10. Experimental part

10.1. Materials and Methods

10.1.1. Chemicals

Polymers:

All poly(ethylene imines) (PEI), polyglycerol (PG), and poly(amidoamine) [G5] dendrimer (PAMAM[G5]) were concentrated and dried under vacuum (50 °C, 1 x 10^{-2} mbar) until loss of weight was lower than 0.025 g per 1.000 g of the dried sample in 5 h drying periods.

PAMAM [G5]	SuperFect®, Qiagen GmbH, Germany; M_n = 14215 g mol^{-1}
PEI_{3600}	Polymin G100, BASF, 50 wt% in water
PEI_{10500}	Polymin G500, BASF, 43 wt% in water
PG_{10000}	M_n = 10,000 g mol^{-1} (pentaerythrit starter, MWD = 1.7) was prepared in our group according to the published procedures. [168,445]
$mPEG_6$	mPEG 350, monomethyl poly(ethylene glycol) ether, Fluka, M_w = 350 g mol^{-1}
$mPEG_{10}$	mPEG 550, monomethyl poly(ethylene glycol) ether, Fluka, M_w = 550 g mol^{-1}
$mPEG_{14}$	mPEG 750, monomethyl poly(ethylene glycol) ether, Fluka, M_w = 750 g mol^{-1}
$mPEG_{22}$	mPEG 1100, monomethyl poly(ethylene glycol) ether, Fluka, M_w = 1100 g mol^{-1}

Chemicals and solvents:

1,18-octadecanoic acid was purchased from Cognis GmbH, Germany. Other chemicals were commercially avaible and were used as delivered. Solvents were purchased as reagent grade and distilled once before use. Anhydrous solvents were either purchased as ultra dry solvents from *Acros Organics*, or dried conventionally.[446]

10.1.2. Analytical methods

NMR spectroscopy

^{1}H NMR and ^{13}C NMR spectra were recorded on following spectrometers: Bruker ARX 300 (300 MHz spectra), Bruker DRX 400 (400 MHz spectra), Bruker DRX 500 (500 MHz spectra), AMX 500 (500 MHz spectra). Typical sample amount: ^{1}H NMR: 10 – 30 mg, ^{13}C NMR: 50 – 100 mg. The deuterated solvents were used for calibration according to the literature.[447] All spectra were recorded at r.t. and were evaluated with the program MestReC from MestReLab Company.

UV/Vis spectroscopy

UV/Vis spectra were recorded on following spectrometers: Jena Analytic Specord S100 (range: 188 – 800 nm; resolution: 745 points) and Scinco S-3150 (range: 187 – 1193 nm; resolution: 1024 points). Typical concentration of the polymer in the solution: 0.0125 – 2.000 g l^{-1} at r.t. Calibration was performed at 360.85 and 453.55 nm with Holmium Oxide Glass. All spectra were recorded at r.t. and were evaluated with the programs LabPro$^®$ Plus from Scinco Co., LTD., Microsoft$^®$ Excel 2000 from Microsoft Corporation, and Origin$^®$ 7.0 from OriginLab Corporation.

Molecular Modelling

Molecular modelling was performed with the program HyperChem release 6.0 from Hypercube, Inc. All calculation was proceeded as molecular mechanic setup with AMBER method with standard settings. Fletcher-Reeves[410] (conjugate gradient) method was used for geometry optimization. The molecular dynamic simulations were run with starting temp. 0 K, simulating temp. 300 K, and final temp. 0 K. Times of heat, run time, and cool time were 2, 8, 2 ps respectively. Time step size was 0.001 ps. Molecular dynamic simulations were performed in vacuo and in periodic box 10 × 10 × 10 nm with water molecules.

Critical Aggregation Concentration (CAC)

The surface tension was measured using the commercially available pendant drop tensiometer PAT1 SINTECH. This instrument was constructed by Surface & Interface Technologies, Germany. The surface tension γ was calculated by fitting the Gauss-Laplace equation to the coordinates of a drop, using γ as the fitting parameter.

Dynamic Light Scattering (DLS)

Dynamic Light Scattering experiments were made by a commercially available equipment Zetasizer Nano from Malvern using a 4 mW He-Ne laser (633 nm wavelength) with a fixed detector angle of 173°. The measurement was performed at 25°C and was started 10 min after the cuvette was placed in the DLS apparatus to allow the temperature to equilibrate. About 1 ml of the sample was transferred to a special dust free light scattering cell. The temperature was controlled to within ± 0.02°C. The differential refractive index increment dn/dc value of multishell nanotransporter in water was measured with a WEG Dr. Bures differential refractometer (model Dn-2010) at a wavelength of 620 nm and 25°C. In a dynamic light scattering experiment, the intensity-intensity time correlation function $g_2(\tau)$ were recorded. If the scattered field obeys Gaussian statistics, the measured correlation function $g_2(\tau)$ could be related to the theoretically amenable first-order field correlation function $g_1(\tau)$ by the Siegert relationship $g_2(\tau) = A\left[1 + \beta\left|g_1(\tau)\right|^2\right]$, where A is the baseline, β is the coherence factor of the experiment, and τ is the delay time. $\left|g_1(\tau)\right|$ is further related to the linewidth (Γ) distribution. The first Γ_1 and second Γ_2 moment of linewidth distribution was extracted by second-order cumulants fitting. If the relaxation is diffusive, Γ_1 can be related to the translation diffusion coefficient D as

$$\frac{\Gamma_1}{q^2} = D\left(1 + k_d c + ..\right)$$ (another quantity, which is often used to specify the polydispersity index

Q, is the normalised variance defined as $Q = \dfrac{\Gamma_2}{\Gamma_1^2}$). Where k_d is the diffusive second virial

coefficient, and $q = \dfrac{4\pi n}{\lambda}\sin(\theta/2)$, n is the solvent refractive index, θ the scattering angle,

and λ is the wavelength of the incident beam. If the translation diffusion coefficient D is known, the hydrodynamic radius R_h can be obtained from the Stokes-Einstein equation

$$\langle R_h \rangle = \frac{k_B T}{6\pi\eta D_0}$$ where, k_B is Boltzmann's constant, T is the absolute temperature and η is the

coefficient of viscosity of the solvent.

Cryo-Transmission Electron Microscopy (CryoTEM)

The samples for CryoTEM were prepared at room temperature by placing a droplet (~5 µL) of the solution on a hydrophilized perforated carbon filmed grid (60s Plasma treatment at 8 W

Michał R. Radowski, Dissertation

using a BALTEC MED 020 device). The excess fluid was blotted off to create an ultra-thin layer (typical thickness of 100 nm) of the solution spanning the holes of the carbon film. The grids were immediately vitrified in liquid ethane at its freezing point (-184 °C) using a standard plunging device. Ultra-fast cooling is necessary for an artifact-free thermal fixation (vitrification) of the aqueous solution avoiding crystallization of the solvent or rearrangement of the assemblies. The vitrified samples were transferred under liquid nitrogen into a Philips CM12 transmission electron microscope using the Gatan cryoholder and -stage (Model 626). Microscopy was carried out at -175 °C sample temperature using the microscopes' low dose protocol at a primary magnification of 58300 ×. The defocus was chosen in all cases to be 2.5 µm.

Negative staining TEM

Aliquots of the aqueous solution (~5 µL) were placed on hydrophilized carbon-coated copper grids and the supernatant fluid was blotted off after incubation of 30s. A droplet of uranyl acetate (1 % w/v) was added for 60 s, subsequently removed, and the sample was allowed to air-dry.

Atomic Force Microscopy (AFM)

Polymers, congo red, and nimodypin were imaged by atomic force microcopy (AFM) using a MultiMode IIIa scanning probe microscope with Extender Modul (Digital Instruments, Inc.,Santa Barbara, CA) in the dynamical modus. Olympus etched silicon cantilevers were used with a typical resonance frequency in the range of 200-400kHz and a sprong constant of 42 N/m. High-resolution-images were obtained with a Super Sharp Silicon tip at low-frequency F_0=260-410 kHz. The set-point amplitude of the cantilever was maintained by the feedback circuitry to 80% of the free oscillation amplitude of the cantilever. The scan angle was maintained at 0 °, and the images were captured in the trace direction with a scan rate from 0.400 to 1.000Hz. All samples were measured at room temperature in air enviroment. The sample was first adjusted with an optical light microscope (Nanoscope, Optical Viewing System). Data-analysis was performed after plane-fit, height measurements based on the cross-sectional profiles and/or particle analysis.

PEI$_{3600}$(C$_{18}$mPEG$_6$)$_{0.7}$ on mica - A 20 µl of polymer solution in water (10^{-5} M, 10^{-6} M) was placed onto the freshly-cleaved mica platelet and the excess of fluid was removed after 15 sec. The fibers were detectable on the mica surface with a heights of 5 nm for the polymer concentration in the solution of 10^{-5} M and between 1.3-1.5 nm for the concentration in the solution of 10^{-6} M.

PEI$_{3600}$(C$_{18}$mPEG$_6$)$_{0.7}$ on HOPG - A 20 µl of polymer solution in water (10^{-5} M) was placed onto the graphite platelet and the excess of fluid was removed after 15 sec.

PEI$_{3600}$(C$_{18}$mPEG$_6$)$_{0.7}$ with encapsulated guest molecules – Polymer was expose on the platelet surface as described abowe. For the polar guest molecules (congo red, vitamin B$_6$) encapsulation were performed *in situ*, when the 20 µl droplet of guest molecules solution (10^{-5} M) was placed on the surface covered with the polymer. In case of nonpolar guest molecules (nimodipine, β-carotene) polymer with encapsulated guest molecules was placed on the platelet surface.

Dialysis

Dialysis was performed with benzoylated cellulose membrane from the Sigma-Aldrich company, MWCO = 1000 or with regenerated cellulose membrane from the Roth company, MWCO = 10000. Typical dialysis time was 24 h.Usually 2 liters of solvent was used for dialysis and was exchanged after first 6 h of the process.

Size Exclusion Chromatography (SEC)

Size Exclusion Chromatography was performed on the Spehadex LH-20 or Sephadex G-25 with water or glucose-phosphate buffer (pH 6) as an eluent, respectively.

TLC and Dry Flash Column Chromatography

TLC was performed on *Merck* aluminium sheets with silica (corn size 60) and fluorescence marker (F$_{254}$). Dry Flash Column Chromatography was performed on *Merck* silica (corn size 60).

10.2. Synthesis of dendritic core-multishell architectures

General procedure for mono-esteryfication reaction of linear aliphatic carboxylic diacids (1,6-hexanedioic acid, 1,12-dodecanedioic acid, or 1,18-octadecanedioic acid) with methoxy-poly(ethylene glycol) (mPEG$_6$, mPEG$_{10}$, mPEG$_{14}$, or mPEG$_{22}$) by azeotropic distillation (C$_{m+4}$mPEG$_{n+1}$).
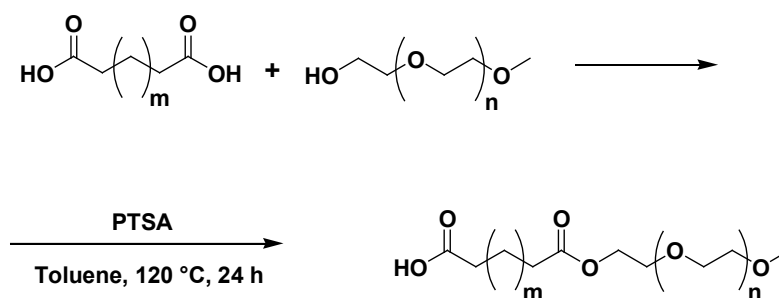
Reaction was performed in an one-neck round-bottom flask equipped with a Dean-Stark trap and a reflux condenser. Methoxy-poly(ethylene glycol) (10.0 mmol, 1 eq.) was dissolved in p.a. toluene (250 ml), solid carboxylic diacid (40.0 mmol, 4 eq.) and *p*-toluenesulfonic acid (4-methylbenzenesulfonic acid) (0.017 g, 0.1 mmol, 0.01 eq.) were added to the solution. The reaction mixture was stirred for 24 h at 120 °C. Reaction control was performed *via* ^{1}H NMR spectroscopy (comparison of the peaks intensity at 4.18 ppm and 3.34 ppm to the ratio 2 : 3). After completion of the reaction, the mixture was concentrated by rotary evaporation *in vacuo*. To the residue toluene was added (100 ml), stirred for 5 minutes and placed in a ice bath for 30 minutes. The resulting suspension was filtrated and the white residue was washed 2 times with cold (0 °C) toluene (2 × 50 ml). The filtrate and washings were combined and concentrated by rotary evaporation *in vacuo*. Crude product, as a yellow wax or oil, was purified by flash dry column chromatography (acidified with AcOH silica gel, eluent: CHCl$_3$ with polarity gradient change to CHCl$_3$:MeOH 5:1) to give a pure product as a white wax or colorless oil (C$_{m+4}$mPEG$_{n+1}$).

6-(Methoxy-poly[ethylene glycol]-oxy)-6-oxohexanoic acid (C$_6$mPEG$_6$). Yield: 61 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.55 (br. m, 24H, $\underline{PEG}$), 3.51 (t, 2H, -C$\underline{H}_2$-O-CH$_3$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 2.35 – 2.25 (m, 4H, HOOC-C$\underline{H}_2$-(CH$_2$)$_2$-C$\underline{H}_2$-COO-), 1.79-1.73 (m, 4H, HOOC-CH$_2$-(C$\underline{H}_2$)$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 176.2 (HOO$\underline{C}$-CH$_2$-), 173.8 (-CH$_2$-$\underline{C}$OO-), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-O-$\underline{C}$H$_3$), 34.2 (-$\underline{C}$H$_2$-COO-), 33.4 (HOOC-$\underline{C}$H$_2$-), 23.9 (HOOC-CH$_2$-$\underline{C}$H$_2$-), 23.6 (-$\underline{C}$H$_2$-CH$_2$-COO-).

Michał R. Radowski, Dissertation

12-(Methoxy-poly[ethylene glycol]-oxy)-12-oxododecanoic acid (C$_{12}$mPEG$_6$). Yield: 67 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.55 (br. m, 24H, $\underline{PEG}$), 3.51 (t, 2H, -C$\underline{H}_2$-O-CH$_3$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 2.30 – 2.20 (m, 4H, HOOC-C$\underline{H}_2$- and -C$\underline{H}_2$-COO-), 1.60 – 1.46 (m, 4H, HOOC-CH$_2$-C$\underline{H}_2$-(CH$_2$)$_6$-C$\underline{H}_2$-CH$_2$-COO-), 1.30 – 1.15 (br. m, 12H, HOOC-CH$_2$-CH$_2$-(C$\underline{H}_2$)$_6$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 176.2 (HOO$\underline{C}$-CH$_2$-), 173.8 (-CH$_2$-$\underline{C}$OO-), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-O-$\underline{C}$H$_3$), 34.1 (-$\underline{C}$H$_2$-COO-), 33.8 (HOOC-$\underline{C}$H$_2$-), 29.2 – 28.9 (HOOC-CH$_2$-CH$_2$-($\underline{C}$H$_2$)$_6$-CH$_2$-CH$_2$-COO-), 24.7 (-$\underline{C}$H$_2$-CH$_2$-COO-), 24.6 (HOOC-CH$_2$-$\underline{C}$H$_2$-).

18-(Methoxy-poly[ethylene glycol]-oxy)-18-oxooctadecanoic acid (C$_{18}$mPEG$_6$). Yield: 77 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3. 45 (br. m, 26H, $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 2.30 – 2.20 (m, 4H, HOOC-C$\underline{H}_2$- and -C$\underline{H}_2$-COO-), 1.60 – 1.46 (m, 4H, HOOC-CH$_2$-C$\underline{H}_2$-(CH$_2$)$_{12}$-C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.15 (br. m, 24H, HOOC-CH$_2$-CH$_2$-(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 176.2 (HOO$\underline{C}$-CH$_2$-), 173.8 (-CH$_2$-$\underline{C}$OO-), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-O-$\underline{C}$H$_3$), 34.1 (-$\underline{C}$H$_2$-COO-), 33.8 (HOOC-$\underline{C}$H$_2$-), 29.7 – 28.9 (HOOC-CH$_2$-CH$_2$-($\underline{C}$H$_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 24.7 (-$\underline{C}$H$_2$-CH$_2$-COO-), 24.6 (HOOC-CH$_2$-$\underline{C}$H$_2$-).

18-(Methoxy-poly[ethylene glycol]-oxy)-18-oxooctadecanoic acid (C$_{18}$mPEG$_{10}$). Yield: 84 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.45 (br. m, 42H, $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 2.30 – 2.20 (m, 4H, HOOC-C$\underline{H}_2$- and -C$\underline{H}_2$-COO-), 1.60 – 1.46 (m, 4H, HOOC-CH$_2$-C$\underline{H}_2$-(CH$_2$)$_{12}$-C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.15 (br. m, 24H, HOOC-CH$_2$-CH$_2$-(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 176.2 (HOO$\underline{C}$-CH$_2$-), 173.8 (-CH$_2$-$\underline{C}$OO-), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-O-$\underline{C}$H$_3$), 34.1 (-$\underline{C}$H$_2$-COO-), 33.8 (HOOC-$\underline{C}$H$_2$-), 29.7 – 28.9 (HOOC-CH$_2$-CH$_2$-($\underline{C}$H$_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 24.7 (-$\underline{C}$H$_2$-CH$_2$-COO-), 24.6 (HOOC-CH$_2$-$\underline{C}$H$_2$-).

18-(Methoxy-poly[ethylene glycol]-oxy)-18-oxooctadecanoic acid (C$_{18}$mPEG$_{14}$). Yield: 79 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.75 – 3.45 (br. m, 68H, $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 2.30 – 2.20 (m, 4H, HOOC-C$\underline{H}_2$- and -C$\underline{H}_2$-COO-), 1.60 – 1.46 (m, 4H, HOOC-CH$_2$-C$\underline{H}_2$-(CH$_2$)$_{12}$-C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.15 (br. m, 24H, HOOC-CH$_2$-CH$_2$-(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 176.2 (HOO$\underline{C}$-CH$_2$-), 173.8 (-CH$_2$-$\underline{C}$OO-), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.8 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-O-$\underline{C}$H$_3$), 34.1 (-$\underline{C}$H$_2$-COO-), 33.8 (HOOC-$\underline{C}$H$_2$-), 29.7 – 28.9 (HOOC-CH$_2$-CH$_2$-($\underline{C}$H$_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 24.7 (-$\underline{C}$H$_2$-CH$_2$-COO-), 24.6 (HOOC-CH$_2$-$\underline{C}$H$_2$-).

18-(Methoxy-poly[ethylene glycol]-oxy)-18-oxooctadecanoic acid (C_{18}mPEG$_{22}$). Yield: 73 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.80 – 3.45 (br. m, 106H, $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 2.30 – 2.20 (m, 4H, HOOC-C$\underline{H}_2$- and -C$\underline{H}_2$-COO-), 1.60 – 1.46 (m, 4H, HOOC-CH$_2$-C$\underline{H}_2$-(CH$_2$)$_{12}$-C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.15 (br. m, 24H, HOOC-CH$_2$-CH$_2$-(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 176.2 (HOO$\underline{C}$-CH$_2$-), 173.8 (-CH$_2$-$\underline{C}$OO-), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 71.2 – 69.6 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-O-$\underline{C}$H$_3$), 34.1 (-$\underline{C}$H$_2$-COO-), 33.8 (HOOC-$\underline{C}$H$_2$-), 29.7 – 28.9 (HOOC-CH$_2$-CH$_2$-($\underline{C}$H$_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 24.7 (-$\underline{C}$H$_2$-CH$_2$-COO-), 24.6 (HOOC-CH$_2$-$\underline{C}$H$_2$-).

General procedure for mono-esteryfication reaction of 1,18-octadecanedioic acid with methoxy-poly(ethylene glycol) (mPEG$_6$, mPEG$_{10}$, mPEG$_{14,}$) by melting reaction (C_{18}mPEG$_{n+1}$).
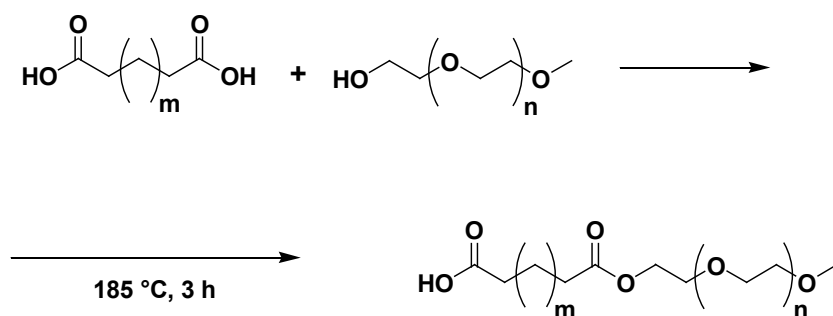
Methoxy-poly(ethylene glycol) (25.0 mmol, 1 eq.) and 1,18-octadecanedioic acid (31.4 g, 100.0 mmol, 4 eq.) were added without solvent into a two-neck round-bottom flask. The reaction mixture was warmed up to 120 °C and stirred for 30 minutes to obtain clear solution of methoxy-poly(ethylene glycol) and melted 1,18-octadecanedioic acid. Than the temperature was increased to 185 °C and the reaction mixture was stirred vigorously for 3 h under vacuum (5 × 10^{-2} mBar). Reaction control was performed *via* ^{1}H NMR spectroscopy (comparison of the peaks intensity at 4.18 ppm and 3.34 ppm to the ratio 2 : 3). After completion of the reaction , the mixture was allowed to cool down to 80 – 90 °C and 150 ml of warm toluene (60 – 70 °C) was added into the flask. Still stirring reaction mixture was slowly cooled down to 0 °C. The resulting suspension was filtrated and white residue was washed twice with cold (0 °C) toluene (2 × 75 ml). The filtrate and washings were combined and concentrated by rotary evaporation *in vacuo*. Crude product (a yellow wax or oil) was purified by flash dry column chromatography (acidified with AcOH silica gel, eluent: CHCl$_3$ to CHCl$_3$:MeOH 5:1) to give pure product, as a white wax or colorless oil (C_{18}mPEG$_{n+1}$).

Michał R. Radowski, Dissertation

18-(Methoxy-poly[ethylene glycol]-oxy)-18-oxooctadecanoic acid (C_{18}mPEG$_6$). Yield: 59 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.45 (br. m, 26H, $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 2.30 – 2.20 (m, 4H, HOOC-C$\underline{H}_2$- and -C$\underline{H}_2$-COO-), 1.60 – 1.46 (m, 4H, HOOC-CH$_2$-C$\underline{H}_2$-(CH$_2$)$_{12}$-C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.15 (br. m, 24H, HOOC-CH$_2$-CH$_2$-(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 176.2 (HOO$\underline{C}$-CH$_2$-), 173.8 (-CH$_2$-$\underline{C}$OO-), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-O-$\underline{C}$H$_3$), 34.1 (-$\underline{C}$H$_2$-COO-), 33.8 (HOOC-$\underline{C}$H$_2$-), 29.7 – 28.9 (HOOC-CH$_2$-CH$_2$-($\underline{C}$H$_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 24.7 (-$\underline{C}$H$_2$-CH$_2$-COO-), 24.6 (HOOC-CH$_2$-$\underline{C}$H$_2$-).

18-(Methoxy-poly[ethylene glycol]-oxy)-18-oxooctadecanoic acid (C_{18}mPEG$_{10}$). Yield: 54 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.45 (br. m, 42H, $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 2.30 – 2.20 (m, 4H, HOOC-C$\underline{H}_2$- and -C$\underline{H}_2$-COO-), 1.60 – 1.46 (m, 4H, HOOC-CH$_2$-C$\underline{H}_2$-(CH$_2$)$_{12}$-C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.15 (br. m, 24H, HOOC-CH$_2$-CH$_2$-(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 176.2 (HOO$\underline{C}$-CH$_2$-), 173.8 (-CH$_2$-$\underline{C}$OO-), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-O-$\underline{C}$H$_3$), 34.1 (-$\underline{C}$H$_2$-COO-), 33.8 (HOOC-$\underline{C}$H$_2$-), 29.7 – 28.9 (HOOC-CH$_2$-CH$_2$-($\underline{C}$H$_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 24.7 (-$\underline{C}$H$_2$-CH$_2$-COO-), 24.6 (HOOC-CH$_2$-$\underline{C}$H$_2$-).

18-(Methoxy-poly[ethylene glycol]-oxy)-18-oxooctadecanoic acid (C_{18}mPEG$_{14}$). Yield: 64 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, - COO-C$\underline{H}_2$-), 3.75 – 3.45 (br. m, 68H, $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 2.30 – 2.20 (m, 4H, HOOC-C$\underline{H}_2$- and -C$\underline{H}_2$-COO-), 1.60 – 1.46 (m, 4H, HOOC-CH$_2$-C$\underline{H}_2$-(CH$_2$)$_{12}$-C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.15 (br. m, 24H, HOOC-CH$_2$-CH$_2$-(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 176.2 (HOO$\underline{C}$-CH$_2$-), 173.8 (-CH$_2$-$\underline{C}$OO-), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.8 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-O-$\underline{C}$H$_3$), 34.1 (-$\underline{C}$H$_2$-COO-), 33.8 (HOOC-$\underline{C}$H$_2$-), 29.7 – 28.9 (HOOC-CH$_2$-CH$_2$-($\underline{C}$H$_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 24.7 (-$\underline{C}$H$_2$-CH$_2$-COO-), 24.6 (HOOC-CH$_2$-$\underline{C}$H$_2$-).

General procedure for activation reaction of mono(methoxy-poly[ethylene glycol]-oxy)-oxo-diotic acids (C_{m+4}mPEG$_{n+1}$) with *N*-hydroxysuccinamide (HONSu). Synthesis of 1-(2,5-dioxopyrrolidin-1-yl) (m+4)-methoxy-poly(ethylene glycol)yl -dioate [(C_{m+4}mPEG$_{n+1}$)-ONSu]
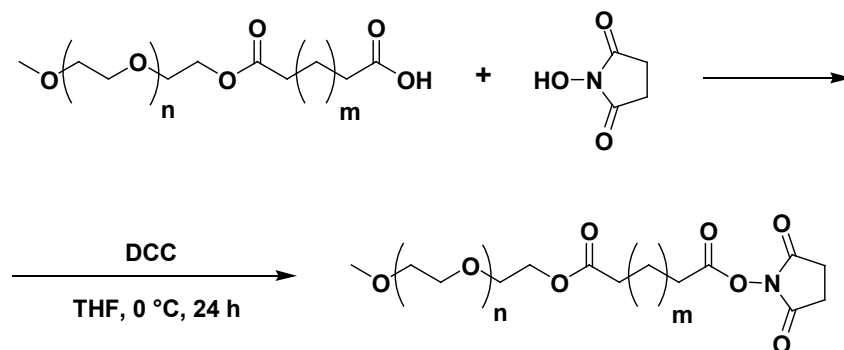
The reaction was performed in a two-neck round-bottom flask equipped with a dropping funnel. Mono(methoxy-poly[ethylene glycol]-oxy)-oxo-diotic acid (C_{m+4}mPEG$_{n+1}$) (5.0 mmol, 1 eq.) was dissolved in p.a. THF (0 °C, 150 ml) and solid *N*-Hydroxysuccinamide (5.25 mmol, 1.05 eq) was added to the solution. The reaction mixture was cooled down with an ice bath and the solution of dicyclohexyl-carbodiimide (DCC) (5.5 mmol, 1.1 eq) in THF (25 ml) was added dropwise (after approximately 30 minutes precipitation occurred). The mixture was stirred at 0 °C for 6 h and left for 18 h at 2 °C (refrigerator). Than the reaction mixture was filtrated (at 0 °C) to remove 1,3-dicyclohexyl-urea (DCU) as white crystals and the residue was washed with a small amount of cold THF (15 ml). The filtrate and the washing were combined and concentrated by rotary evaporation *in vacuo* to one-third of the initial volume. The obtained solution was left for 24 h at 2 °C (refrigerator) to precipitate the rest of the DCU. The mixture was filtrated again and the residue of 1,3-dicyclohexyl-urea was washed with 5 ml of cold THF (0 °C). The filtrate and the washing were combined and concentrated by rotary evaporation *in vacuo* to give crude product as a white wax with a purity of ~95 % which was used in the next step reaction without further purification.

1-(2,5-dioxopyrrolidin-1-yl) 6-methoxy-poly(ethylene glycol)yl hexanedioate [(C_6mPEG$_6$)-ONSu]. Yield: 92 %; purity: 93 %; ^{1}H NMR (500 MHz, CDCl$_3$, sample contains residue of 1,3-dicyclohexyl-urea at δ (ppm) = 3.20 – 3.13, 1.92 – 1.50): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.55 (br. m, 24H, P$\underline{EG}$), 3.51 (t, 2H, -C$\underline{H}_2$-O-CH$_3$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 2.86 – 2.76 (m, 4H, -N'-C(O)-C$\underline{H}_2$-C$\underline{H}_2$-C(O)-N'- from -ONSu), 2.67 – 2.59 (m, 2H, -N-O-C(O)-C$\underline{H}_2$-), 2.39 – 2.30 (m, 2H, -C$\underline{H}_2$-COO-), 1.92 – 1.60 (m, 4H, -N-O-C(O)-CH$_2$-(C$\underline{H}_2$)$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$, sample contains residue of 1,3-dicyclohexyl-urea at δ (ppm) = 34.8, 33.7, 30.3, 25.4, 23.5): δ (ppm) = 172.9 (-CH$_2$-$\underline{C}$OO-), 169.0 (-CH$_2$-$\underline{C}$(O)-N-), 168.2 (-N-O-$\underline{C}$(O)-CH$_2$-), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$),

Michał R. Radowski, Dissertation

70.6 – 70.0 (<u>PEG</u>), 69.2 (-COO-CH$_2$-<u>CH$_2$</u>-), 63.3 (-COO-<u>CH$_2$</u>-), 59.0 (-O-<u>CH$_3$</u>), 33.7 (-<u>CH$_2$</u>-COO-), 30.5 (-N-OC(O)-<u>CH$_2$</u>-), 25.5 (-N'-C(O)-<u>CH$_2$</u>-<u>CH$_2$</u>-C(O)-N'-), 24.2 (-N-O-C(O)-CH$_2$-<u>CH$_2$</u>-), 23.9 (-<u>CH$_2$</u>-CH$_2$-COO-).

1-(2,5-dioxopyrrolidin-1-yl) 12-methoxy-poly(ethylene glycol)yl dodecanedioate [(C$_{12}$mPEG$_6$)-ONSu]. Yield: 91 %; purity: 93 %; ^{1}H NMR (500 MHz, CDCl$_3$, sample contains residue of 1,3-dicyclohexyl-urea at δ (ppm) = 3.20 – 3.13, 1.92 – 1.50): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C<u>H$_2$</u>-), 3.70 – 3.45 (br. m, 26H, <u>PEG</u>), 3.34 (s, 3H, -O-C<u>H$_3$</u>), 2.86 – 2.76 (m, 4H, -N'-C(O)-C<u>H$_2$</u>-C<u>H$_2$</u>-C(O)-N'-), 2.60 – 2.51 (m, 2H, -N-O-C(O)-C<u>H$_2$</u>-), 2.31 – 2.24 (m, 2H, -C<u>H$_2$</u>-COO-), 1.85 – 1.50 (br. m, 4H, -N-O-C(O)-CH$_2$-C<u>H$_2$</u>-(CH$_2$)$_6$-C<u>H$_2$</u>-CH$_2$-COO-), 1.30 – 1.15 (br. m, 12H, , -N-O-C(O)-CH$_2$-CH$_2$-(C<u>H$_2$</u>)$_6$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$, sample contains residue of 1,3-dicyclohexyl-urea at δ (ppm) = 34.8, 33.7, 30.3, 25.4, 23.5): δ (ppm) = 173.7 (-CH$_2$-<u>C</u>OO-), 169.1 (-CH$_2$-<u>C</u>(O)-N-), 168.6 (-N-O-<u>C</u>(O)-CH$_2$-), 71.9 (-<u>CH$_2$</u>-O-CH$_3$), 70.6 – 70.0 (<u>PEG</u>), 69.2 (-COO-CH$_2$-<u>CH$_2$</u>-), 63.3 (-COO-<u>CH$_2$</u>-), 59.0 (-O-<u>CH$_3$</u>), 34.1 (-N-O-C(O)-<u>CH$_2$</u>-), 30.9 (-<u>CH$_2$</u>-COO-), 29.2 – 28.9 (-N-O-C(O)-CH$_2$-CH$_2$-(<u>CH$_2$</u>)$_6$-CH$_2$-CH$_2$-COO-), 25.5, 25.4 (-N'-C(O)-<u>CH$_2$</u>-<u>CH$_2$</u>-C(O)-N'-), 24.8 (-<u>CH$_2$</u>-CH$_2$-COO-), 24.6 (-NO-C(O)-CH$_2$-<u>CH$_2$</u>-).

1-(2,5-dioxopyrrolidin-1-yl) 18-methoxy-poly(ethylene glycol)yl octadecanedioate [(C$_{18}$mPEG$_6$)-ONSu]. Yield: 96 %; purity: 94 %; ^{1}H NMR (500 MHz, CDCl$_3$, sample contains residue of 1,3-dicyclohexyl-urea at δ (ppm) = 3.20 – 3.13, 1.92 – 1.50): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C<u>H$_2$</u>-), 3.70 – 3.45 (br. m, 26H, <u>PEG</u>), 3.34 (s, 3H, -O-C<u>H$_3$</u>), 2.86 – 2.76 (m, 4H, -N'-C(O)-C<u>H$_2$</u>-C<u>H$_2$</u>-C(O)-N'-), 2.60 – 2.51 (m, 2H, -N-O-C(O)-C<u>H$_2$</u>-), 2.31 – 2.24 (m, 2H, -C<u>H$_2$</u>-COO-), 1.85 – 1.50 (br. m, 4H, -N-O-C(O)-CH$_2$-C<u>H$_2$</u>-(CH$_2$)$_{12}$-C<u>H$_2$</u>-CH$_2$-COO-), 1.30 – 1.15 (br. m, 24H, , -N-O-C(O)-CH$_2$-CH$_2$-(C<u>H$_2$</u>)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$, sample contains residue of 1,3-dicyclohexyl-urea at δ (ppm) = 34.8, 33.7, 30.3, 25.4, 23.5): δ (ppm) = 173.7 (-CH$_2$-<u>C</u>OO-), 169.1 (-CH$_2$-<u>C</u>(O)-N-), 168.6 (-N-O-<u>C</u>(O)-CH$_2$-), 71.9 (-<u>CH$_2$</u>-O-CH$_3$), 70.6 – 70.0 (<u>PEG</u>), 69.2 (-COO-CH$_2$-<u>CH$_2$</u>-), 63.3 (-COO-<u>CH$_2$</u>-), 59.0 (-O-<u>CH$_3$</u>), 34.1 (-N-O-C(O)-<u>CH$_2$</u>-), 30.9 (-<u>CH$_2$</u>-COO-), 29.2 – 28.9 (-N-O-C(O)-CH$_2$-CH$_2$-(<u>CH$_2$</u>)$_{12}$-CH$_2$-CH$_2$-COO-), 25.5, 25.4 (-N'-C(O)-<u>CH$_2$</u>-<u>CH$_2$</u>-C(O)-N'-), 24.8 (-<u>CH$_2$</u>-CH$_2$-COO-), 24.6 (-NO-C(O)-CH$_2$-<u>CH$_2$</u>-).

1-(2,5-dioxopyrrolidin-1-yl) 18-methoxy-poly(ethylene glycol)yl octadecanedioate [(C$_{18}$mPEG$_{10}$)-ONSu]. Yield: 96 %; purity: 95 %; ^{1}H NMR (500 MHz, CDCl$_3$, sample contains residue of 1,3-dicyclohexyl-urea at δ (ppm) = 3.20 – 3.13, 1.92 – 1.50): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C<u>H$_2$</u>-), 3.70 – 3.45 (br. m, 42H, <u>PEG</u>), 3.34 (s, 3H, -O-C<u>H$_3$</u>), 2.86 – 2.76 (m, 4H, -N'-C(O)-C<u>H$_2$</u>-C<u>H$_2$</u>-C(O)-N'-), 2.60 – 2.51 (m, 2H, -N-O-C(O)-C<u>H$_2$</u>-), 2.31 – 2.24 (m, 2H, -C<u>H$_2$</u>-COO-), 1.85 – 1.50 (br. m, 4H, -N-O-C(O)-CH$_2$-C<u>H$_2$</u>-(CH$_2$)$_{12}$-C<u>H$_2$</u>-CH$_2$-COO-), 1.30 – 1.15 (br. m, 24H, , -N-O-C(O)-CH$_2$-CH$_2$-(C<u>H$_2$</u>)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$, sample contains residue of 1,3-dicyclohexyl-urea at δ (ppm) = 34.8, 33.7, 30.3, 25.4,

23.5): δ (ppm) = 173.7 (-CH$_2$-$\underline{C}$OO-), 169.1 (-CH$_2$-$\underline{C}$(O)-N-), 168.6 (-N-O-$\underline{C}$(O)-CH$_2$-), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-O-$\underline{C}$H$_3$), 34.1 (-N-O-C(O)-$\underline{C}$H$_2$-), 30.9 (-$\underline{C}$H$_2$-COO-), 29.2 – 28.9 (-N-O-C(O)-CH$_2$-CH$_2$-($\underline{C}$H$_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 25.5, 25.4 (-N'-C(O)-$\underline{C}$H$_2$-$\underline{C}$H$_2$-C(O)-N'-), 24.8 (-$\underline{C}$H$_2$-CH$_2$-COO-), 24.6 (-NO-C(O)-CH$_2$-$\underline{C}$H$_2$-).

1-(2,5-dioxopyrrolidin-1-yl) 18-methoxy-poly(ethylene glycol)yl octadecanedioate [(C$_{18}$mPEG$_{14}$)-ONSu]. Yield: 96 %; purity: 94 ; ^{1}H NMR (500 MHz, CDCl$_3$, sample contains residue of 1,3-dicyclohexyl-urea at δ (ppm) = 3.20 – 3.13, 1.92 – 1.50): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.45 (br. m, 68H, $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 2.86 – 2.76 (m, 4H, -N'-C(O)-C$\underline{H}_2$-C$\underline{H}_2$-C(O)-N'-), 2.60 – 2.51 (m, 2H, -N-O-C(O)-C$\underline{H}_2$-), 2.31 – 2.24 (m, 2H, -C$\underline{H}_2$-COO-), 1.85 – 1.50 (br. m, 4H, -N-O-C(O)-CH$_2$-C$\underline{H}_2$-(CH$_2$)$_{12}$-C$\underline{H}_2$-CH$_2$-COO-), 1.30 – 1.15 (br. m, 24H, , -N-O-C(O)-CH$_2$-CH$_2$-(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$, sample contains residue of 1,3-dicyclohexyl-urea at δ (ppm) = 34.8, 33.7, 30.3, 25.4, 23.5): δ (ppm) = 173.7 (-CH$_2$-$\underline{C}$OO-), 169.1 (-CH$_2$-$\underline{C}$(O)-N-), 168.6 (-N-O-$\underline{C}$(O)-CH$_2$-), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-O-$\underline{C}$H$_3$), 34.1 (-N-O-C(O)-$\underline{C}$H$_2$-), 30.9 (-$\underline{C}$H$_2$-COO-), 29.2 – 28.9 (-N-O-C(O)-CH$_2$-CH$_2$-($\underline{C}$H$_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 25.5, 25.4 (-N'-C(O)-$\underline{C}$H$_2$-$\underline{C}$H$_2$-C(O)-N'-), 24.8 (-$\underline{C}$H$_2$-CH$_2$-COO-), 24.6 (-NO-C(O)-CH$_2$-$\underline{C}$H$_2$-).

1-(2,5-dioxopyrrolidin-1-yl) 18-methoxy-poly(ethylene glycol)yl octadecanedioate [(C$_{18}$mPEG$_{22}$)-ONSu]. Yield: 96 %; purity: 94 %; ^{1}H NMR (500 MHz, CDCl$_3$, sample contains residue of 1,3-dicyclohexyl-urea at δ (ppm) = 3.20 – 3.13, 1.92 – 1.50): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.45 (br. m, 112H, $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 2.86 – 2.76 (m, 4H, -N'-C(O)-C$\underline{H}_2$-C$\underline{H}_2$-C(O)-N'-), 2.60 – 2.51 (m, 2H, -N-O-C(O)-C$\underline{H}_2$-), 2.31 – 2.24 (m, 2H, -C$\underline{H}_2$-COO-), 1.85 – 1.50 (br. m, 4H, -N-O-C(O)-CH$_2$-C$\underline{H}_2$-(CH$_2$)$_{12}$-C$\underline{H}_2$-CH$_2$-COO-), 1.30 – 1.15 (br. m, 24H, , -N-O-C(O)-CH$_2$-CH$_2$-(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$, sample contains residue of 1,3-dicyclohexyl-urea at δ (ppm) = 34.8, 33.7, 30.3, 25.4, 23.5): δ (ppm) = 173.7 (-CH$_2$-$\underline{C}$OO-), 169.1 (-CH$_2$-$\underline{C}$(O)-N-), 168.6 (-N-O-$\underline{C}$(O)-CH$_2$-), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-O-$\underline{C}$H$_3$), 34.1 (-N-O-C(O)-$\underline{C}$H$_2$-), 30.9 (-$\underline{C}$H$_2$-COO-), 29.2 – 28.9 (-N-O-C(O)-CH$_2$-CH$_2$-($\underline{C}$H$_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 25.5, 25.4 (-N'-C(O)-$\underline{C}$H$_2$-$\underline{C}$H$_2$-C(O)-N'-), 24.8 (-$\underline{C}$H$_2$-CH$_2$-COO-), 24.6 (-NO-C(O)-CH$_2$-$\underline{C}$H$_2$-).

General procedure for synthesis of core-multishell architectures with a PEI core. Amide formation by activation with HONSu.
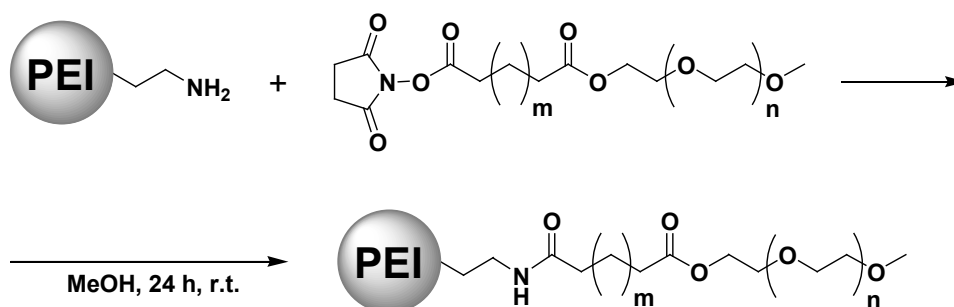
The Reaction was performed in an one-neck round-bottom flask equipped with a dropping funnel. Poly(ethylene imine) [PEI_{3600}: M_n = 3600 g mol^{-1}, 0.144 g, 0.04 mmol, 1.24 (31 × 0.04) mmol -NH$_2$ (T) groups; or PEI_{10500}: M_n = 10500 g mol^{-1}, 0.210 g, 0.02 mmol, 1.52 (76 × 0.02) mmol -NH$_2$ (T) groups] was dissolved in p.a. MeOH (100 ml) and a solution of ($C_{m+4}mPEG_{n+1}$)-ONSu (for PEI_{3600}: 31 × DF × 0.042 mmol, 1.05 eq. per -NH$_2$ group, or for PEI_{10500}: 76 × DF × 0.021 mmol, 1.05 eq. per -NH$_2$ group) in MeOH (25 ml) was added dropwise. The reaction mixture was stirred for 24 h at r.t. and than concentrated by rotary evaporation *in vacuo*. The obtained crude product was dissolved and dialyzed twice in MeOH. After drying under high vacuum a white or light-yellow solid was obtained.

$PEI_{3600}(C_6mPEG_6)_{0.9}$. Conversion (DF): 88 % (90 %); yield: 81 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.50 (br. m, 26H, $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 3.50 – 3.05 (br. m, -C$\underline{H}_2$-N$\underline{H}$CO- from functionalized PEI backbone), 3.00 – 2.38 (br. m, $\underline{PEI}$ backbone), 2.38 – 2.25 (m, 2H, -C$\underline{H}_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}_2$-), 1.68 – 1.52 (m, 4H, -(C$\underline{H}_2$)$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 174.1 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-O-$\underline{C}$H$_3$), 55.0 – 40.0 ($\underline{PEI}$ backbone), 36.5 (-$\underline{C}$H$_2$-NHCO- from functionalized PEI backbone), 34.6 (-NHCO-$\underline{C}$H$_2$-), 34.2 (-$\underline{C}$H$_2$-COO-), 24.1 (-NHCO-CH$_2$-$\underline{C}$H$_2$-), 23.6 (-$\underline{C}$H$_2$-CH$_2$-COO-).

$PEI_{10500}(C_6mPEG_6)_{0.9}$. Conversion (DF): 96 % (90 %); yield: 74 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.50 (br. m, 26H, $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 3.50 – 3.05 (br. m, -C$\underline{H}_2$-N$\underline{H}$CO- from functionalized PEI backbone), 3.00 – 2.38 (br. m, $\underline{PEI}$ backbone), 2.38 – 2.25 (m, 2H, -C$\underline{H}_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}_2$-), 1.68 – 1.52 (m, 4H, -(C$\underline{H}_2$)$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 174.1 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-O-$\underline{C}$H$_3$), 55.0 – 40.0 ($\underline{PEI}$ backbone),

Michał R. Radowski, Dissertation

36.8 – 36.5 (-$\underline{C}H_2$-NHCO- from functionalized PEI backbone), 34.6 (-NHCO-$\underline{C}H_2$-), 34.2 (-$\underline{C}H_2$-COO-), 24.1 (-NHCO-CH$_2$-$\underline{C}H_2$-), 23.6 (-$\underline{C}H_2$-CH$_2$-COO-).

PEI$_{3600}$(C$_{12}$mPEG$_6$)$_{0.9}$. Conversion (DF): 87 % (90 %); yield: 65 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.50 (br. m, 26H, $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 3.50 – 3.05 (br. m, -C$\underline{H}_2$-NHCO- from functionalized PEI backbone), 3.00 – 2.38 (br. m, $\underline{PEI}$ backbone), 2.36 – 2.26 (m, 2H, -C$\underline{H}_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}_2$-), 1.66 – 1.52 (m, 4H, -NHCO-CH$_2$-C$\underline{H}_2$-(CH$_2$)$_6$-C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.20 (m, 12H, -(C$\underline{H}_2$)$_6$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 174.1 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 71.9 (-$\underline{C}H_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}H_2$-), 63.3 (-COO-$\underline{C}H_2$-), 59.0 (-O-$\underline{C}H_3$), 55.0 – 40.0 ($\underline{PEI}$ backbone), 36.8 – 36.5 (-$\underline{C}H_2$-NHCO- from functionalized PEI backbone), 34.9 (-NHCO-$\underline{C}H_2$-), 34.2 (-$\underline{C}H_2$-COO-), 29.7 – 29.1 (-(C$\underline{H}_2$)$_6$-CH$_2$-CH$_2$-COO-), 25.1 (-NHCO-CH$_2$-$\underline{C}H_2$-), 24.9 (-$\underline{C}H_2$-CH$_2$-COO-).

PEI$_{10500}$(C$_{12}$mPEG$_6$)$_{0.9}$. Conversion (DF): 92 % (90 %); yield: 72 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.50 (br. m, 26H, $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 3.50 – 3.05 (br. m, -C$\underline{H}_2$-NHCO- from functionalized PEI backbone), 3.00 – 2.38 (br. m, $\underline{PEI}$ backbone), 2.36 – 2.26 (m, 2H, -C$\underline{H}_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}_2$-), 1.66 – 1.52 (m, 4H, -NHCO-CH$_2$-C$\underline{H}_2$-(CH$_2$)$_6$-C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.20 (m, 12H, -(C$\underline{H}_2$)$_6$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 174.1 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 71.9 (-$\underline{C}H_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}H_2$-), 63.3 (-COO-$\underline{C}H_2$-), 59.0 (-O-$\underline{C}H_3$), 55.0 – 40.0 ($\underline{PEI}$ backbone), 36.8 – 36.5 (-$\underline{C}H_2$-NHCO- from functionalized PEI backbone), 34.9 (-NHCO-$\underline{C}H_2$-), 34.2 (-$\underline{C}H_2$-COO-), 29.7 – 29.1 (-(C$\underline{H}_2$)$_6$-CH$_2$-CH$_2$-COO-), 25.1 (-NHCO-CH$_2$-$\underline{C}H_2$-), 24.9 (-$\underline{C}H_2$-CH$_2$-COO-).

PEI$_{3600}$(C$_{18}$mPEG$_6$)$_{0.25}$. Conversion: unknown; yield: 74 %; *Comment: sample after drying under vacuum was not soluble in any solvent. Therefore no NMR spectrum was recorded.*

PEI$_{3600}$(C$_{18}$mPEG$_6$)$_{0.3}$. Conversion: unknown; yield: 75 %; *Comment: sample after drying under vacuum was not soluble in any solvent, repetition of the reaction results in similar situation. Therefore no NMR spectrum was recorded.*

PEI$_{3600}$(C$_{18}$mPEG$_6$)$_{0.5}$. Conversion: unknown; yield: 78 %; *Comment: sample after drying under vacuum was not soluble in any solvent. Therefore no NMR spectrum was recorded.*

PEI$_{3600}$(C$_{18}$mPEG$_6$)$_{0.7}$. Conversion (DF): 71 % (70 %); yield: 79 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.50 (br. m, 26H, $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 3.50 – 3.05 (br. m, -C$\underline{H}_2$-NHCO- from functionalized PEI backbone), 3.00 – 2.38 (br. m, $\underline{PEI}$ backbone), 2.36 – 2.26 (m, 2H, -C$\underline{H}_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}_2$-), 1.66 – 1.52 (m, 4H, -NHCO-CH$_2$-C$\underline{H}_2$-(CH$_2$)$_{12}$-C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.20 (m, 24H, -(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 174.1 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 71.9 (-$\underline{C}H_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}H_2$-), 63.3 (-COO-$\underline{C}H_2$-), 59.0 (-O-$\underline{C}H_3$), 55.0 – 40.0 ($\underline{PEI}$ backbone), 36.8 – 36.5 (-$\underline{C}H_2$-NHCO- from

Michał R. Radowski, Dissertation

functionalized PEI backbone), 34.9 (-NHCO-$\underline{C}H_2$-), 34.2 (-$\underline{C}H_2$-COO-), 29.7 – 29.1 (-($\underline{C}H_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 25.1 (-NHCO-CH$_2$-$\underline{C}H_2$-), 24.9 (-$\underline{C}H_2$-CH$_2$-COO-).

PEI$_{3600}$(C$_{18}$mPEG$_6$)$_{0.9}$. Conversion (DF): 87 % (90 %); yield: 72 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.50 (br. m, 26H, $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 3.50 – 3.05 (br. m, -C$\underline{H}_2$-N$\underline{H}$CO- from functionalized PEI backbone), 3.00 – 2.38 (br. m, $\underline{PEI}$ backbone), 2.36 – 2.26 (m, 2H, -C$\underline{H}_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}_2$-), 1.66 – 1.52 (m, 4H, -NHCO-CH$_2$-C$\underline{H}_2$-(CH$_2$)$_{12}$-C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.20 (m, 24H, -(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 174.1 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 71.9 (-$\underline{C}H_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}H_2$-), 63.3 (-COO-$\underline{C}H_2$-), 59.0 (-O-$\underline{C}H_3$), 55.0 – 40.0 ($\underline{PEI}$ backbone), 36.8 – 36.5 (-$\underline{C}H_2$-NHCO- from functionalized PEI backbone), 34.9 (-NHCO-$\underline{C}H_2$-), 34.2 (-$\underline{C}H_2$-COO-), 29.7 – 29.1 (-($\underline{C}H_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 25.1 (-NHCO-CH$_2$-$\underline{C}H_2$-), 24.9 (-$\underline{C}H_2$-CH$_2$-COO-).

PEI$_{3600}$(C$_{18}$mPEG$_6$)$_{1.0}$. Conversion (DF): 94 % (100 %); yield: 62 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.50 (br. m, 26H, $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 3.50 – 3.05 (br. m, -C$\underline{H}_2$-N$\underline{H}$CO- from functionalized PEI backbone), 3.00 – 2.38 (br. m, $\underline{PEI}$ backbone), 2.36 – 2.26 (m, 2H, -C$\underline{H}_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}_2$-), 1.66 – 1.52 (m, 4H, -NHCO-CH$_2$-C$\underline{H}_2$-(CH$_2$)$_{12}$-C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.20 (m, 24H, -(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 174.1 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 71.9 (-$\underline{C}H_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}H_2$-), 63.3 (-COO-$\underline{C}H_2$-), 59.0 (-O-$\underline{C}H_3$), 55.0 – 40.0 ($\underline{PEI}$ backbone), 36.8 – 36.5 (-$\underline{C}H_2$-NHCO- from functionalized PEI backbone), 34.9 (-NHCO-$\underline{C}H_2$-), 34.2 (-$\underline{C}H_2$-COO-), 29.7 – 29.1 (-($\underline{C}H_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 25.1 (-NHCO-CH$_2$-$\underline{C}H_2$-), 24.9 (-$\underline{C}H_2$-CH$_2$-COO-).

PEI$_{3600}$(C$_{18}$mPEG$_{10}$)$_{0.3}$. Conversion: unknown; yield: 77 %; *Comment: sample after drying under vacuum was not soluble in any solvent. Therefore no NMR spectrum was recorded.*

PEI$_{3600}$(C$_{18}$mPEG$_{10}$)$_{0.5}$. Conversion (DF): 45 % (50 %); yield: 92 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.50 (br. m, 42H, $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 3.50 – 3.05 (br. m, -C$\underline{H}_2$-N$\underline{H}$CO- from functionalized PEI backbone), 3.00 – 2.38 (br. m, $\underline{PEI}$ backbone), 2.36 – 2.26 (m, 2H, -C$\underline{H}_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}_2$-), 1.66 – 1.52 (m, 4H, -NHCO-CH$_2$-C$\underline{H}_2$-(CH$_2$)$_{12}$-C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.20 (m, 24H, -(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 174.1 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 71.9 (-$\underline{C}H_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}H_2$-), 63.3 (-COO-$\underline{C}H_2$-), 59.0 (-O-$\underline{C}H_3$), 55.0 – 40.0 ($\underline{PEI}$ backbone), 36.8 – 36.5 (-$\underline{C}H_2$-NHCO- from functionalized PEI backbone), 34.9 (-NHCO-$\underline{C}H_2$-), 34.2 (-$\underline{C}H_2$-COO-), 29.7 – 29.1 (-($\underline{C}H_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 25.1 (-NHCO-CH$_2$-$\underline{C}H_2$-), 24.9 (-$\underline{C}H_2$-CH$_2$-COO-).

PEI$_{3600}$(C$_{18}$mPEG$_{10}$)$_{0.7}$. Conversion (DF): 64 % (70 %); yield: 69 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.50 (br. m, 42H, $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 3.50 – 3.05 (br. m, -C$\underline{H}_2$-N$\underline{H}$CO- from functionalized PEI backbone), 3.00 – 2.38

(br. m, $\underline{P}$EI backbone), 2.36 – 2.26 (m, 2H, -C$\underline{H}_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}_2$-), 1.66 – 1.52 (m, 4H, -NHCO-CH$_2$-C$\underline{H}_2$-(CH$_2$)$_{12}$-C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.20 (m, 24H, -(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 174.1 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-O-$\underline{C}$H$_3$), 55.0 – 40.0 ($\underline{PEI}$ backbone), 36.8 – 36.5 (-$\underline{C}$H$_2$-NHCO- from functionalized PEI backbone), 34.9 (-NHCO-$\underline{C}$H$_2$-), 34.2 (-$\underline{C}$H$_2$-COO-), 29.7 – 29.1 (-($\underline{C}$H$_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 25.1 (-NHCO-CH$_2$-$\underline{C}$H$_2$-), 24.9 (-$\underline{C}$H$_2$-CH$_2$-COO-).

PEI$_{3600}$(C$_{18}$mPEG$_{14}$)$_{0.3}$. Conversion: unknown; yield: 78 %; *Comment: sample after drying under vacuum was not soluble in any solvent. Therefore no NMR spectrum was recorded.*

PEI$_{3600}$(C$_{18}$mPEG$_{14}$)$_{0.5}$. Conversion (DF): 43 % (50 %); yield: 75 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.50 (br. m, 68H, $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 3.50 – 3.05 (br. m, -C$\underline{H}_2$-NHCO- from functionalized PEI backbone), 3.00 – 2.38 (br. m, $\underline{P}$EI backbone), 2.36 – 2.26 (m, 2H, -C$\underline{H}_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}_2$-), 1.66 – 1.52 (m, 4H, -NHCO-CH$_2$-C$\underline{H}_2$-(CH$_2$)$_{12}$-C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.20 (m, 24H, -(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 174.1 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-O-$\underline{C}$H$_3$), 55.0 – 40.0 ($\underline{PEI}$ backbone), 36.8 – 36.5 (-$\underline{C}$H$_2$-NHCO- from functionalized PEI backbone), 34.9 (-NHCO-$\underline{C}$H$_2$-), 34.2 (-$\underline{C}$H$_2$-COO-), 29.7 – 29.1 (-($\underline{C}$H$_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 25.1 (-NHCO-CH$_2$-$\underline{C}$H$_2$-), 24.9 (-$\underline{C}$H$_2$-CH$_2$-COO-).

PEI$_{3600}$(C$_{18}$mPEG$_{14}$)$_{0.7}$. Conversion (DF): 66 % (70 %); yield: 88 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.50 (br. m, 68H, $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 3.50 – 3.05 (br. m, -C$\underline{H}_2$-N$\underline{H}$CO- from functionalized PEI backbone), 3.00 – 2.38 (br. m, $\underline{P}$EI backbone), 2.36 – 2.26 (m, 2H, -C$\underline{H}_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}_2$-), 1.66 – 1.52 (m, 4H, -NHCO-CH$_2$-C$\underline{H}_2$-(CH$_2$)$_{12}$-C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.20 (m, 24H, -(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 174.1 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-O-$\underline{C}$H$_3$), 55.0 – 40.0 ($\underline{PEI}$ backbone), 36.8 – 36.5 (-$\underline{C}$H$_2$-NHCO- from functionalized PEI backbone), 34.9 (-NHCO-$\underline{C}$H$_2$-), 34.2 (-$\underline{C}$H$_2$-COO-), 29.7 – 29.1 (-($\underline{C}$H$_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 25.1 (-NHCO-CH$_2$-$\underline{C}$H$_2$-), 24.9 (-$\underline{C}$H$_2$-CH$_2$-COO-).

PEI$_{3600}$(C$_{18}$mPEG$_{14}$)$_{1.0}$. Conversion (DF): 94 % (100 %); yield: 78 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.50 (br. m, 68H, $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 3.50 – 3.05 (br. m, -C$\underline{H}_2$-N$\underline{H}$CO- from functionalized PEI backbone), 3.00 – 2.38 (br. m, $\underline{P}$EI backbone), 2.36 – 2.26 (m, 2H, -C$\underline{H}_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}_2$-), 1.66 – 1.52 (m, 4H, -NHCO-CH$_2$-C$\underline{H}_2$-(CH$_2$)$_{12}$-C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.20 (m, 24H, -(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 174.1 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-O-$\underline{C}$H$_3$), 55.0 – 40.0 ($\underline{PEI}$ backbone), 36.8 – 36.5 (-$\underline{C}$H$_2$-NHCO- from

Michał R. Radowski, Dissertation

functionalized PEI backbone), 34.9 (-NHCO-$\underline{C}H_2$-), 34.2 (-$\underline{C}H_2$-COO-), 29.7 – 29.1 (-($\underline{C}H_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 25.1 (-NHCO-CH$_2$-$\underline{C}H_2$-), 24.9 (-$\underline{C}H_2$-CH$_2$-COO-).

PEI$_{10500}$(C$_{18}$mPEG$_6$)$_{0.25}$. Conversion: unknown; yield: 65 %; *Comment: sample after drying under vacuum was not soluble in any solvent. Therefore no NMR spectrum was recorded.*

PEI$_{10500}$(C$_{18}$mPEG$_6$)$_{0.5}$. Conversion: unknown; yield: 86 %; *Comment: sample after drying under vacuum was not soluble in any solvent. Therefore no NMR spectrum was recorded.*

PEI$_{10500}$(C$_{18}$mPEG$_6$)$_{0.7}$. Conversion (DF): 73 % (70 %); yield: 81 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.50 (br. m, 26H, $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 3.50 – 3.05 (br. m, -C$\underline{H}_2$-N$\underline{H}$CO- from functionalized PEI backbone), 3.00 – 2.38 (br. m, $\underline{PEI}$ backbone), 2.36 – 2.26 (m, 2H, -C$\underline{H}_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}_2$-), 1.66 – 1.52 (m, 4H, -NHCO-CH$_2$-C$\underline{H}_2$-(CH$_2$)$_{12}$-C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.20 (m, 24H, -(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 174.1 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 71.9 (-$\underline{C}H_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}H_2$-), 63.3 (-COO-$\underline{C}H_2$-), 59.0 (-O-$\underline{C}H_3$), 55.0 – 40.0 ($\underline{PEI}$ backbone), 36.8 – 36.5 (-$\underline{C}H_2$-NHCO- from functionalized PEI backbone), 34.9 (-NHCO-$\underline{C}H_2$-), 34.2 (-$\underline{C}H_2$-COO-), 29.7 – 29.1 (-($\underline{C}H_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 25.1 (-NHCO-CH$_2$-$\underline{C}H_2$-), 24.9 (-$\underline{C}H_2$-CH$_2$-COO-).

PEI$_{10500}$(C$_{18}$mPEG$_6$)$_{0.9}$. Conversion (DF): 82 % (90 %); yield: 78 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.50 (br. m, 26H, $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 3.50 – 3.05 (br. m, -C$\underline{H}_2$-N$\underline{H}$CO- from functionalized PEI backbone), 3.00 – 2.38 (br. m, $\underline{PEI}$ backbone), 2.36 – 2.26 (m, 2H, -C$\underline{H}_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}_2$-), 1.66 – 1.52 (m, 4H, -NHCO-CH$_2$-C$\underline{H}_2$-(CH$_2$)$_{12}$-C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.20 (m, 24H, -(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 174.1 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 71.9 (-$\underline{C}H_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}H_2$-), 63.3 (-COO-$\underline{C}H_2$-), 59.0 (-O-$\underline{C}H_3$), 55.0 – 40.0 ($\underline{PEI}$ backbone), 36.8 – 36.5 (-$\underline{C}H_2$-NHCO- from functionalized PEI backbone), 34.9 (-NHCO-$\underline{C}H_2$-), 34.2 (-$\underline{C}H_2$-COO-), 29.7 – 29.1 (-($\underline{C}H_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 25.1 (-NHCO-CH$_2$-$\underline{C}H_2$-), 24.9 (-$\underline{C}H_2$-CH$_2$-COO-).

PEI$_{10500}$(C$_{18}$mPEG$_6$)$_{1.0}$. Conversion (DF): 99 % (100 %); yield: 74 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.50 (br. m, 26H, $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 3.50 – 3.05 (br. m, -C$\underline{H}_2$-N$\underline{H}$CO- from functionalized PEI backbone), 3.00 – 2.38 (br. m, $\underline{PEI}$ backbone), 2.36 – 2.26 (m, 2H, -C$\underline{H}_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}_2$-), 1.66 – 1.52 (m, 4H, -NHCO-CH$_2$-C$\underline{H}_2$-(CH$_2$)$_{12}$-C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.20 (m, 24H, -(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 174.1 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 71.9 (-$\underline{C}H_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}H_2$-), 63.3 (-COO-$\underline{C}H_2$-), 59.0 (-O-$\underline{C}H_3$), 55.0 – 40.0 ($\underline{PEI}$ backbone), 36.8 – 36.5 (-$\underline{C}H_2$-NHCO- from functionalized PEI backbone), 34.9 (-NHCO-$\underline{C}H_2$-), 34.2 (-$\underline{C}H_2$-COO-), 29.7 – 29.1 (-($\underline{C}H_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 25.1 (-NHCO-CH$_2$-$\underline{C}H_2$-), 24.9 (-$\underline{C}H_2$-CH$_2$-COO-).

PEI$_{10500}$(C$_{18}$mPEG$_{10}$)$_{0.5}$. Conversion (DF): 49 % (50 %); yield: 71 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.50 (br. m, 42H, $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 3.50 – 3.05 (br. m, -C$\underline{H}_2$-NHCO- from functionalized PEI backbone), 3.00 – 2.38 (br. m, $\underline{PEI}$ backbone), 2.36 – 2.26 (m, 2H, -C$\underline{H}_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}_2$-), 1.66 – 1.52 (m, 4H, -NHCO-CH$_2$-C$\underline{H}_2$-(CH$_2$)$_{12}$-C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.20 (m, 24H, -(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 174.1 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-O-$\underline{C}$H$_3$), 55.0 – 40.0 ($\underline{PEI}$ backbone), 36.8 – 36.5 (-$\underline{C}$H$_2$-NHCO- from functionalized PEI backbone), 34.9 (-NHCO-$\underline{C}$H$_2$-), 34.2 (-$\underline{C}$H$_2$-COO-), 29.7 – 29.1 (-(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 25.1 (-NHCO-CH$_2$-$\underline{C}$H$_2$-), 24.9 (-$\underline{C}$H$_2$-CH$_2$-COO-).

PEI$_{10500}$(C$_{18}$mPEG$_{10}$)$_{1.0}$. Conversion (DF): >100 % (100 %); yield: 86 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.50 (br. m, 42H, $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 3.50 – 3.05 (br. m, -C$\underline{H}_2$-NHCO- from functionalized PEI backbone), 3.00 – 2.38 (br. m, $\underline{PEI}$ backbone), 2.36 – 2.26 (m, 2H, -C$\underline{H}_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}_2$-), 1.66 – 1.52 (m, 4H, -NHCO-CH$_2$-C$\underline{H}_2$-(CH$_2$)$_{12}$-C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.20 (m, 24H, -(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 174.1 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-O-$\underline{C}$H$_3$), 55.0 – 40.0 ($\underline{PEI}$ backbone), 36.8 – 36.5 (-$\underline{C}$H$_2$-NHCO- from functionalized PEI backbone), 34.9 (-NHCO-$\underline{C}$H$_2$-), 34.2 (-$\underline{C}$H$_2$-COO-), 29.7 – 29.1 (-(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 25.1 (-NHCO-CH$_2$-$\underline{C}$H$_2$-), 24.9 (-$\underline{C}$H$_2$-CH$_2$-COO-).

PEI$_{10500}$(C$_{18}$mPEG$_{14}$)$_{1.0}$. Conversion (DF): 101 % (100 %); yield: 67 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.50 (br. m, 68H, $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 3.50 – 3.05 (br. m, -C$\underline{H}_2$-N$\underline{H}$CO- from functionalized PEI backbone), 3.00 – 2.38 (br. m, $\underline{PEI}$ backbone), 2.36 – 2.26 (m, 2H, -C$\underline{H}_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}_2$-), 1.66 – 1.52 (m, 4H, -NHCO-CH$_2$-C$\underline{H}_2$-(CH$_2$)$_{12}$-C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.20 (m, 24H, -(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 174.1 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-O-$\underline{C}$H$_3$), 55.0 – 40.0 ($\underline{PEI}$ backbone), 36.8 – 36.5 (-$\underline{C}$H$_2$-NHCO- from functionalized PEI backbone), 34.9 (-NHCO-$\underline{C}$H$_2$-), 34.2 (-$\underline{C}$H$_2$-COO-), 29.7 – 29.1 (-(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 25.1 (-NHCO-CH$_2$-$\underline{C}$H$_2$-), 24.9 (-$\underline{C}$H$_2$-CH$_2$-COO-).

PEI$_{10500}$(C$_{18}$mPEG$_{22}$)$_{1.0}$. Conversion (DF): 103 % (100 %); yield: 74 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.50 (br. m, 116H, $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 3.50 – 3.05 (br. m, -C$\underline{H}_2$-N$\underline{H}$CO- from functionalized PEI backbone), 3.00 – 2.38 (br. m, $\underline{PEI}$ backbone), 2.36 – 2.26 (m, 2H, -C$\underline{H}_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}_2$-), 1.66 – 1.52 (m, 4H, -NHCO-CH$_2$-C$\underline{H}_2$-(CH$_2$)$_{12}$-C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.20 (m, 24H, -(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 174.1 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3

(-COO-C$\underline{H}_2$-), 59.0 (-O-C$\underline{H}_3$), 55.0 – 40.0 (P$\underline{EI}$ backbone), 36.8 – 36.5 (-C$\underline{H}_2$-NHCO- from functionalized PEI backbone), 34.9 (-NHCO-C$\underline{H}_2$-), 34.2 (-C$\underline{H}_2$-COO-), 29.7 – 29.1 (-(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 25.1 (-NHCO-CH$_2$-C$\underline{H}_2$-), 24.9 (-C$\underline{H}_2$-CH$_2$-COO-).

General procedure for synthesis of core-multishell architecture with PEI core. Amide formation by activation with CDI.
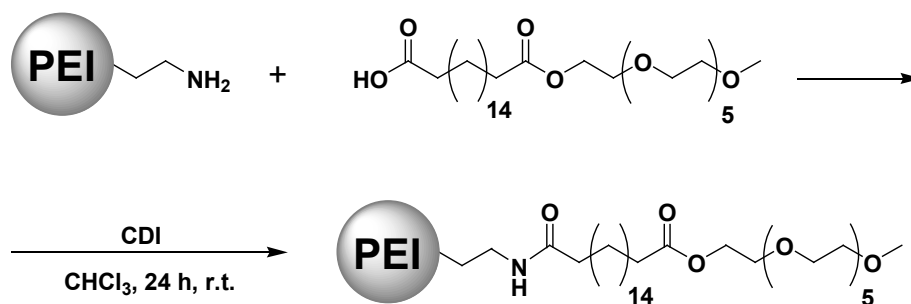
The reaction was performed under inert gas atmosphere in a two-neck round-bottom flask equipped with a dropping funnel. Poly(ethylene imine) [PEI$_{3600}$: M_n = 3600 g mol^{-1}, 0.144 g, 0.04 mmol, 1.24 (31 × 0.04) mmol -NH$_2$ (T) groups; or PEI$_{10500}$: M_n = 10500 g mol^{-1}, 0.210 g, 0.02 mmol, 1.52 (76 × 0.02) mmol -NH$_2$ (T) groups] and C$_{18}$mPEG$_6$ (for PEI$_{3600}$: 0.840 g, 1.30 mmol, 1.05 eq. per -NH$_2$ group; or for PEI$_{10500}$: 1.031 g, 1.60 mmol, 1.05 eq. per -NH$_2$ group) were dissolved in dry CHCl$_3$ (50 ml) and a solution of CDI (for PEI$_{3600}$: 0.210 g, 1.30 mmol, 1.05 eq. per -NH$_2$ group; for PEI$_{10500}$: 0.259 g, 1.60 mmol, 1.05 eq. per -NH$_2$ group) in dry CHCl$_3$ (10 ml) was added dropwise. The reaction mixture was stirred for 24 h at r.t. and the solvent was removed by rotary evaporation *in vacuo*. The obtained crude product was dissolved and dialyzed twice in MeOH. After drying under high vacuum a yellow, glassy solid was obtained.

PEI$_{3600}$(C$_{18}$mPEG$_6$)$_{1.0}$. Conversion (DF): 99 % (100 %); yield: 41 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.50 (br. m, 26H, P$\underline{EG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 3.50 – 3.05 (br. m, -C$\underline{H}_2$-NHCO- from functionalized PEI backbone), 3.00 – 2.38 (br. m, P$\underline{EI}$ backbone), 2.36 – 2.26 (m, 2H, -C$\underline{H}_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}_2$-), 1.66 – 1.52 (m, 4H, -NHCO-CH$_2$-C$\underline{H}_2$-(CH$_2$)$_{12}$-C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.20 (m, 24H, -(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 174.1 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 (P$\underline{EG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-C$\underline{H}_2$-), 59.0 (-O-C$\underline{H}_3$), 55.0 – 40.0 (P$\underline{EI}$ backbone), 36.8 – 36.5 (-C$\underline{H}_2$-NHCO- from functionalized PEI backbone), 34.9 (-NHCO-C$\underline{H}_2$-), 34.2 (-C$\underline{H}_2$-COO-), 29.7 – 29.1 (-(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 25.1 (-NHCO-CH$_2$-C$\underline{H}_2$-), 24.9 (-C$\underline{H}_2$-CH$_2$-COO-).

PEI$_{10500}$(C$_{18}$mPEG$_6$)$_{1.0}$. Conversion (DF): 85 % (100 %); yield: 45 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.50 (br. m, 26H, P$\underline{EG}$), 3.34 (s,

3H, -O-C$\underline{H}_3$), 3.50 – 3.05 (br. m, -C$\underline{H}_2$-N$\underline{H}$CO- from functionalized PEI backbone), 3.00 – 2.38 (br. m, $\underline{PEI}$ backbone), 2.36 – 2.26 (m, 2H, -C$\underline{H}_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}_2$-), 1.66 – 1.52 (m, 4H, -NHCO-CH$_2$-C$\underline{H}_2$-(CH$_2$)$_{12}$-C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.20 (m, 24H, -(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 173.7 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-O-$\underline{C}$H$_3$), 55.0 – 40.0 ($\underline{PEI}$ backbone), 36.8 – 36.5 (-$\underline{C}$H$_2$-NHCO- from functionalized PEI backbone), 34.5 (-NHCO-$\underline{C}$H$_2$-), 34.2 (-$\underline{C}$H$_2$-COO-), 29.7 – 29.1 (-(-$\underline{C}$H$_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 25.6 (-NHCO-CH$_2$-$\underline{C}$H$_2$-) 24.9 (-$\underline{C}$H$_2$-CH$_2$-COO-).

**General procedure for the synthesis of *O*-Mesylpolyglycerol (PG-OMs) (established by S. Roller).**
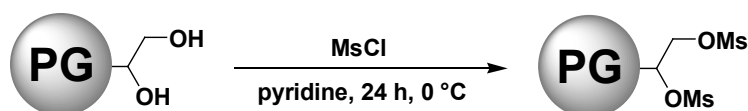
The reaction was performed in a two-neck round-bottom flask equipped with a dropping funnel and a thermometer. Polyglycerol (M_n = 10000 g mol^{-1}, 20.0 g, 2.00 mmol, 256 mmol -OH groups) was dissolved in p.a. pyridine (150 ml) and the solution was cooled down to 0 °C. Than, under vigorous stirring, a solution of MsCl (320 mmol × DF$_{NH2}$, 1.25 eq per -OH group) in pyridine (30 ml) was added dropwise that the temperature did not exceed 10 °C. The brown reaction mixture was stirred for 16 h at 0°C. Than to the reaction mixture ice was added, and a dark brown solid precipitated. After decantation of the liquid phase, the obtained solid was washed with H$_2$O and dissolved as well as dialyzed in acetone to give a brown honey-like product.

PG$_{10000}$(-OMs)$_{0.5}$. Conversion (DF = DF-$_{NH2}$): 47 % (50 %); yield: 59 %; ^{1}H NMR (500 MHz, (CD$_3$)$_2$CO): δ (ppm) = 5.12 – 4.68 (br. m, functionalized sec. $\underline{PG}$ groups), 4.63 – 4.12 (br. m, functionalized prim. $\underline{PG}$ groups), 4.10 – 3.40 (br. m, $\underline{PG}$ backbone), 3.25 – 3.10 (m, -O-SO$_2$-C$\underline{H}_3$), 0.98 – 0.84 (m, $\underline{PG}$ starter unit); ^{13}C NMR (125 MHz, (CD$_3$)$_2$CO): δ (ppm) = 81.2 – 66.5 ($\underline{PG}$ backbone), 37.9 (-O-SO$_2$-$\underline{C}$H$_3$ on sec. PG groups), 36.7 (-O-SO$_2$-$\underline{C}$H$_3$ on prim. PG groups).

PG$_{10000}$(-OMs)$_{0.7}$. Conversion (DF = DF-$_{NH2}$): 94 % (90 %); yield: 72 %; ^{1}H NMR (500 MHz, (CD$_3$)$_2$CO): δ (ppm) = 5.08 – 4.80 (br. m, functionalized sec. $\underline{PG}$ groups), 4.59 – 4.18 (br. m, functionalized prim. $\underline{PG}$ groups), 4.12 – 3.46 (br. m, $\underline{PG}$ backbone), 3.29 – 3.10 (m, -O-SO$_2$-C$\underline{H}_3$), 0.98 – 0.84 (m, $\underline{PG}$ starter unit); ^{13}C NMR (125 MHz, (CD$_3$)$_2$CO): δ (ppm) = 81.2 – 66.5 ($\underline{PG}$ backbone), 37.9 (-O-SO$_2$-$\underline{C}$H$_3$ on sec. PG groups), 36.7 (-O-SO$_2$-$\underline{C}$H$_3$ on prim. PG groups).

PG$_{10000}$(-OMs)$_{0.9}$. Conversion (DF = DF-$_{NH2}$): 73 % (70 %); yield: 63 %; ^{1}H NMR (500 MHz, (CD$_3$)$_2$CO): δ (ppm) = 5.12 – 4.77 (br. m, functionalized sec. <u>PG</u> groups), 4.59 – 4.20 (br. m, functionalized prim. <u>PG</u> groups), 4.10 – 3.45 (br. m, <u>PG</u> backbone), 3.30 – 3.08 (m, -O-SO$_2$-C<u>H</u>$_3$), 0.98 – 0.84 (m, <u>PG</u> starter unit); ^{13}C NMR (125 MHz, (CD$_3$)$_2$CO): δ (ppm) = 81.2 – 66.5 (<u>PG</u> backbone), 37.9 (-O-SO$_2$-<u>C</u>H$_3$ on sec. PG groups), 36.7 (-O-SO$_2$-<u>C</u>H$_3$ on prim. PG groups).

General procedure for synthesis of polyglycerylazide (PG-N$_3$) (established by S. Roller).
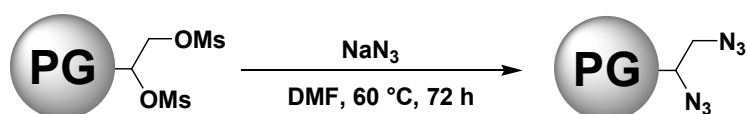
CAUTION: Risk of explosion. Reaction was performed in an one-neck round-bottom flask equipped with a reflux condenser. *O*-mesylpolyglycerol (1.00 mmol, 128 × DF$_{NH2}$ × 1.00 mmol -OMs groups) was dissolved in p.a. DMF (200 ml) and solid NaN$_3$ (128 × DF$_{NH2}$ × 5.00 mmol, 5 eq. per -OMs group) was added. The resulting suspension was stirred for 72 h at 60 °C. After cooling to r.t. the reaction mixture was filtrated to give a red filtrate and a white residue of excess of NaN$_3$, which was washed with a small amount of DMF (15 ml). The filtrate and washing were combined and concentrated by rotary evaporation *in vacuo* below 50 °C. The residue was dissolved in CHCl$_3$ and extracted four times with water. After drying over MgSO$_4$, the organic phase was concentrated by rotary evaporation *in vacuo* to give a brown, honey-like crude product which was used in the next reaction step without further purification.

PG$_{10000}$(-N$_3$)$_{0.5}$. Conversion (DF = DF-$_{NH2}$): quant. (50 %); yield: 92 %; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) = 5.36 – 5.09 (br. m, <u>PG</u> backbone), 4.93 – 4.39 (br. m, <u>PG</u> backbone), 4.11 – 3.06 (br. m, <u>PG</u> backbone), 1.39 – 1.22 (m, <u>PG</u> starter unit), 0.87 – 0.77 (m, <u>PG</u> starter unit); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm) = 81.9 – 67.9 (<u>PG</u> backbone), 60.5 – 60.1 (functionalized sec. <u>PG</u> groups), 50.9 – 50.7 (functionalized prim. <u>PG</u> groups).

PG$_{10000}$(-N$_3$)$_{0.7}$. Conversion (DF = DF-$_{NH2}$): quant. (70 %); yield: 96 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 5.39 – 5.06 (br. m, <u>PG</u> backbone), 4.91 – 4.41 (br. m, <u>PG</u> backbone), 4.11 – 3.09 (br. m, <u>PG</u> backbone), 1.41 – 1.22 (m, <u>PG</u> starter unit), 0.89 – 0.77 (m, <u>PG</u> starter unit); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 81.9 – 67.9 (<u>PG</u> backbone), 60.5 – 60.1 (functionalized sec. <u>PG</u> groups), 50.9 – 50.7 (functionalized prim. <u>PG</u> groups).

PG$_{10000}$(-N$_3$)$_{0.9}$. Conversion (DF = DF-$_{NH2}$): quant. (90 %); yield: 93 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 3.97 – 3.17 (br. m, <u>PG</u> backbone), 1.41 – 1.22 (m, <u>PG</u> starter unit), 0.89 –

0.77 (m, <u>PG</u> starter unit); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 81.9 – 67.9 (<u>PG</u> backbone), 60.5 – 60.1 (functionalized sec. <u>PG</u> groups), 50.9 – 50.7 (functionalized prim. <u>PG</u> groups).

General procedure for the synthesis of polyglycerylamine (PG-NH$_2$) (established by S. Roller).
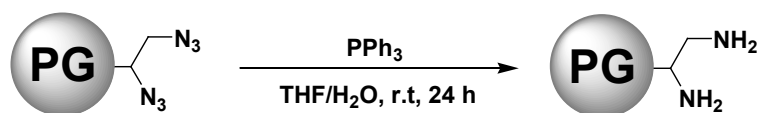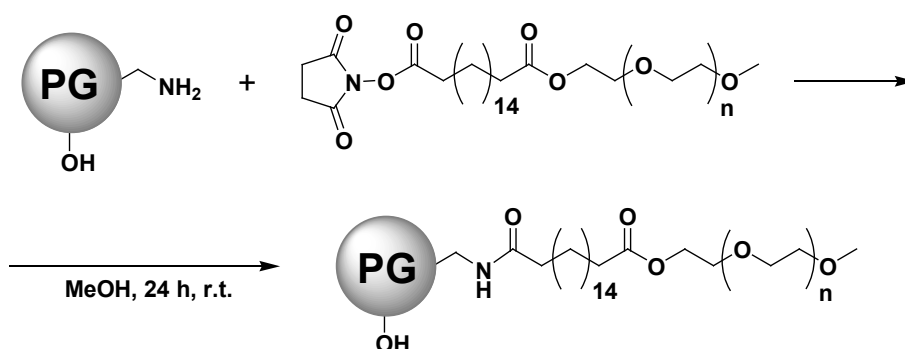

The reaction was performed in an one-neck round-bottom flask equipped with a dropping funnel. Polyglycerylazide (0.50 mmol, 128 × DF$_{NH2}$ × 0.50 mmol -N$_3$ groups) was dissolved in THF/H$_2$O (10 : 1 v/v) mixture (150 ml) and a solution of PPh$_3$ (128 × DF$_{NH2}$ × 0.55 mmol, 1.1 eq. per -N$_3$ group) in p.a. THF (25 ml) was added dropwise. Formation of N$_2$ was observed. The reaction mixture was stirred for 24 h at r.t., in the meantime H$_2$O was added to the reaction mixture in small portions (5 × 10 ml) to avoid precipitation of the partially reduced product. The mixture was concentrated by rotary evaporation *in vacuo* to remove THF. The aqueous phase was extracted with CHCl$_3$ four times (4 × 50 ml) and than concentrated by rotary evaporation *in vacuo*. The obtained crude product was dissolved and dialyzed in MeOH (2 × 2 dm^3) to give after drying under the high vacuum brown, honey-like product.

PG$_{10000}$(-NH$_2$)$_{0.5}$. Conversion (DF-$_{NH2}$): 93% (50 %); yield: 87 %; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) = 3.90 – 3.25 (br. m, <u>PG</u> backbone), 3.15 – 2.35 (functionalized <u>PG</u> groups); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm) = 83.0 – 65.5 (<u>PG</u> backbone), 55.5 – 43.6 (functionalized <u>PG</u> groups).

PG$_{10000}$(-NH$_2$)$_{0.7}$. Conversion (DF-$_{NH2}$): 89 % (70 %); yield: 93 %; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) = 3.90 – 3.25 (br. m, <u>PG</u> backbone), 3.15 – 2.35 (functionalized <u>PG</u> groups); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm) = 83.0 – 65.5 (<u>PG</u> backbone), 55.5 – 43.6 (functionalized <u>PG</u> groups).

PG$_{10000}$(-NH$_2$)$_{0.9}$. Conversion (DF-$_{NH2}$): 86 % (90 %); yield: 84 %; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) = 3.90 – 3.25 (br. m, <u>PG</u> backbone), 3.15 – 2.35 (functionalized <u>PG</u> groups); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm) = 83.0 – 65.5 (<u>PG</u> backbone), 55.5 – 43.6 (functionalized <u>PG</u> groups).

Michał R. Radowski, Dissertation

General procedure for synthesis of core-multishell architecture with PG-NH$_2$ core. Amide formation by activation with HONSu.

The reaction was performed in an one-neck round-bottom flask equipped with a dropping funnel. PG-NH$_2$ (M_n = 10000 g mol^{-1}, 0.200 g, 0.02 mmol, 128 × DF$_{NH2}$ × 0.02 mmol -NH$_2$ groups) was dissolved in p.a. MeOH (100 ml) and a solution of (C$_{18}$mPEG$_{n+1}$)-ONSu (128 × DF$_{NH2}$ × 0.022 mmol, 1.1 eq. per -NH$_2$ group) in MeOH (25 ml) was added dropwise. The reaction mixture was stirred for 24 h at r.t. and than concentrated by rotary evaporation *in vacuo*. The obtained crude product was dissolved and dialyzed twice in MeOH. After drying *in vacuo* a white solid was obtained as a product.

PG$_{10000}$(-NH$_2$)$_{0.5}$(C$_{18}$mPEG$_6$)$_{1.0}$. Conversion (DF$_{-NH2}$): ~100% (50 %); yield: 67 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 4.90 – 3.10 (br. m, $\underline{PG}$ backbone and –C$\underline{H}_2$-N$\underline{H}$CO- from PG overlapping with $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 2.30 (m, 2H, -C$\underline{H}_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}_2$-), 1.66 – 1.52 (m, 4H, -NHCO-CH$_2$-C$\underline{H}_2$- and -C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.20 (m, 24H, -NHCO-CH$_2$-CH$_2$-(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 174.1 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 83.0 – 65.5 ($\underline{PG}$ backbone), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-O-$\underline{C}$H$_3$), 36.9 – 36.1 (-$\underline{C}$H$_2$-NHCO- from functionalized $\underline{PG}$ backbone), 34.9 (-NHCO-$\underline{C}$H$_2$-), 34.2 (-$\underline{C}$H$_2$-COO-), 29.7 – 29.1 (-(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 25.1 (-NHCO-CH$_2$-$\underline{C}$H$_2$-), 24.9 (-$\underline{C}$H$_2$-CH$_2$-COO-).

PG$_{10000}$(-NH$_2$)$_{0.7}$(C$_{18}$mPEG$_6$)$_{1.0}$. Conversion (DF$_{-NH2}$): ~100% (70 %); yield: 78 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 4.90 – 3.10 (br. m, $\underline{PG}$ backbone and –C$\underline{H}_2$-N$\underline{H}$CO- from PG overlapping with $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 2.30 (m, 2H, -C$\underline{H}_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}_2$-), 1.66 – 1.52 (m, 4H, -NHCO-CH$_2$-C$\underline{H}_2$- and -C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.20 (m, 24H, -(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 174.1 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 83.0 – 65.5 ($\underline{PG}$ backbone), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-O-$\underline{C}$H$_3$), 36.9 – 36.1 (-$\underline{C}$H$_2$-NHCO- from functionalized $\underline{PG}$ backbone), 34.9 (-NHCO-$\underline{C}$H$_2$-),

Michał R. Radowski, Dissertation

34.2 (-C$\underline{H}_2$-COO-), 29.7 – 29.1 (-(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 25.1 (-NHCO-CH$_2$-C$\underline{H}_2$-), 24.9 (-C$\underline{H}_2$-CH$_2$-COO-).

PG$_{10000}$(-NH$_2$)$_{0.9}$(C$_{18}$mPEG$_6$)$_{0.7}$. Conversion (DF$_{-NH2}$): ~70% (90 %); yield: 77 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 4.90 – 3.10 (br. m, $\underline{PG}$ backbone and –C$\underline{H}_2$-N$\underline{H}$CO- from PG overlapping with $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 2.30 (m, 2H, -C$\underline{H}_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}_2$-), 1.66 – 1.52 (m, 4H, -NHCO-CH$_2$-C$\underline{H}_2$- and -C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.20 (m, 24H, -(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-);^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 174.1 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 83.0 – 65.5 ($\underline{PG}$ backbone), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-O-$\underline{C}$H$_3$), 36.9 – 36.1 (-$\underline{C}$H$_2$-NHCO- from functionalized $\underline{PG}$ backbone), 34.9 (-NHCO-$\underline{C}$H$_2$-), 34.2 (-$\underline{C}$H$_2$-COO-), 29.7 – 29.1 (-(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 25.1 (-NHCO-CH$_2$-$\underline{C}$H$_2$-), 24.9 (-$\underline{C}$H$_2$-CH$_2$-COO-).

PG$_{10000}$(-NH$_2$)$_{0.9}$(C$_{18}$mPEG$_6$)$_{1.0}$. Conversion (DF$_{-NH2}$): ~100% (90 %); yield: 66 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 4.90 – 3.10 (br. m, $\underline{PG}$ backbone and –C$\underline{H}_2$-N$\underline{H}$CO- from PG overlapping with $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 2.30 (m, 2H, -C$\underline{H}_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}_2$-), 1.66 – 1.52 (m, 4H, -NHCO-CH$_2$-C$\underline{H}_2$- and -C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.20 (m, 24H, -(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-);^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 174.1 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 83.0 – 65.5 ($\underline{PG}$ backbone), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-CH$_2$-O-$\underline{C}$H$_3$), 36.9 – 36.1 (-$\underline{C}$H$_2$-NHCO- from functionalized $\underline{PG}$ backbone), 34.9 (-NHCO-$\underline{C}$H$_2$-), 34.2 (-$\underline{C}$H$_2$-COO-), 29.7 – 29.1 (-(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 25.1 (-NHCO-CH$_2$-$\underline{C}$H$_2$), 24.9 (-$\underline{C}$H$_2$-CH$_2$-COO-).

PG$_{10000}$(-NH$_2$)$_{0.5}$(C$_{18}$mPEG$_{14}$)$_{1.0}$. Conversion (DF$_{-NH2}$): ~100% (50 %); yield: 75 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 4.90 – 3.10 (br. m, $\underline{PG}$ backbone and –C$\underline{H}_2$-N$\underline{H}$CO- from PG overlapping with $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 2.30 (m, 2H, -C$\underline{H}_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}_2$-), 1.66 – 1.52 (m, 4H, -NHCO-CH$_2$-C$\underline{H}_2$- and -C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.20 (m, 24H, -(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-);^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 174.1 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 83.0 – 65.5 ($\underline{PG}$ backbone), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-O-$\underline{C}$H$_3$), 36.9 – 36.1 (-$\underline{C}$H$_2$-NHCO- from functionalized $\underline{PG}$ backbone), 34.9 (-NHCO-$\underline{C}$H$_2$-), 34.2 (-$\underline{C}$H$_2$-COO-), 29.7 – 29.1 (-(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 25.1 (-NHCO-CH$_2$-C$\underline{H}_2$-), 24.9 (-$\underline{C}$H$_2$-CH$_2$-COO-).

PG$_{10000}$(-NH$_2$)$_{0.7}$(C$_{18}$mPEG$_{14}$)$_{1.0}$. Conversion (DF$_{-NH2}$): ~100% (70 %); yield: 62 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 4.90 – 3.10 (br. m, $\underline{PG}$ backbone and –C$\underline{H}_2$-N$\underline{H}$CO- from PG overlapping with $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 2.30 (m, 2H, -C$\underline{H}_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}_2$-), 1.66 – 1.52 (m, 4H, -NHCO-CH$_2$-C$\underline{H}_2$- and -C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.20 (m, 24H, -(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-);^{13}C NMR (125 MHz,

CDCl$_3$): δ (ppm) = 174.1 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 83.0 – 65.5 ($\underline{PG}$ backbone), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-CH$_2$-O-$\underline{C}$H$_3$), 36.9 – 36.1 (-$\underline{C}$H$_2$-NHCO- from functionalized $\underline{PG}$ backbone), 34.9 (-NHCO-$\underline{C}$H$_2$-), 34.2 (-$\underline{C}$H$_2$-COO-), 29.7 – 29.1 (-($\underline{C}$H$_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 25.1 (-NHCO-CH$_2$-$\underline{C}$H$_2$-), 24.9 (-$\underline{C}$H$_2$-CH$_2$-COO-).

PG$_{10000}$(-NH$_2$)$_{0.9}$(C$_{18}$mPEG$_{14}$)$_{0.7}$. Conversion (DF$_{-NH2}$): ~70% (90 %); yield: 73 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}$$_2$-), 4.90 – 3.10 (br. m, $\underline{PG}$ backbone and –C$\underline{H}$$_2$-N$\underline{H}$CO- from PG overlapping with $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}$$_3$), 2.30 (m, 2H, -C$\underline{H}$$_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}$$_2$-), 1.66 – 1.52 (m, 4H, -NHCO-CH$_2$-C$\underline{H}$$_2$- and -C$\underline{H}$$_2$-CH$_2$-COO-), 1.35 – 1.20 (m, 24H, -(C$\underline{H}$$_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 174.1 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 83.0 – 65.5 ($\underline{PG}$ backbone), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-O-$\underline{C}$H$_3$), 36.9 – 36.1 (-$\underline{C}$H$_2$-NHCO- from functionalized $\underline{PG}$ backbone), 34.9 (-NHCO-$\underline{C}$H$_2$-), 34.2 (-$\underline{C}$H$_2$-COO-), 29.7 – 29.1 (-($\underline{C}$H$_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 25.1 (-NHCO-CH$_2$-$\underline{C}$H$_2$-), 24.9 (-$\underline{C}$H$_2$-CH$_2$-COO-).

PG$_{10000}$(-NH$_2$)$_{0.9}$(C$_{18}$mPEG$_{14}$)$_{1.0}$. Conversion (DF$_{-NH2}$): ~100% (90 %); yield: 60 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}$$_2$-), 4.90 – 3.10 (br. m, $\underline{PG}$ backbone and –C$\underline{H}$$_2$-N$\underline{H}$CO- from PG overlapping with $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}$$_3$), 2.30 (m, 2H, -C$\underline{H}$$_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}$$_2$-), 1.66 – 1.52 (m, 4H, -NHCO-CH$_2$-C$\underline{H}$$_2$- and -C$\underline{H}$$_2$-CH$_2$-COO-), 1.35 – 1.20 (m, 24H, -(C$\underline{H}$$_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 174.1 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 83.0 – 65.5 ($\underline{PG}$ backbone), 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-O-$\underline{C}$H$_3$), 36.9 – 36.1 (-$\underline{C}$H$_2$-NHCO- from functionalized $\underline{PG}$ backbone), 34.9 (-NHCO-$\underline{C}$H$_2$-), 34.2 (-$\underline{C}$H$_2$-COO-), 29.7 – 29.1 (-($\underline{C}$H$_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 25.1 (-NHCO-CH$_2$-$\underline{C}$H$_2$-) 24.9 (-$\underline{C}$H$_2$-CH$_2$-COO-).

Michał R. Radowski, Dissertation

General procedure for synthesis of core-multishell architecture with PAMAM [G5] core. Amide formation by activation with HONSu.
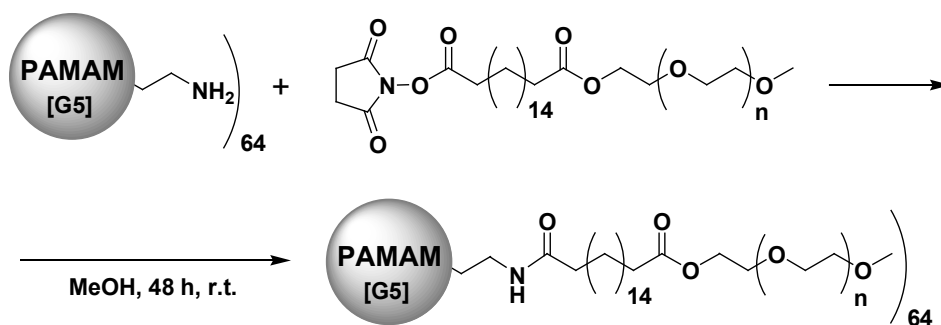
The reaction was performed in an one-neck round-bottom flask equipped with a dropping funnel. PAMAM[G5] dendrimer (0.284 g, 0.02 mmol, 1.28 mmol -NH$_2$ groups) was dissolved in p.a. MeOH (100 ml) and a solution of (C$_{18}$mPEG$_{n+1}$)-ONSu (1.66 mmol, 1.3 eq. per -NH$_2$ group) in MeOH (25 ml) was added dropwise. The reaction mixture was stirred for 48 h at r.t. and than concentrated by rotary evaporation *in vacuo*. The obtained crude product was dissolved and dialyzed twice in MeOH. After drying under high vacuum a white solid was obtained as a product.

PAMAM[G5](C$_{18}$mPEG$_6$)$_{1.0}$. Conversion (DF): ~95 % (100 %); yield: 83 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.50 (br. m, 26H, $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 3.45 – 3.05 (br. m, -C$\underline{H}_2$-NHCO- from functionalized $\underline{PAMAM}$ term. groups and $\underline{PAMAM}$ backbone), 2.85 – 2.08 (br. m, $\underline{PAMAM}$ backbone), 2.36 – 2.26 (m, 2H, -C$\underline{H}_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}_2$-), 1.66 – 1.52 (m, 4H, -NHCO-CH$_2$-C$\underline{H}_2$- and -C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.20 (m, 24H, -(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 174.1 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 173.3 – 172.5 ($\underline{PAMAM}$ backbone) 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 ($\underline{PEG}$), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-O-$\underline{C}$H$_3$), 52.0 – 48.0 ($\underline{PAMAM}$ backbone), 42.0 – 35.0 ($\underline{PAMAM}$ backbone), 36.8 – 36.5 (-$\underline{C}$H$_2$-NHCO- from functionalized $\underline{PAMAM}$ backbone), 34.9 (-NHCO-$\underline{C}$H$_2$-), 34.2 (-$\underline{C}$H$_2$-COO-), 29.7 – 29.1 (-(CH$_2$)$_{12}$-$\underline{C}$H$_2$-CH$_2$-COO-), 25.1 (-NHCO-CH$_2$-$\underline{C}$H$_2$-), 24.9 (-$\underline{C}$H$_2$-CH$_2$-COO-).

PAMAM[G5](C$_{18}$mPEG$_{14}$)$_{1.0}$. Conversion (DF): ~95 % (100 %); yield: 79 %; ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) = 4.21 – 4.17 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 – 3.50 (br. m, 26H, $\underline{PEG}$), 3.34 (s, 3H, -O-C$\underline{H}_3$), 3.45 – 3.05 (br. m, -C$\underline{H}_2$-NHCO- from functionalized $\underline{PAMAM}$ term. groups and $\underline{PAMAM}$ backbone), 2.85 – 2.08 (br. m, $\underline{PAMAM}$ backbone), 2.36 – 2.26 (m, 2H, -C$\underline{H}_2$-COO-), 2.24 – 2.10 (m, 2H, -NHCO-C$\underline{H}_2$-), 1.66 – 1.52 (m, 4H, -NHCO-CH$_2$-C$\underline{H}_2$- and -C$\underline{H}_2$-CH$_2$-COO-), 1.35 – 1.20 (m, 24H, -(C$\underline{H}_2$)$_{12}$-CH$_2$-CH$_2$-COO-); ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) = 174.1 (-NH$\underline{C}$O-CH$_2$-), 173.4 (-CH$_2$-$\underline{C}$OO-), 173.3 – 172.5 ($\underline{PAMAM}$

Michał R. Radowski, Dissertation

backbone) 71.9 (-$\underline{C}$H$_2$-O-CH$_3$), 70.6 – 70.0 (PEG), 69.2 (-COO-CH$_2$-$\underline{C}$H$_2$-), 63.3 (-COO-$\underline{C}$H$_2$-), 59.0 (-O-$\underline{C}$H$_3$), 52.0 – 48.0 (PAMAM backbone), 42.0 – 35.0 (PAMAM backbone), 36.8 – 36.5 (-$\underline{C}$H$_2$-NHCO- from functionalized PAMAM backbone), 34.9 (-NHCO-$\underline{C}$H$_2$-), 34.2 (-$\underline{C}$H$_2$-COO-), 29.7 – 29.1 (-($\underline{C}$H$_2$)$_{12}$-CH$_2$-CH$_2$-COO-), 25.1 (-NHCO-CH$_2$-$\underline{C}$H$_2$-), 24.9 (-$\underline{C}$H$_2$-CH$_2$-COO-).

Michał R. Radowski, Dissertation