C_q), 7.03 (s, 2H, CNCC*H*), 6.99 (d, J^3 = 8.9 Hz, 4H, SCCHC*H*), 6.55 (s, 2H, C$H^{Ar,His}$), 6.53 (s, 2H, C$H^{Ar,His}$), 4.54 - 4.30 (m, 14H, OCH_2CO/ α-C*H*), 3.57 - 3.35 (m, 108H, CH_2O), 3.25 - 3.15 (m, 30H, CH_3^{TEG}/ NCH_2CH_2O), 3.09 - 2.59 (m, 24H, β-CH_2^{Phe}/ β-CH_2^{His}/ NCH_2[CH_2]$_4^{Ahx}$), 2.29 (t, J^3 = 6.4 Hz, 12H, CH_2C=O), 2.01 (t, J^3 = 7.5 Hz, 4H, N[CH_2]$_4$CH_2^{Ahx}), 1.41 - 1.31 (m, 4H, NCH_2CH_2^{Ahx}), 1.29 - 1.17 (m, 4H, N[CH_2]$_3$CH_2^{Ahx}), 1.15 - 1.02 (m, 4H, N[CH_2]$_2$CH_2^{Ahx}).

Compound 11[9]



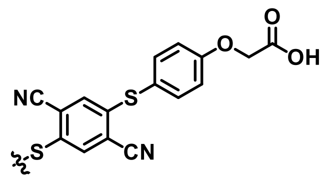
2,5-dibromophthalonitrile (400 mg, 1.4 mmol, 1.0 eq.), 4-hydroxy-thiophenol (818 mg, 6.5 mmol, 4.6 eq.) and potassium carbonate (1.16 g, 8.4 mmol, 6.0 eq.) were added to a 100 mL round bottom flask. The flask was evacuated and purged with argon. After this evacuation-argon-filling operation was repeated once, dry DMF (15 mL) was added to the flask. The solution was stirred at 45 °C for 6 h. Then the reaction was very carefully quenched with 4 M HCl (50 mL), the product precipitated as yellow-green solid. This solid was separated by filtration and washed with copious amounts of distilled water. The product was purified by recrystallization from DMF. Finally, the product was dried *in vacuo* and obtained as a yellow-green solid.
Yield: 524 mg (1.39 mmol, 99%).
Molecular Formula: $C_{20}H_{12}N_2O_2S_2$.
ESI-HRMS (*m/z*): Calculated for $[M-H]^-$: 375.0267, found: 374.9821.
^{1}H-NMR (300 MHz, DMSO-*d*$_6$): δ/ ppm = 10.15 (s, 2H, O*H*), 7.41 (d, J^3 = 8.6 Hz, 4H, CH^{Ar}), 7.27 (s, 2H, CH^{Ar}), 6.90 (d, J^3 = 8.5 Hz, 4H, CH^{Ar}).
^{13}C{^{1}H}-NMR (75 MHz, DMSO-*d*$_6$): δ/ ppm = 159.20, 141.13, 136.40, 132.04, 117.11, 116.34, 114.99, 114.31.
ATR-IR (cm^{-1}): 3385, 3082, 3010, 2928, 2869, 2788, 2725, 2668, 2607, 2361, 2239, 2224, 1668, 1599, 1579, 1494, 1447, 1390, 1345, 1264, 1230, 1170, 1147, 1095, 1056, 1011, 881, 840, 767, 723, 709, 671, 656.

Compound 12[9]



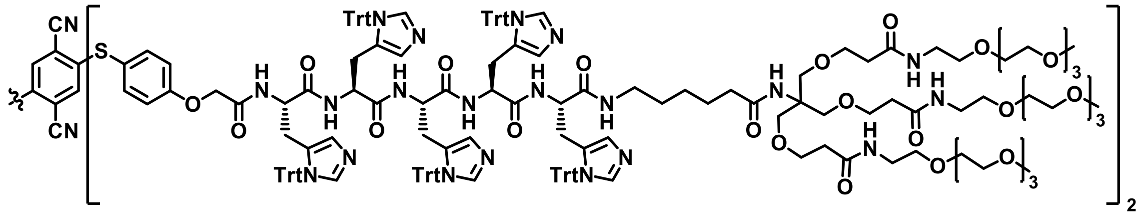
11 (200 mg, 530 µmol, 1.0 eq.) and potassium carbonate (442 mg, 3.2 mmol, 6.0 eq.) were added to a 100 mL round bottom flask. Dry DMF (12 mL) and *tert*-butyl bromoacetate (312 mg, 1.6 mmol, 3.0 eq.) was added to the flask. The solution was stirred at 40°C for 1 d. Then the reaction was quenched with distilled water and the product precipitated as a colorless solid. This solid was separated by filtration and washed with copious amounts of distilled water. Finally, the product was dried i*n vacuo*.
Yield: 297 mg, (490 µmol, 92%); colorless solid.
Molecular Formula: $C_{32}H_{32}N_2O_6S_2$.
ESI-HRMS (*m*/*z*): Calculated for $[M+Na]^+$:627.1594, found: 627.1607.
^{1}H-NMR (300 MHz, DMSO-*d*$_6$): δ/ ppm = 7.52 (d, J^3= 8.8 Hz, 4H, CH^{Ar}), 7.38 (s, 2H, CH^{Ar}), 7.05 (d, J^3 = 8.9 Hz, 4H, CH^{Ar}), 4.75 (s, 4H, CH_2), 1.42 (s, 18H, CH_3).
^{13}C{^{1}H}-NMR (75 MHz, DMSO-*d*$_6$): δ/ ppm = 167.45 (C=O), 159.15, 140.84, 135.96, 133.13, 119.99, 116.48, 115.36, 115.17, 81.56, 65.04, 27.65.
ATR-IR (cm^{-1}): 2987, 2938, 2871, 2225, 1749, 1724, 1681, 1589, 1573, 1492, 1448, 1409, 1393, 1367, 1345, 1310, 1292, 1262, 1223, 1177, 1151, 1105, 1092, 1065, 1008, 943, 921, 884, 837, 802, 781.

Compound 13[9]



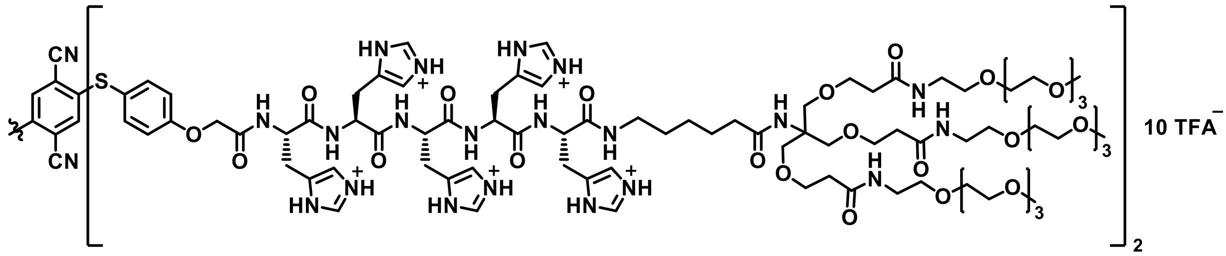
12 (150 mg, 250 µmol) was deprotected according to **SOP 2**.
Yield: 112 mg (230 µmol, 92%); colorless solid.
Molecular Formula: $C_{24}H_{16}N_2O_6S_2$.
ESI-HRMS (*m*/*z*): Calculated for $[M-H]^-$: 491.0366, found: 491.0367.
^{1}H-NMR (300 MHz, DMSO-*d*$_6$): δ/ ppm = 13.11 (bs, 2H, CO$_2$*H*), 7.52 (d, J^3= 8.8 Hz, 4H, CH^{Ar}), 7.41 (s, 2H, CH^{Ar}), 7.06 (d, J^3= 8.9 Hz, 4H, CH^{Ar}), 4.76 (s, 4H, CH_2).
^{13}C{^{1}H}-NMR (75 MHz, DMSO-*d*$_6$): δ/ ppm = 169.80 (C=O), 159.24, 140.85, 136.00, 133.27, 119.92, 116.47, 115.43, 115.26, 64.58.
ATR-IR(cm^{-1}):3060, 2323, 2224, 1733, 1707, 1570, 1655, 1593, 1576, 1542, 1494, 1474, 1455, 1427, 1408, 1372, 1341, 1315, 1305, 1286, 1267, 1242, 1179, 1150, 1108, 1094, 1081,1010, 916, 896, 833, 813, 800, 725, 712, 652.

Compound 14



PyBOP (23.5 mg, 45.2 µmol, 3.0 eq.) was added to a solution of **13** (7.7 mg, 15.1 µmol, 1.0 eq.), **5** (110 mg, 37.7 µmol, 2.5 eq.) and DIPEA (27 µL, 151 µmol, 10.0 eq.) in DMF (1 mL). The reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and the residue was purified *via* size exclusion chromatography (Sephadex® LH 20, MeOH).
Yield: 104 mg (14.1 µmol, quant.); colorless, amorphous solid.
Molecular formula: $C_{366}H_{404}N_{42}O_{52}S_2$.
MALDI-MS (ACN/ H_2O) (*m/z*): Calculated for $[M+Na]^+$: 6310.6, found: 6311.4.
^{1}H-NMR (400 MHz, DMSO-d$_6$, 298 K): δ/ ppm = 8.53 (d, J^3 = 7.1 Hz, 2H, NH^{His}), 8.44 (d, J^3 = 3.4 Hz, 2H, NH^{His}), 8.24 (d, J^3 = 7.6 Hz, 2H, NH^{His}), 8.13 (d, J^3 = 5.8 Hz, 2H, NH^{His}), 7.98 (d, J^3 = 6.9 Hz, 2H, NH^{His}), 7.94 (t, J^3 = 5.6 Hz, 6H, N*H*CH_2), 7.81 (t, J^3 = 5.6 Hz, 2H, NH^{Ahx}), 7.53 (d, J^3 = 8.6 Hz, 4H, SCC*H*CH), 7.32 - 6.94 (m, 168H, CNCC*H*/ CH^{Trt}/ C$H^{Ar,His}$/ N*H*C_q/ SCCHC*H*), 6.69 (s, 2H, C$H^{Ar,His}$), 6.67 (s, 2H, C$H^{Ar,His}$), 6.65 (s, 2H, C$H^{Ar,His}$), 6.60 (s, 2H, C$H^{Ar,His}$), 6.59 (s, 2H, C$H^{Ar,His}$), 4.52 (s, 4H, OCH_2CO), 4.46 - 4.38 (m, 2H, *α*-CH^{His}), 4.34 - 4.27 (m, 2H, *α*-CH^{His}), 4.24 - 4.14 (m, 6H, *α*-CH^{His}), 3.64 - 3.37 (m, 108H, CH_2O), 3.28 - 3.13 (m, 30H, CH_3^{TEG}/ NCH_2CH_2O), 3.06 - 2.90 (m, 4H, NCH_2[CH_2]$_4^{Ahx}$), 2.89 - 2.53 (m, 20H, β-CH_2^{His}), 2.29 (t, J^3 = 6.4 Hz, 12H, CH_2C=O), 2.05 (t, J^3 = 7.3 Hz, 4H, N[CH_2]$_4$CH_2^{Ahx}), 1.55 - 1.37 (m, 8H, NCH_2CH_2^{Ahx}/ N[CH_2]$_3$CH_2^{Ahx}), 1.35 - 1.16 (m, 4H, N[CH_2]$_2$CH_2^{Ahx}).

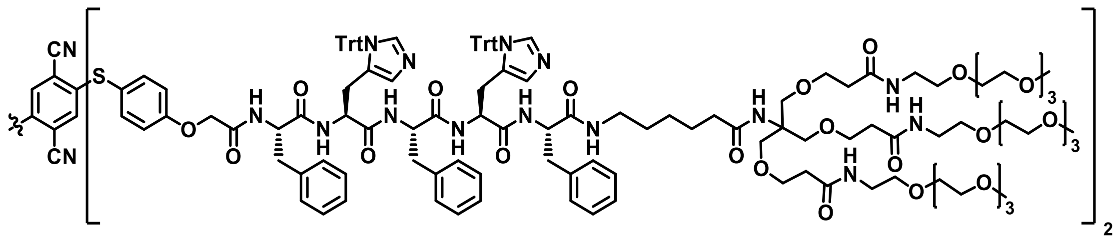
Compound II



14 (100 mg, 14 µmol) was deprotected according to **SOP 2**.
Yield: 65 mg (13 µmol, 93%); colorless, amorphous solid.
Molecular formula: $C_{195}H_{277}N_{42}O_{52}S_2$ * 10 TFA.
MALDI-MS (ACN/ H_2O) (*m/z*): Calculated for $[M+Na]^+$: 3886.9, found: 3887.5.
^{1}H-NMR (400 MHz, DMSO-*d*$_6$, 298 K): δ/ ppm = 14.35 (s, 20H, NH^{His}), 8.52 (d, J^3 = 7.2 Hz, 2H, NH^{His}), 8.44 (d, J^3 = 3.4 Hz, 2H, NH^{His}), 8.24 (d, J^3 = 7.6 Hz, 2H, NH^{His}), 8.12 (d, J^3 = 5.8 Hz, 2H, NH^{His}), 7.99 (d, J^3 = 6.9 Hz, 2H, NH^{His}), 7.94 (t, J^3 = 5.6 Hz, 6H, N*H*CH_2), 7.81 (t, J^3 = 5.6 Hz, 2H, NH^{Ahx}), 7.53 (d, J^3 = 8.6 Hz, 4H, SCC*H*CH), 7.34 (s, 5H, C*H*NH_2^{His}), 7.27 (s, 5H, C*H*NH_2^{His}), 7.05 (s, 2H, N*H*C_q), 7.03 (s, 2H, CNCC*H*), 6.99 (d, J^3 = 8.9 Hz, 4H, SCCHC*H*), 6.69 (s, 2H, C$H^{Ar,His}$), 6.67 (s, 2H, C$H^{Ar,His}$), 6.65 (s, 2H, C$H^{Ar,His}$), 6.60 (s, 2H, C$H^{Ar,His}$), 6.59 (s, 2H, C$H^{Ar,His}$), 4.52 (s, 4H, OCH_2CO), 4.46 - 4.38 (m, 2H, *α*-CH^{His}), 4.34 - 4.27 (m, 2H, *α*-CH^{His}), 4.24 - 4.14 (m, 6H, *α*-CH^{His}),

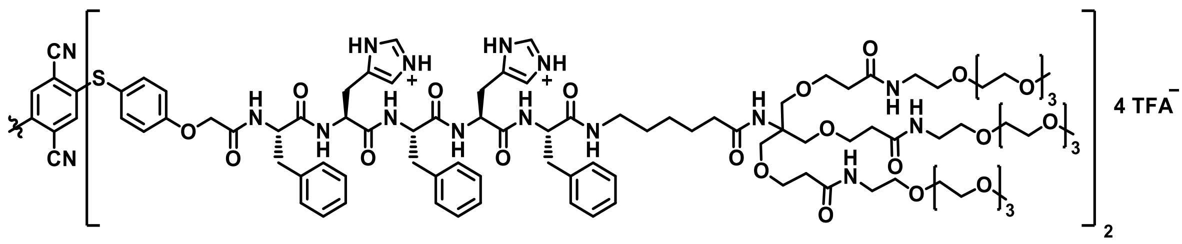
3.64 - 3.37 (m, 108H, C*H*2O), 3.28 - 3.13 (m, 30H, C*H*$_3^{TEG}$/ NCH2C*H*2O), 3.06 - 2.90 (m, 4H, NC*H*2[CH2]$_4^{Ahx}$), 2.89 - 2.53 (m, 20H, β-C*H*$_2^{His}$), 2.29 (t, J^3 = 6.4 Hz, 12H, C*H*2C=O), 2.05 (t, J^3 = 7.3 Hz, 4H, N[CH2]4C*H*$_2^{Ahx}$), 1.55 - 1.37 (m, 8H, NCH2C*H*$_2^{Ahx}$/ N[CH2]3C*H*$_2^{Ahx}$), 1.35 - 1.16 (m, 4H, N[CH2]2C*H*$_2^{Ahx}$).

Compound 15



PyBOP (37 g, 72 µmol, 3.0 eq.) was added to a solution of **13** (12 mg, 24 µmol, 1.0 eq.), **2** (175 mg, 60 µmol, 2.5 eq.) and DIPEA (42 µL, 240 µmol, 10.0 eq.) in DMF (1 mL). The reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and the residue was purified *via* size exclusion chromatography (Sephadex® LH 20, MeOH).
Yield: 108 mg (22 µmol, 92%); colorless, amorphous solid.
Molecular formula: $C_{270}H_{332}N_{30}O_{52}S_2$.
MALDI-MS (ACN/ H_2O) (*m/z*): Calculated for $[M+Na]^+$: 4915.37, found: 4914.73.
^{1}H-NMR (400 MHz, DMSO-*d*$_6$, 298 K): δ/ ppm = 8.72 (d, J^3 = 7.6 Hz, 2H, α-N*H*), 8.56 (d, J^3 = 7.8 Hz, 2H, α-N*H*^{Phe}), 8.24 (d, J^3 = 7.4 Hz, 2H, α-N*H*), 8.21 - 8.12 (m, 4H, α-N*H*), 8.05 - 7.98 (m, 2H, N*H*CH$_2^{Ahx}$), 7.91 (t, J^3 = 5.6 Hz, 6H, N*H*CH2), 7.84 (d, J^3 = 8.1 Hz, 2H, α-N*H*), 7.53 (d, J^3 = 8.6 Hz, 4H, SCC*H*CH), 7.42 - 7.00 (m, 100H, C*H*^{Ar,Phe}/ CNCC*H*/ C*H*^{Trt}/ C*H*^{Ar,His}/ SCCHC*H*), 6.98 (s, 2H, N*H*C$_q$), 6.55 (s, 2H, C*H*^{Ar,His}), 6.53 (s, 2H, C*H*^{Ar,His}), 4.54 - 4.30 (m, 14H, OC*H*2CO/ α-C*H*), 3.57 - 3.35 (m, 108H, C*H*2O), 3.25 - 3.15 (m, 30H, C*H*$_3^{TEG}$/ NCH2C*H*2O), 3.09 - 2.59 (m, 24H, β-CH$_2^{Phe}$/ β-CH$_2^{His}$/ NC*H*2[CH2]$_4^{Ahx}$), 2.29 (t, J^3 = 6.4 Hz, 12H, C*H*2C=O), 2.01 (t, J^3 = 7.5 Hz, 4H, N[CH2]4C*H*$_2^{Ahx}$), 1.41 - 1.31 (m, 4H, NCH2C*H*$_2^{Ahx}$), 1.29 - 1.17 (m, 4H, N[CH2]3C*H*$_2^{Ahx}$), 1.15 - 1.02 (m, 4H, N[CH2]2C*H*$_2^{Ahx}$).

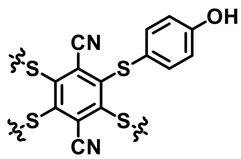
Compound V



15 (45 mg, 9.0 µmol) was deprotected according to **SOP 2**.
Yield: 38 mg (8.6 µmol, 96%); colorless, amorphous solid.
Molecular formula: $C_{194}H_{276}N_{30}O_{52}S_2$ * 4 TFA.

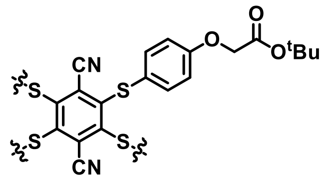
MALDI-MS (ACN/ H_2O) (*m/z*): Calculated for $[M+Na]^+$: 3947.61, found: 3948.32.
^{1}H-NMR (400 MHz, DMSO-*d*$_6$, 298 K): δ/ ppm = 14.35 (s, 8H, NH^{His}), 8.72 (d, J^3 = 7.6 Hz, 2H, α-N*H*), 8.56 (d, J^3 = 7.8 Hz, 2H, α-NH^{Phe}), 8.24 (d, J^3 = 7.4 Hz, 2H, α-N*H*), 8.21 - 8.12 (m, 4H, α-N*H*), 8.05 - 7.98 (m, 2H, N*H*CH_2^{Ahx}), 7.91 (t, J^3 = 5.6 Hz, 6H, N*H*CH_2), 7.84 (d, J^3 = 8.1 Hz, 2H, α-N*H*), 7.53 (d, J^3 = 8.6 Hz, 4H, SCC*H*CH), 7.34 (s, 2H, C*H*NH_2^{His}), 7.27 (s, 2H, C*H*NH_2^{His}), 7.32 - 7.11 (m, 30H, C$H^{Ar,Phe}$), 7.05 (s, 2H, NHC_q), 7.03 (s, 2H, CNCC*H*), 6.99 (d, J^3 = 8.9 Hz, 4H, SCCHC*H*), 6.55 (s, 2H, C$H^{Ar,His}$), 6.53 (s, 2H, C$H^{Ar,His}$), 4.54 - 4.30 (m, 14H, OCH_2CO/ α-C*H*), 3.57 - 3.35 (m, 108H, CH_2O), 3.25 - 3.15 (m, 30H, CH_3^{TEG}/ NCH$_2$CH_2O), 3.09 - 2.59 (m, 24H, β-CH_2^{Phe}/ β-CH_2^{His}/ NCH_2[CH$_2$]$_4^{Ahx}$), 2.29 (t, J^3 = 6.4 Hz, 12H, CH_2C=O), 2.01 (t, J^3 = 7.5 Hz, 4H, N[CH$_2$]$_4$CH_2^{Ahx}), 1.41 - 1.31 (m, 4H, NCH$_2$CH_2^{Ahx}), 1.29 - 1.17 (m, 4H, N[CH$_2$]$_3$CH_2^{Ahx}), 1.15 - 1.02 (m, 4H, N[CH$_2$]$_2$CH_2^{Ahx}).

Compound 16[9]



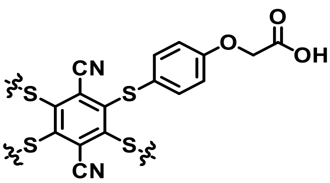
Tetrachloroterephthalonitrile (500 mg, 1.9 mmol, 1.0 eq.) 4-hydroxy-thiophenol (1.39 g, 11 mmol, 5.8 eq.) and potassium carbonate (3.18 g, 23 mmol, 12.1 eq.) were added to a 100 mL round bottom flask. The flask was evacuated and filled with argon 3x). After that dry DMF (30 mL) was added to the flask and solution was stirred at 45°C for 6 h. Then the reaction was quenched with quenched very slowly with 4 M HCl (50 mL) and the product precipitated as a red-orange solid. This solid was separated by filtration and washed with copious amounts of distilled water. The product was dried *in vacuum*.
Yield: 1.11 g (1.78 mmol, 94%), red-orange solid.
Molecular Formula: $C_{32}H_{20}N_2O_4S_4$.
ESI-HRMS (*m/z*): Calculated for $[M-H]^-$: 623.0233, found: 622.9556
^{1}H-NMR (300 MHz, DMSO-*d*$_6$): δ/ ppm = 9.77 (s, 4H, CH^{Ar}), 7.05 (d, *J3* = 9.8 Hz, 8H, CH^{Ar}), 6.69 (d, J^3 = 13.5 Hz, CH^{Ar}).
^{13}C{^{1}H}-NMR (75 MHz, DMSO-*d*$_6$): δ/ ppm = 157.55, 145.89, 132.30, 127.11, 122.34, 116.58, 114.42.
ATR-IR (cm^{-1}): 3365, 3259, 3019, 2361, 2241, 2100, 2089, 1600, 1581, 1492, 1434, 1362, 1309, 1271, 1236, 1213, 1170, 1147, 1101, 1009, 825, 752, 697.

Compound 17[9]



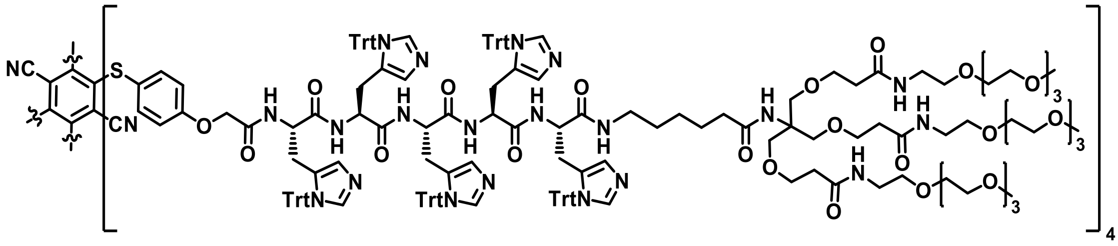
16 (100 mg, 160 µmol, 1.0 eq.) and potassium carbonate (1.16 g, 8.4 mmol, 53.0 eq.) were added to a 100 mL round bottom flask. Dry DMF (15 mL) and ^{t}butyl bromoacetate (267 mg 1.93 mmol, 12.0 eq.) was added to the flask and the solution was stirred at 40°C for 1 d followed by quenching of the reaction mixture with distilled water. The product precipitates as yellow solid. This solid was separated by filtration and washed with copious amounts of distilled water. The product was dried *in vacuo*.
Yield: 176 mg (158 µmol, 99%); yellow solid.
Molecular Formula: $C_{56}H_{60}N_2O_{12}S_4$.
ESI-HRMS (*m/z*): Calculated for $[M+Na]^+$:1103.2921, found: 1103.2962.
^{1}H-NMR (300 MHz, DMSO-*d$_6$*): δ/ ppm = 7.17 (d, J^3 = 8.8 Hz, 8H, CH^{Ar}), 6.85 (d, J^3 = 8.8 Hz, 8H, CH^{Ar}), 4.64 (s, 8H, CH_2), 1.41 (s, 36H, CH_3).
^{13}C{^{1}H}-NMR (75 MHz, DMSO-*d$_6$*): δ/ ppm = 167.55 (C=O), 157.41, 145.71, 131.55, 127.82, 125.36, 115.79, 114.40, 81.50, 65.07, 27.66.
ATR-IR (cm^{-1}): 2983, 2359, 1744, 1592, 1490, 1440 1394, 1368, 1310, 1293, 1255, 1217, 1161, 1108, 1076, 950, 763, 735, 688.

Compound 18[9]



17 (150 mg, 138 µmol) was deprotected according to **SOP 2**.
Yield: 117 mg (135 µmol, 99%), orange solid.
Molecular Formula: $C_{40}H_{28}N_2O_{12}S_4$.
ESI-HRMS (*m/z*): Calculated for $[M-H]^-$: 855.0441, found: 855.0426.
^{1}H-NMR (300 MHz, DMSO-*d$_6$*): δ/ ppm = 13.12 (bs, 4H, CO_2H), 7.17 (d, J^3 = 8.2 Hz, 8H, CH^{Ar}), 6.87 (d, J^3= 8.3 Hz, 8H, CH^{Ar}), 4.66 (s, 8H, CH_2).
^{13}C{^{1}H}-NMR (75 MHz, DMSO-*d$_6$*): δ/ ppm 169.92 (C=O), 157.55, 145.78, 131.63, 127.89, 125.15, 115.77, 114.45, 64.63.
ATR-IR (cm^{-1}): 2915,2337, 2114, 2084,1991, 1882, 1689, 1593, 1490, 1434, 1408, 1290, 1225, 1174, 1151, 1065, 1024, 1003, 927, 816, 761, 698.

Compound 19



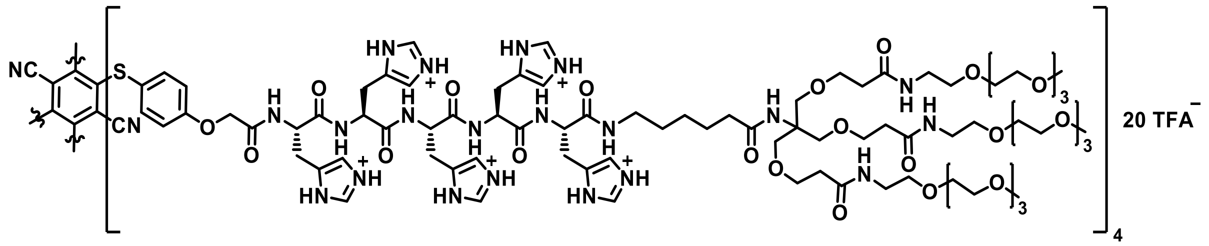
PyBOP (28.9 mg, 55.5 µmol, 7.4 eq.) was added to a solution of **18** (6.4 mg, 7.5 µmol, 1.0 eq.), **5** (110 mg, 37.7 µmol, 2.5 eq.) and DIPEA (23 µL, 135 µmol, 18.0 eq.) in DMF (8 mL). The reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and the residue was purified *via* size exclusion chromatography (Sephadex® LH 20, MeOH).

Yield: 73 mg (5.6 µmol, 78%); yellow, amorphous solid.

Molecular formula: $C_{724}H_{804}N_{82}O_{104}S_4$.

^{1}H-NMR (400 MHz, DMSO-*d*$_6$, 298 K): δ/ ppm = 8.53 (d, J^3 = 7.1 Hz, 4H, α-N*H*^{His}), 8.44 (d, J^3 = 3.4 Hz, 4H, α-N*H*^{His}), 8.24 (d, J^3 = 7.6 Hz, 4H, α-N*H*^{His}), 8.13 (d, J^3 = 5.8 Hz, 4H, α-N*H*^{His}), 7.98 (d, J^3 = 6.9 Hz, 4H, α-N*H*^{His}), 7.94 (t, J^3 = 5.6 Hz, 12H, N*H*CH$_2$), 7.81 (t, J^3 = 5.6 Hz, 4H, N*H*^{Ahx}), 7.53 (d, J^3 = 8.6 Hz, 8H, SCC*H*CH), 7.32 - 6.94 (m, 334H, C*H*^{Trt}/ C*H*^{Ar,His}/ N*H*C$_q$/ SCCHC*H*), 6.69 (s, 4H, C*H*^{Ar,His}), 6.67 (s, 4H, C*H*^{Ar,His}), 6.65 (s, 4H, C*H*^{Ar,His}), 6.60 (s, 4H, C*H*^{Ar,His}), 6.59 (s, 4H, C*H*^{Ar,His}), 4.52 (s, 8H, OC*H*$_2$CO), 4.46 - 4.38 (m, 4H, α-C*H*^{His}), 4.34 - 4.27 (m, 4H, α-C*H*^{His}), 4.24 - 4.14 (m, 12H, α-C*H*^{His}), 3.64 - 3.37 (m, 216H, C*H*$_2$O), 3.29 - 3.13 (m, 60H, C*H*$_3$^{TEG}/ NCH$_2$C*H*$_2$O), 3.06 - 2.90 (m, 8H, NC*H*$_2$[CH$_2$]$_4$^{Ahx}), 2.89 - 2.53 (m, 40H, β-C*H*$_2$^{His}), 2.29 (t, J^3 = 6.4 Hz, 24H, C*H*$_2$C=O), 2.05 (t, J^3 = 7.3 Hz, 8H, N[CH$_2$]$_4$C*H*$_2$^{Ahx}), 1.53 - 1.35 (m, 16H, NCH$_2$C*H*$_2$^{Ahx}/ N[CH$_2$]$_3$C*H*$_2$^{Ahx}), 1.34 - 1.15 (m, 8H, N[CH$_2$]$_2$C*H*$_2$^{Ahx}).

Compound III



19 (30 mg, 2.3 µmol) was deprotected according to **SOP 2**.

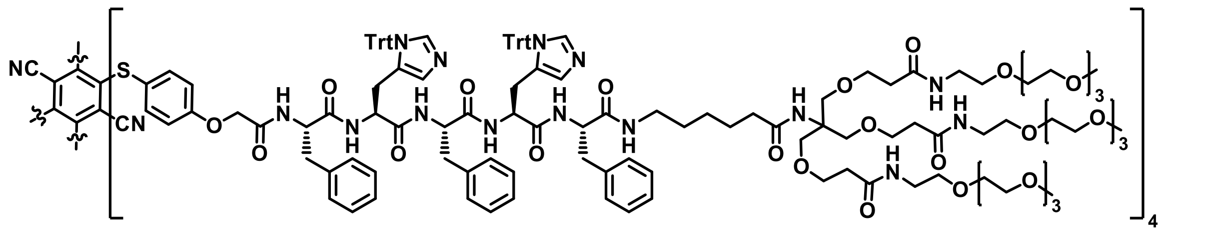
Yield: 21 mg (2.1 µmol, 91%); yellow, amorphous solid.

Molecular formula: $C_{344}H_{524}N_{82}O_{104}S_4$ * 20 TFA.

MALDI-MS (ACN/ H$_2$O) (*m/z*): Calculated for [M+Na]$^+$: 7623.7, found: 7622.5.

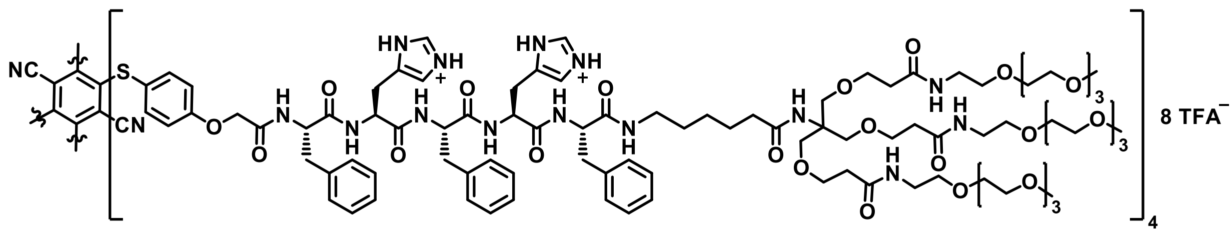
^{1}H-NMR (400 MHz, DMSO-*d*$_6$, 298 K): δ/ ppm = 14.35 (s, 40H, N*H*^{His}), 8.52 (d, J^3 = 7.2 Hz, 4H, α-N*H*^{His}), 8.44 (d, J^3 = 3.4 Hz, 4H, α-N*H*^{His}), 8.24 (d, J^3 = 7.6 Hz, 4H, α-N*H*^{His}), 8.12 (d, J^3 = 5.8 Hz, 4H, α-N*H*^{His}), 7.99 (d, J^3 = 6.9 Hz, 4H, α-N*H*^{His}), 7.94 (t, J^3 = 5.6 Hz, 12H, N*H*CH$_2$), 7.81 (t, J^3 = 5.6 Hz, 4H, N*H*^{Ahx}), 7.53 (d, J^3 = 8.6 Hz, 8H, SCC*H*CH), 7.34 (s, 10H, C*H*NH$_2$^{His}), 7.28 (s, 10H, C*H*NH$_2$^{His}), 7.05 (s, 4H, N*H*C$_q$), 6.99 (d, J^3 = 8.9 Hz, 8H, SCCHC*H*), 6.69 (s, 4H, C*H*^{Ar,His}), 6.67 (s, 4H, C*H*^{Ar,His}), 6.65 (s, 4H, C*H*^{Ar,His}), 6.60 (s, 4H, C*H*^{Ar,His}), 6.59 (s, 4H, C*H*^{Ar,His}), 4.53 (s, 8H, OC*H*$_2$CO), 4.46 - 4.38 (m, 4H, α-C*H*^{His}), 4.34 - 4.26 (m, 4H, α-C*H*^{His}), 4.24 - 4.14 (m, 12H, α-C*H*^{His}), 3.64 - 3.37 (m, 216H, C*H*$_2$O), 3.28 - 3.13 (m, 60H, C*H*$_3$$^{\mathrm{TEG}}$/ NCH$_2$C*H*$_2$O), 3.08 - 2.90 (m, 8H, NC*H*$_2$[CH$_2$]$_4$$^{\mathrm{Ahx}}$), 2.89 - 2.53 (m, 40H, β-C*H*$_2$$^{\mathrm{His}}$), 2.29 (t, J^3 = 6.4 Hz, 24H, C*H*$_2$C=O), 2.05 (t, J^3 = 7.3 Hz, 8H, N[CH$_2$]$_4$C*H*$_2$$^{\mathrm{Ahx}}$), 1.55 - 1.37 (m, 16H, NCH$_2$C*H*$_2$$^{\mathrm{Ahx}}$/ N[CH$_2$]$_3$C*H*$_2$$^{\mathrm{Ahx}}$), 1.36 - 1.16 (m, 8H, N[CH$_2$]$_2$C*H*$_2$$^{\mathrm{Ahx}}$).

Compound 20



PyBOP (29 mg, 56 µmol, 7.4 eq.) was added to a solution of **18** (6.4 mg, 7.5 µmol, 1.0 eq.), **2** (100 mg, 45 µmol, 6.0 eq.) and DIPEA (23 µL, 135 µmol, 18.0 eq.) in DMF (1 mL). The reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and the residue was purified *via* size exclusion chromatography (Sephadex® LH 20, MeOH).
Yield: 63 mg (6.5 µmol, 86%); yellow, amorphous solid.
Molecular formula: $C_{409}H_{502}N_{44}O_{81}S_4$.
MALDI-MS (ACN/ H$_2$O) (*m/z*): Calculated for [M+Na]$^+$: 9682.66, found: 9682.73.
^{1}H-NMR (400 MHz, DMSO-*d*$_6$, 298 K): δ/ ppm = 8.72 (d, J^3 = 7.6 Hz, 4H, α-N*H*), 8.56 (d, J^3 = 7.8 Hz, 4H, α-N*H*$^{\mathrm{Phe}}$), 8.24 (d, J^3 = 7.4 Hz, 4H, α-N*H*), 8.21 - 8.12 (m, 8H, α-N*H*), 8.05 - 7.98 (m, 4H, N*H*CH$_2$$^{\mathrm{Ahx}}$), 7.91 (t, J^3 = 5.6 Hz, 12H, N*H*CH$_2$), 7.84 (d, J^3 = 8.1 Hz, 4H, α-N*H*), 7.53 (d, J^3 = 8.6 Hz, 8H, SCC*H*CH), 7.42 - 7.00 (m, 196H, C*H*$^{\mathrm{Ar,Phe}}$/ C*H*$^{\mathrm{Trt}}$/ C*H*$^{\mathrm{Ar,His}}$/ SCCHC*H*), 6.98 (s, 4H, N*H*C$_q$), 6.55 (s, 4H, C*H*$^{\mathrm{Ar,His}}$), 6.53 (s, 4H, C*H*$^{\mathrm{Ar,His}}$), 4.54 - 4.30 (m, 28H, OC*H*$_2$CO/ α-C*H*), 3.57 - 3.35 (m, 216H, C*H*$_2$O), 3.25 - 3.15 (m, 60H, C*H*$_3$$^{\mathrm{TEG}}$/ NCH$_2$C*H*$_2$O), 3.09 - 2.59 (m, 48H, β-CH$_2$$^{\mathrm{Phe}}$/ β-CH$_2$$^{\mathrm{His}}$/ NC*H*$_2$[CH$_2$]$_4$$^{\mathrm{Ahx}}$), 2.29 (t, J^3 = 6.4 Hz, 24H, C*H*$_2$C=O), 2.01 (t, J^3 = 7.5 Hz, 8H, N[CH$_2$]$_4$C*H*$_2$$^{\mathrm{Ahx}}$), 1.41 - 1.31 (m, 8H, NCH$_2$C*H*$_2$$^{\mathrm{Ahx}}$), 1.29 - 1.17 (m, 8H, N[CH$_2$]$_3$C*H*$_2$$^{\mathrm{Ahx}}$), 1.15 - 1.02 (m, 8H, N[CH$_2$]$_2$C*H*$_2$$^{\mathrm{Ahx}}$).

Compound VI



20 (14.4 mg, 1.5 µmol) was deprotected according to **SOP 2**.
Yield: 12.9 mg (1.5 µmol, 99%); yellow, amorphous solid.
Molecular formula: $C_{380}H_{548}N_{58}O_{104}S_4$ * 8 TFA.
MALDI-MS (ACN/ H$_2$O) (*m/z*): Calculated for [M+Na]$^+$: 7744.1, found: 7744.9.
^{1}H-NMR (400 MHz, DMSO-*d*$_6$, 298 K): δ/ ppm = 14.35 (s, 16H, N*H*$^{\mathrm{His}}$), 8.72 (d, J^3 = 7.6 Hz, 4H, α-N*H*), 8.56 (d, J^3 = 7.8 Hz, 4H, α-N*H*$^{\mathrm{Phe}}$), 8.24 (d, J^3 = 7.4 Hz, 4H, α-N*H*), 8.21 - 8.12 (m, 8H,

α-N*H*), 8.05 - 7.98 (m, 4H, N*H*CH_2^{Ahx}), 7.91 (t, *J*^{3} = 5.6 Hz, 12H, N*H*CH_2), 7.84 (d, *J*^{3} = 8.1 Hz, 4H, α-N*H*), 7.53 (d, *J*^{3} = 8.6 Hz, 8H, SCC*H*CH), 7.34 (s, 4H, C*H*NH_2^{His}), 7.27 (s, 4H, C*H*NH_2^{His}), 7.32 - 7.11 (m, 60H, C*H*^{Ar,Phe}), 7.05 (s, 4H, N*H*C_q), 7.03 (s, 4H, CNCC*H*), 6.99 (d, *J*^{3} = 8.9 Hz, 8H, SCCHC*H*), 6.55 (s, 4H, C*H*^{Ar,His}), 6.53 (s, 4H, C*H*^{Ar,His}), 4.54 - 4.30 (m, 28H, OCH_2CO/ α-C*H*), 3.57 - 3.35 (m, 216H, CH_2O), 3.25 - 3.15 (m, 60H, CH_3^{TEG}/ N$CH_2$$CH_2$O), 3.09 - 2.59 (m, 48H, β-CH_2^{Phe}/ β-CH_2^{His}/ NCH_2[CH_2]$_4$^{Ahx}), 2.29 (t, *J*^{3} = 6.4 Hz, 24H, CH_2C=O), 2.01 (t, *J*^{3} = 7.5 Hz, 8H, N[CH_2]$_4$$CH_2$^{Ahx}), 1.41 - 1.31 (m, 8H, N$CH_2$$CH_2$^{Ahx}), 1.29 - 1.17 (m, 8H, N[CH_2]$_3$$CH_2$^{Ahx}), 1.15 - 1.02 (m, 8H, N[CH_2]$_2$$CH_2$^{Ahx}).

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