Directed Interactions of Block Copolypept(o)ides with Mannose-Binding Receptors: PeptoMicelles Targeted to Cells of the Innate Immune System[a]

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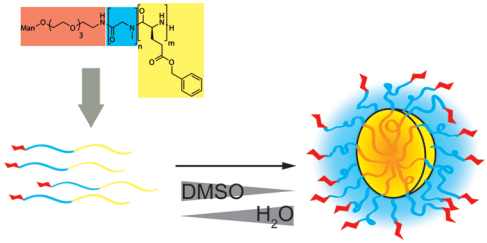
Core-shell structures based on polypept(o)ides combine stealth-like properties of the corona material polysarcosine with adjustable functionalities of the polypeptidic core. Mannose-bearing block copolypept(o)ides (PSar-block-PGlu(OBn)) have been synthesized using 11-amino-3,6,9-trioxa-undecyl-2,3,4,6-tetra-O-acetyl-O-α-D-mannopyranoside as initiator in the sequential ring-opening polymerization of α-amino acid N-carboxyanhydrides. These amphiphilic block copolypept(o)ides self-assemble into multivalent PeptoMicelles and bind to mannose-binding receptors as expressed by dendritic cells. Mannosylated micelles showed enhanced cell uptake in DC 2.4 cells and in bone marrow-derived dendritic cells (BMDCs) and therefore appear to be a suitable platform for immune modulation.



1. Introduction

In nature, glycoproteins are involved in a multitude of essential biological processes, which range from phagocytosis and inflammation to cell–cell interactions and signal transduction.[1,2] Many researchers have investigated multivalent binding of carbohydrates presented on polymers, polymeric micelles, polymersomes or inorganic nanoparticles to cytosolic, extracellular or transmembrane proteins.[3–9] In general, the interaction between a single carbohydrate moiety and its receptor is comparatively weak (nM to mM affinities). Thus, to enhance the binding strength of carbohydrates for directed interaction (drug targeting) multivalence turned out to be of particular importance. This phenomenon is known as the cluster glycoside effect[10] and has been studied in depth for decades.

Besides binding to model proteins like concavalin A[7] or inhibition of hemagglutination (HIA),[3,4] glycoside functionalization of nanoparticles can be used to direct their binding to transmembrane proteins and to trigger specific internalization; prominent examples involve the targeting of the transferrin receptor[11,12] and mannose-binding receptors (e.g., MMR family, DC-SIGN).[13] Activation of

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cellular uptake pathways like clathrin-mediated endocytosis by targeting transmembrane proteins is of major interest to enhance internalization and to alter intracellular distribution, as clearly demonstrated in studies on uptake kinetics, pathway specificity and intracellular distribution of viruses.[14,15]

In this context, mannose-functionalized nanoparticles have been investigated to target dendritic cells (DCs) as well as macrophages.[13,16] Both cell types play important roles in the first line of immune defense and are interesting cellular targets to induce/alter immune responses. In case of DCs, especially vaccination with mannose-modified carriers against various model antigens or diseases has been studied.[17–20] Macrophages, on the other hand, are potential targets for the delivery of drugs, e.g., against tuberculosis[21] or leishmaniasis.[22,23] While after i.v. injection ending up in macrophages is likely to be the fate of mannosylated nanoparticles, subcutaneous administration has already demonstrated that macrophage uptake can be minimized because transfer to the lymphatic system occurs rapidly and therefore exposes particles almost exclusively to dendritic cells.[24]

Nearly all targeted delivery systems have a common feature: a directed interaction of a recognition structure on the particle corona with a cellular transmembrane protein. Therefore, the recognition structure needs to be exposed on the particle surface. The self-assembly of amphiphilic block copolymers in a block-selective solvent offers a versatile route to the formation of core–shell structures. Provided that the amphiphilic polymers already contain the recognition structure, micelles that present these structures on the corona can be directly obtained through this process.[25] Therefore, the targeting moiety—for example peptide or glycoside—needs to be attached to the hydrophilic block by conjugation to the side chain or the end group. BIND therapeutics is currently investigating the concept of end group modification for block copolymer-based nanoparticles containing the chemotherapeutic docetaxel in clinical trials. These nanoparticles are decorated with a small molecule ligand (ACUPA) targeting a prostate cancer specific antigen as expressed specifically by prostate cancer cells.[26]

While functionalization of the polymer side chains can be achieved by polymer-analogous reactions,[27,28] functional end groups are accessible by the use of appropriate initiators for polymerization. The ring-opening polymerization of *N*-substituted α-amino acid *N*-carboxyanhydrides, e.g., sarcosine NCA, leads to polymers with high end group integrity in one step. Thus, the use of bioactive initiators represents a straight forward approach for the synthesis of end group-functionalized polypept(o)ides such as glycosylated polypept(o) ides presented in this work. Other strategies for the synthesis of glycopolypeptides involve the polymerization of glycosylated NCAs or postpolymerization glycosylation.[2,9,29–32]

Almost 40 years after the first report on the living polymerization of the polypeptoide polysarcosine (PSar),[33] scientists have rediscovered this type of polymer as a very interesting material being—amongst others[34]—a potential alternative to PEG for biomedical applications. Polysarcosine is a typical example for a material, which behaves accordingly to the Whitesides rules for protein–resistant surfaces,[35,36] since it is non-ionic, hydrophilic, and a weak hydrogen bond acceptor. Therefore, it is not much of a surprise, that PSar has already demonstrated protein–resistant properties[35,37] as well as non-immunogenic properties[38] and thus meets the requirements for biocompatibility. While Guo and Zhang used PSar for the synthesis of cyclic polypeptoides,[39] the group of Luxenhofer applied it for the synthesis of block copolypeptoides.[40] Sun and Zuckermann derived monodisperse PSar by solid phase synthesis.[41] Surprisingly, so far only the groups of Kimura and Barz used PSar as substitute of PEG in amphiphilic di-[42] and tri-block[43] copolymers designed for biomedical applications: for example Lactosomes,[44,45] microspheres,[46] Nanosheets,[38] PeptoMicelles,[42] and PeptoPlexes.[47]

2. Experimental Section

2.1. Materials

DMF was purchased from VWR, dried over BaO and subsequently distilled in vacuo onto pre-dried molecular sieves (3 Å). THF and hexane were purchased from Sigma–Aldrich and distilled from Na/K. Diethylether was distilled prior to use to remove the stabilizer. Other solvents were used as received. Diphosgene was purchased from Alfa Aesar and used as provided. Neopentylamine was purchased from TCI Europe, dried over NaOH and fractionally distilled. Sarcosine was purchased from Sigma–Aldrich, all protected amino Acids were purchased from ORPEGEN. Oregon Green® 488-X, Succinimidyl Ester, 6-isomer was purchased from Life Technologies. Cell culture media were bought from Lonza. CellTiter 96® Non-Radioactive Cell Proliferation Assay kit (for MTT assay) was purchased from Promega (Madison, USA). Mannan from Saccharomyces cerevisiae was purchased from Sigma–Aldrich. Dynasore-OH was kindly provided by Prof. T. Kirchhausen from Harvard Medical School, Boston.

2.2. Characterization

400 MHz ^{1}H NMR spectra were recorded on a Bruker Avance II 400. HSQC and DOSY NMR measurements were performed on a Bruker Avance III HD 400. All spectra were recorded at room temperature and analyzed with MestReNova software. Degrees of polymerization as well as molecular weights were calculated by comparing the integrals of the neopentylamine initiator peak or the mannose initiator acetyl peaks either with the integrals of the side chain amide protons (for PLys(TFA)), with the protective group protons (for PGlu(OBn)) or with the average of integrals of methyl group and

α-protons (for PSar) in the 400 MHz 1H NMR spectra. Attenuated total reflectance Fourier transformed infrared (ATR-FTIR) spectroscopy was performed on an FT/IR-4100 (JASCO) with an ATR sampling accessory (MIRacleTM, Pike Technologies). IR Spectra were analyzed using Spectra Manager 2.0 (JASCO). Melting points were determined using a METTLER FP62 (METTLER WAAGEN GMBH).

Hexafluoroisopropanol (HFIP) gel permeation chromatography (GPC) was performed with HFIP containing 3 g · L^{-1} potassium trifluoroacetate as eluent at 40 °C and a flow rate of 0.8 mL · min^{-1}. The columns were packed with modified silica (PFG columns; particle size: 7 μm, porosity: 100 and 1 000 Å). Polymer was detected with a refractive index detector (G 1362 A RID, JASCO) and a UV/Vis Detector (UV-2075 Plus, JASCO). Molecular weights were calculated using a calibration performed with PMMA standards (Polymer Standards Services GmbH) and toluene as internal standard. Elution diagrams were analyzed using WinGPC UniChrome 8.00 (Build 994) software from Polymer Standards Services.

Preparative reverse phase-HPLC was performed with a Knauer HPLC-System (Berlin, Germany), consisting of two HPLC pumps (Smartline 1000), an UV/Vis-detector (Smartline 2500), a RI-detector (Smartline 2400), and a Phenomenex (Torrance, USA) Luna-column (10 μm, C18(2), 100 A, 250 × 30 mm^2) run at a flow of 10.0 mL · min^{-1} and loaded with a 2 mL injection loop. The system was operated and samples analyzed with D-7000 HPLC-System-Manager software (version 4.1). For the preparative runs a mixture of THF and Millipore water with a gradient from 40/60 to 90/10 (THF/water) over 50 min was utilized. The UV/Vis-detector was set to a wavelength of 488 nm.

For dynamic light scattering experiments micelle solutions were prepared with concentrations as described. After transfer to a dust free flow box, all samples were filtered (Millex HV 0.45 μm) into dust free cylindrical scattering cells (Suprasil, 20 mm diameter, Hellma, Mühlheim, Germany). Then, dynamic light scattering (DLS) measurements were performed using a Uniphase He/Ne Laser ($l = 632.8$ nm, 22 mW), a ALV-SP125 Goniometer, a ALV/High QE APD-Avalanche photo-diode with fiber optical detection, an ALV 5000/E/PCI-correlator and a Lauda RC-6 thermostat unit at 20 °C. Angular dependent measurements of typically 15° steps were carried out in the range $30° \leq q \leq 150°$. For data evaluation experimental intensity correlation functions were transformed into amplitude correlation functions applying the Siegert relation extended to include negative values after baseline subtraction by calculation $g1(t) = \mathrm{SIGN}(\mathrm{G2}(t)) \cdot \mathrm{SQRT}(\mathrm{ABS}((\mathrm{G2}(t) - A)/A)$. All field correlation functions usually showed monomodal decay and were fitted by a sum of two exponentials $g1(t) = a \cdot \exp(-t/b) + c \cdot \exp(-t/d)$ to take polydispersity into account. Average apparent diffusion coefficients D_{app} were calculated by applying $q^2 \cdot D_{app} = (a \cdot b - 1 + c \cdot d - 1)/(a + c)$ resulting in an angular-dependent diffusion coefficient D_{app} or reciprocal hydrodynamic radius $<1/R_{h,app}>$, according to formal application of Stokes–Einstein law. By extrapolation of $<1/R_{h,app}>$ to $q = 0$ z-average hydrodynamic radii $R_h = <1/R_h>_{(z^{-1})}$ were obtained (uncorrected for c-dependency).

Labeling efficiency for polymers was determined by measuring the Oregon Green 488-related absorbance at 488 nm with a Nanodrop® ND-1000.

(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay measurements were performed using a Dynex MRX TC Revelation microtiter plate reader (Dynex Technologies, Chantilly, VA).

FACS experiments were executed on a FACS Canto II flow cytometer equipped with BD FACS Diva software (both from BD Biosciences, San Diego, CA). Data were analyzed using FlowJo software (FLOWJO, Ashland, OR).

2.3. Methods and Experimental Protocols

2.3.1. Tetraethylene Glycol Monotosylate

To a mixture of 4.11 g (103 mmol) sodium hydroxide in 23 mL of water and 23 mL THF tetraethylene glycol was added. The solution was cooled to 0 °C and a mixture of 12.15 g (65 mmol) *p*-toluenesulfonic acid in 100 mL THF was added dropwise. The reaction mixture was stirred for 2 h at 0 °C. After stirring 2 h at room temperature the mixture was poured into ice-cold water and extracted with dichloromethane (4 × 150 mL). The organic layers were washed with water (2 × 75 mL) and dried over magnesium sulfate. Rotary evaporation gave a pale yellow oil which was used in the next reaction step without further purification. Yield: 22 g (63 mmol, 97%), Lit.: 90%[48]. $R_f = 0.24$ (cyclohexane/EtOAc 1:4).

^{1}H NMR (300 MHz, $CDCl_3$): δ(ppm) = 7.77 (2H, d, $J = 8.5$ Hz, $-SO_2-o$–Ar–H), 7.32 (2H, d, $J = 8.5$ Hz, $-SO_2-m$–Ar–H), 4.14 (2H, t, $J = 4.5$ Hz, $-CH_2-$OTos), 3.71–3.51 (14H, m, $-CH_2-CH_2-$O), 2.42 (3H, s, $-CH_3$).

ESI-MS: (m/z) = 371.13 ($[M + Na]^+$, ber.: 371.11).

$C_{15}H_{24}O_7S$ (348.41 g · mol^{-1})

2.3.2. *N*-(11-hydroxy-3,6,9-trioxaundecyl)phthalimide

A mixture of 22 g (63 mmol) of tetraethylene glycol monotosylate, 14 g (76 mmol) potassium phthalimide and 3 g molecular sieve (3 Å) in 160 mL dimethylformamide was stirred for 10 h at 150 °C and 8 h at room temperature. The suspension was filtered through Celite and washed with DMF. The solvent was evaporated using high vacuum. The crude product was co-distilled with toluene (4 × 70 mL) and purified by column chromatography (eluent: EtOAc) to obtain a pale yellow oil. Yield: 18.2 g (56 mmol, 89%), Lit.: 57%.[48] $R_f = 0.34$ (EtOAc).

^{1}H NMR (300 MHz, $CDCl_3$): δ(ppm) = 7.85–7.81 (2H, m, Ar–H), 7.72–7.68 (2H, m, Ar–H), 3.88 (2H, t, $J = 5.5$ Hz, NPhth–CH_2-CH_2-O–), 3.73 (2H, t, $J = 5.8$ Hz, NPhth–CH_2-CH_2-O–), 3.75–3.52 (m, 12H, $-CH_2-CH_2-$O–).

ESI-MS: (m/z) = 346.14 ($[M + Na]^+$, ber.: 346.13).

$C_{16}H_{21}NO_6$ (348.41 g · mol^{-1})

2.3.3. 1-Amino-11-hydroxy-3,6,9-trioxaundecan

To a solution of 18.2 g (56 mmol) *N*-(11-hydroxy-3,6,9-trioxaundecyl) phthalimide in 100 mL ethanol 3.5 mL (110 mmol) hydrazine hydrate was added and stirred for 2.5 h at 80 °C. A colorless solid precipitates and after cooling to room temperature 1.0 m HCl was added until pH = 1 was achieved. The solvent was evaporated under vacuum and the residue was taken up in 200 mL of water. After filtration through Celite sodium hydroxide solution was added until a pH of 11 was reached. The basic filtrate was stirred for 20 min at room temperature and the water was removed by rotary evaporation. The crude product was dissolved in 300 mL chloroform, dried over $MgSO_4$ and filtrated through Celite. The solvent

was removed by rotary evaporation to obtain an orange oil, which was used in the next reaction step without further purification. Yield: 7.8 g (40 mmol, 71%).

^{1}H NMR (300 MHz, $CDCl_3$): δ(ppm) = 3.71–3.50 (14H, m, $-CH_2-CH_2-O$), 2.87–2.84 (2H, t, J = 4.2 Hz, $-CH_2-NH_2$).

ESI-MS: (m/z) = 194.14 ($[M+H]^+$, ber.: 194.14).

$C_8H_{19}NO_4$ (193.24 g · mol^{-1})

2.3.4. 1-(Benzoyloxycarbonylamino-11-hydroxy-3,6,9-trioxa) undecan[49]

To a solution of 7.8 g (40 mmol) 1-amino-11-hydroxy-3,6,9-trioxaundecan in 270 mL water 6.7 g (80 mmol) $NaHCO_3$ was dissolved and the mixture was stirred at 0 °C. 8.6 mL (60 mmol) of benzyl chloroformate were dissolved in 50 mL dioxan at 5 °C and added dropwise to the reaction mixture. The solution was stirred for 2 h at 0 °C and overnight at room temperature 50 mL water was added and the water phase was extracted with ethyl acetate (2 × 150 mL). The organic layers were collected and extracted with saturated $NaHCO_3$ solution (2 × 150 mL). The aqueous layers were acidified by 1.0 m HCl to pH = 1 and extracted with ethyl acetate (3 × 100 mL). The organic layers were collected, dried over $MgSO_4$, filtrated, and the solvent was removed by rotary evaporation. The yellow oil was purified by column chromatography (eluent: EtOAc). Yield: 8.5 g (26 mmol, 65%). R_f = 0.29 (EtOAc).

^{1}H NMR (300 MHz, $CDCl_3$): δ(ppm) = 7.35–7.29 (5H, m, Ar–H), 5.10 (2H, s, $-O-CH_2-Ar$), 3.66–3.53 (14H, m, $-CH_2-CH_2-O-$), 3.35 (2H, t, J = 9.5 Hz, $-NH-CH_2-CH_2-O$).

ESI-MS: (m/z) = 350.17 ($[M+Na]^+$, ber.: 350.22).

$C_{16}H_{25}NO_6$ (327.24 g · mol^{-1})

2.3.5. 1,2,3,4,6-Penta-*O*-acetyl-D-mannopyranoside

To a solution of 5 g (27.7 mmol) D-mannose in 200 mL pyridine 39 mL (415 mmol) acetic anhydride was added and stirring was continued overnight at room temperature. After co-evaporation with toluene (3 × 40 mL), the residue was taken up in 200 mL ethyl acetate, washed with 2 n HCl (3 × 50 mL), water (1 × 40 mL), saturated sodium bicarbonate (3 × 50 mL), and brine (1 × 40 mL). The organic layers were dried with Na_2SO_4 and rotary evaporation gave an α,β mixture as a pale yellow oil, which was used in the next reaction without further purification. Yield 10 g (25.6 mmol, 92%). Lit.: 100%.[50] R_f = 0.57 (*n*-hexane/EtOAc 1:1).

^{1}H NMR (300 MHz, $CDCl_3$): δ(ppm) = 6.06 (1H, d, J = 1.8 Hz, 1α), 5.84 (1H, d, J = 1.2 Hz, 1β), 5.46 (1H, dd, J = 1.1 Hz, 3.3 Hz, 2β), 5.24–5.34 (3H, m, 2α, 3α, 4α), 5.12 (1H, dd, J = 3.3 Hz, 9.9 Hz, 3β), 4.23–4.32 (2H, m, 4β, 5α), 4.12–4.02 (4H, m, 6aα, 6bα, 6aβ, 6bβ), 3.78 (1H, ddd, J = 2.4 Hz, 5.3 Hz, 9.8 Hz, 5β), 2.19–1.94 (5s, 15H, $-CH_3$).

ESI-MS: (m/z) = 413.52 ($[M+Na]^+$, ber.: 413.11), 803.24 ($[M-M+Na]^+$, ber.: 803.23).

$C_{16}H_{22}O_{11}$ (390.12 g · mol^{-1})

2.3.6. 2,3,4,6-Tetra-*O*-acteyl-D-mannopyranosyl bromide

10 g (25.6 mmol) 1,2,3,4,6-Penta-*O*-acetyl-D-mannopyranoside was dissolved in 25 mL dichloromethane and stirred at room temperature under argon. 20 mL of 33% HBr/acetic acid was added via syringe and the reaction was stirred at room temperature for 2 h. 100 mL of dichloromethane was added and the reaction mixture was extracted with ice-cold water (3 × 200 mL). The organic layer was neutralized by a saturated sodium bicarbonate solution, washed with brine, dried over sodium sulfate and concentrated under reduced pressure to give a yellow oil which was used in the next reaction without further purification. Yield 9.8 g (24 mmol, 93%). Lit.: 81%.[50] R_f = 0.3 (*n*-hexane/EtOAc (6:4)).

^{1}H NMR (300 MHz, $CDCl_3$): δ(ppm) = 6.26 (1H, d, J = 1.1 Hz, 1), 5.67 (1H, dd, J = 3.4 Hz, 10.2 Hz, 3), 5.40 (1H, dd, J = 1.6 Hz, 3.4 Hz, 2), 5.31 (1H, t, J = 10.1 Hz, 4), 4.29 (1H, dd, J = 4.8 Hz, 12.4 Hz, 5), 4.18 (1H, ddd, J = 1.9 Hz, 4.9 Hz, 10.1 Hz, 6a), 4.09 (1H, dd, J = 2.1 Hz, 12.4 Hz, 6b), 2.23–2.03 (12H, 4s, $-CH_3$).

ESI-MS: (m/z) = 412.16 ($[M+H]^+$, ber.: 412.20).

$C_{14}H_{19}O_5Br$ (411.20 g · mol^{-1})

2.3.7. 1-Benzyloxycarbonylamino-3,6,9-trioxaundecan-2,3,4,6-tetra-*O*-acetyl-β-d-mannopyranoside

2.4 g (5.9 mmol) 1,2,3,4,6-Penta-*O*-acetyl-D-mannopyranosyl bromide and 2.9 g (8.9 mmol) 1-(benzoyloxycarbonylamino-11-hydroxy-3,6,9-trioxa) undecan were dissolved in 100 mL dichloromethane. 3 g molecular sieve (3 Å) was added and the reaction mixture was stirred for 30 min at −20 °C and under argon atmosphere in the absence of light. 1.9 g (7.5 mmol) silver trifluoromethane sulfonate was added and the reaction was stirred at room temperature overnight. After neutralizing with triethylamine (pH = 8) the mixture was filtered through Celite, washed with dichloromethane, and dried over $MgSO_4$. The solvent was removed by rotary evaporation and the crude product was purified by column chromatography to obtain a pale yellow oil. Yield 2.7 g (4.1 mmol, 70%). R_f = 0.6 (EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm) = 7.34 (5H, m, Ar–H), 5.34 (1H, dd, J = 3.2 Hz, 10 Hz, 4), 5.28 (1H, d, J = 9.6 Hz, 3), 5.25 (1H, t, J = 1.6 Hz, 2), 5.08 (2H, s, $-O-CH_2-Ar$), 4.85 (1H, d, J = 1.6, 1), 4.28 (1H, dd, J = 5.2 Hz, 12 Hz, 5), 4.18 (1H, t, J = 4.4 Hz, 6a), 4.06 (1H, m, 6b), 3.63–3.53 (14H, m, $-CH_2-CH_2-O-$), 3.38 (2H, q, J = 5.2 Hz, $-CH_2-O-$man), 2.13 (3H, s, $-CH_3$), 2.08 (3H, s, $-CH_3$), 2.02 (3H, s, $-CH_3$), 1.97 (3H, s, $-CH_3$).

ESI-MS: (m/z) = 680.28 ($[M+Na]^+$, ber.: 680.64).

$C_{30}H_{43}NO_{15}$ (657.66 g · mol^{-1})

2.3.8. 11-Amino-3,6,9-trioxa-undecyl-2,3,4,6-tetra-*O*-acetyl-*O*-α-D-mannopyranoside

0.5 g (0.76 mmol) 1-Benzyloxycarbonylamino-3,6,9-trioxaundecan-2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranoside was dissolved in 10 mL methanol. Acetic acid was added until a pH of 4–5 was reached and the reaction mixture was degassed by a slight vacuum. The reaction vessel was flushed with argon and approximately 0.5 g of $Pd(OH)_2/C$ (20%) as catalyst were added in counterflow. After degassing with a slight vacuum the reaction vessel was flooded with H_2 and the mixture was stirred at room temperature overnight. The H_2 atmosphere was changed into an argon atmosphere and the catalyst was removed by filtration. The mixture was neutralized by saturated $NaHCO_3$ solution until a pH value of 8. The solution was concentrated by rotary evaporation. Water was added and lyophilization yielded a pale yellow oil. Yield: 0.4 g (0.76 mmol, 100%). R_f = 0.2 (cyclohexane/EtOH/TEA (2:3:0.15)).

^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm) = 6.61 (br, $-NH_2$), 5.34 (1H, dd, J = 3.2 Hz, 10 Hz, 4), 5.28 (1H, d, J = 9.6 Hz, 3), 5.25 (1H, t, J = 1.6 Hz, 2),

4.87 (1H, d, $J = 1.6$, 1), 4.28 (1H, dd, $J = 5.2$ Hz, 12 Hz, 5), 4.22 (1H, t, $J = 4.4$ Hz, 6a), 4.10 (1H, m, 6b), 3.63–3.53 (14H, m, CH_2–CH_2–O–), 3.05 (2H, m, –CH_2–NH_2), 2.15 (3H, s, –CH_3), 2.09 (3H,s, –CH_3), 2.03 (3H, s, –CH_3), 1.98 (3H,s, –CH_3).

ESI-MS: (m/z) = 524.65 ($[M + H]^+$, ber.: 524.23; 546.20 $[M + Na]^+$, ber.: 546.21).

$C_{22}H_{37}NO_{13}$ (523.23 g · mol^{-1})

2.3.9. Sarcosine *N*-Carboxyanhydride

The synthesis of sarcosine NCA was adapted from literature and modified.[40] 14.92 g (167.4 mmol) of sarcosine were weighed into a three neck-flask and dried in vacuo for 1 h. 300 mL of absolute THF were added under a steady flow of dry nitrogen and 16.2 mL (134 mmol) of diphosgene were added slowly via syringe and the nitrogen stream was reduced. The colorless suspension was mildly refluxed for 3 h yielding a clear solution. Overnight, a steady flow of dry nitrogen was lead through the solution into two gas washing bottles filled with aqueous NaOH solution removing excess phosgene and HCl. The solvent was evaporated under reduced pressure yielding a brownish oil as crude reaction product. The oil was heated to 50 °C and dried under reduced pressure (20 mbar, then $<10^{-2}$ mbar for 2 h) to obtain an amorphous solid free of phosgene and HCl. The crude product was redissolved in 40 mL of absolute THF and precipitated with 300 mL of dry hexane. The solution was cooled to –18 °C overnight to complete precipitation. The solid was filtered under dry nitrogen atmosphere and dried under a stream of dry nitrogen for 60–90 min and afterwards in high vacuum for 2 h in a sublimation apparatus. The crude product was sublimated at 80–85 °C and $<10^{-2}$ mbar. The product was collected from the sublimation apparatus in a glove box on the same day. 12.64 g of purified product (110 mmol; 65% yield; colorless crystallites; melting point: 102–104 °C (lit: 102–105 °C)[51,52]) were stored in a Schlenk tube at –80 °C, only handled in a glove box and used up quickly.

^{1}H NMR (300 MHz, $CDCl_3$): δ(ppm) = 4.22 (2 H, s, –N(CH_3)–CH_2–CO–), 2.86 (3H, s, –CH_3).

2.4.1. *N*-ϵ-Trifluoroacetyl-L-Lysine *N*-Carboxyanhydride

12.5 g (51.6 mmol) of *N*-ϵ-trifluoroacetyl-protected lysine were weighed into a three-neck flask and dried in vacuo for 1 h. The solid was suspended in 300 mL of absolute THF under a steady flow of dry nitrogen. Then, 5.50 mL (45.6 mmol) of diphosgene were added slowly via syringe and the nitrogen stream was reduced. The colorless suspension was mildly refluxed for 2 h, yielding a cloudy solution. Overnight, a steady flow of dry nitrogen was lead through the solution into two gas washing bottles filled with aqueous NaOH solution removing excess phosgene and HCl. The next day, the solution was filtered under dry nitrogen atmosphere. The solvent was evaporated under reduced pressure and absolute THF was added to completely dissolve the crude reaction product. Excess absolute hexane was added to the solution. After 1 h storage at 4 °C, the solid was collected by filtration under dry nitrogen atmosphere and washed with hexane. The crude product was recrystallized twice with absolute THF/hexane. 10.43 g of the purified product (39.0 mmol; 75% yield; colorless needles; melting point: 101 °C (lit: 92–93 °C)[53]) were obtained and stored in a Schlenk tube at –80 °C.

^{1}H NMR (300 MHz, DMSO-D_6): δ(ppm) = 9.40 (1H, s, –NH–CO–CF_3), 9.09 (1H, s, –NH–CO–O–CO–), 4.43 (1H, t, $^3J_{H,H} = 6.3$ Hz, –CO–CH–CH_2–), 3.17 (2H, q, $^3J_{H,H} = 6.3$ Hz, –CH_2–NH–), 1.79–1.61 (2H, m, –CH–CH_2–CH_2–CH_2–CH_2–NH–), 1.53–1.46 (2H, quin, $^3J_{H,H} = 7.13$ Hz, –CH–CH_2–CH_2–CH_2–CH_2–NH–), 1.42–1.24 (2H, m, –CH–CH_2–CH_2–CH_2–CH_2–NH–).

2.4.2. γ-Benzyl-L-Glutamate *N*-Carboxyanhydride

The synthesis was adapted from literature and modified.[54] 10.01 g (42.1 mmol) of benzyl-ester protected glutamine were weighed into a three-neck flask and dried under vacuum for 1 h. The solid was suspended in 200 mL of absolute THF under a steady flow of nitrogen. Then 4.07 mL (33.7 mmol) of diphosgene were added slowly via syringe and the nitrogen stream was reduced. The colorless suspension was mildly refluxed for 1.5 h, yielding a yellowish solution. After the last particles were dissolved a steady flow of dry nitrogen was lead throw the solution for 3 h. The condenser outlet was connected to two gas washing bottles filled with aqueous NaOH, removing excess HCl and phosgene.

The remaining solvent was evaporated under reduced pressure and absolute THF was added to completely dissolve the reaction product. Excess absolute hexane was added to the solution and the latter kept at 4 °C overnight to crystallize. The solid was filtered under dry nitrogen atmosphere and the crude product was recrystallized from THF/hexane twice, washed with hexane, dried in a dry nitrogen flow, and finally in high vacuum. A total of 10.52 g of the purified product (40 mmol; 95% yield; colorless needles; melting point: 93–94 °C (lit: 9394 °C)[51]) was obtained and stored in a Schlenk tube at –80 °C.

^{1}H NMR (300 MHz, $CDCl_3$): δ(ppm) = 9.11 (1H, s, –NH–), 7.42–7.20 (5H, m, –C_6H_5), 5.10 (2H, s, –CH_2–C_6H_5), 4.52–4.42 (1H, dd ($^3J_{H,H} = 7.9$), 5.5 Hz, –CO–CH–NH–), 2.52–2.48 (2H, t ($^3J_{H,H} = 7.9$ Hz), BnO–CO–CH_2–), 2.15–1.85 (2H, m, –CH_2–CH–).

2.4.3. (Man)-PSar$_n$

N eq. of PSar NCA were weighed into a Schlenk tube, dried under vacuum for 1 h and dissolved in absolute DMF. A stock solution of either mannose initiator or neopentylamine in absolute DMF was prepared in a separate Schlenk tube. Both solutions were joined via syringe to yield a final monomer concentration of 0.1 g · mL^{-1}. The polymerization solution was stirred at room temperature for 3 d and kept at a constant pressure of 1.25 bar of dry nitrogen via the Schlenk line to prevent impurities from entering the reaction vessel while allowing CO_2 to escape. Completion of the reaction was confirmed by IR spectroscopy (disappearance of the NCA peaks (1 853 and 1 786 cm^{-1})). Directly after completion of the reaction the polymer was precipitated in cold diethyl ether and centrifuged (4 500 rpm at 4 °C for 15 min). After discarding the liquid fraction, new ether was added and the polymer was resuspended using a sonic bath. The suspension was centrifuged again and the procedure was repeated. After DMF removal by the resuspension steps, the polymer was dissolved in water and lyophilized to obtain a stiff, porous, colorless solid. Yield: 88.9%.

^{1}H NMR of Man-PSar$_n$ (400 MHz, D_2O): δ(ppm) = 5.65 (1H, d, $^3J_{H-H} = 2.29$ Hz, man.ini), 5.35 (1H, dd, $^3J_{H-H} = 4.17$ Hz, $^3J_{H-H} = 5.87$ Hz, man.ini), 5.10 (1H, t, $^3J_{H-H} = 10.16$ Hz, man.ini), 4.52–3.95 (2nH, br, –NCH_3–CH_2–CO–), 3.70–3.47 (14H, br, man.ini), 3.19–2.60 ((3n + 2) H, br, –NCH3– and man.ini), 2.10–1.95 (12H, br, –CO–CH_3 of man. ini).

2.4.4. (Man)-PSar$_n$-*b*-PGlu(OBn)$_m$

1 eq. of previously prepared homopolymer was weighed into a Schlenk tube, dried under vacuum for 1 h and dissolved in absolute DMF (0.1 g · mL^{-1}). In a separate Schlenk tube m eq. of Glu(OBn) NCA were dried under vacuum and dissolved in absolute DMF (0.1 g · mL^{-1}). Both solutions were joined via syringe. Polymerization was carried out for 3 d at 0 °C under dry nitrogen atmosphere. Completion of the reaction was monitored by IR spectroscopy (disappearance of the NCA peaks (1 853 and 1 786 cm^{-1})). Directly after completion of the reaction the polymer was precipitated to cold diethyl ether and centrifuged (4 500 rpm at 4 °C for 15 min). After discarding the liquid fraction, new ether was added and the polymer was resuspended using a sonic bath. The suspension was centrifuged again and the procedure was repeated. After DMF removal by the resuspension steps, the polymer was suspended in water and lyophilized to obtain a stiff, porous, colorless solid. Yield: 93.7%.

^{1}H NMR of Man-PSar$_n$-*b*-PGlu(OBn)$_m$ (400 MHz, DMSO-D$_6$): δ (ppm) = 7.41–7.07 (5mH, br, –C$_6$H$_5$), 5.65 (1H, d, $^3J_{H\text{-}H}$ = 2.31 Hz, man.ini), 5.34 (1H, dd, $^3J_{H\text{-}H}$ = 3.90 Hz, $^3J_{H\text{-}H}$ = 6.11 Hz, man.ini), 5.16–4.85 ((2m + 1)H, br, –CH$_2$–C$_6$H$_5$ and man.ini), 4.60–3.65 ((2n + 1m)H, br, –NCH$_3$–CH$_2$–CO– and –CO–CH–NH–), 3.54–3.47 (14H, br, man.ini), 3.17–2.62 ((3n + 2)H, br, –NCH3– and man.ini), 2.31–1.66 ((4m + 12)H, br, –CH$_2$–CH$_2$–COOBn and –CO–CH$_3$ of man. ini).

2.4.5. (Man)-PSar$_n$-*b*-PLys(TFA)$_m$

(Man)-PSar$_n$-b-PLys(TFA)$_m$ was prepared similarly to the synthesis of (Man)-PSar$_n$-*b*-PGlu(OBn)$_m$ by using Lys(TFA) NCA instead of Glu (OBn) NCA. Yield: 90.0%.

^{1}H NMR of Man-PSar$_n$-*b*-PLys(TFA)$_m$ (400 MHz, DMSO-D$_6$): δ (ppm) = 9.49–9.21 (1mH, br, –NH–CO–CF$_3$), 5.65 (1H, d, $^3J_{H\text{-}H}$ = 2.29 Hz, man.ini), 5.35 (1H, dd, $^3J_{H\text{-}H}$ = 3.94 Hz, $^3J_{H\text{-}H}$ = 6.07 Hz, man.ini), 5.05 (1H, t, $^3J_{H\text{-}H}$ = 9.92 Hz, man.ini), 4.72–3.62 ((2n + 1m)H, br, –NCH$_3$–CH$_2$–CO– and –CO–CH–NH–), 3.50 (14H, br, man.ini), 3.23–3.05 (2mH, br, –CH$_2$–NH–CO–CF$_3$), 3.04–2.56 ((3n + 2)H, br, –NCH3– and man.ini), 2.15–0.90 ((6m + 12)H, br, –CH–CH$_2$–CH$_2$–CH$_2$–CH$_2$–NH– and –CO–CH$_3$ of man.ini).

2.4.6. End Group Labeling of (Man)-PSar$_n$-*b*-PGlu(OBn)$_m$ with Oregon Green 488

In a glass vial, 42.06 mg of Man-PSar$_{277}$-*b*-PGlu(OBn)$_{28}$ were dissolved in 1 mL of DMSO under dry nitrogen atmosphere. 1.2 eq. of Oregon Green 488 NHS ester (with respect to the terminal amino group of the polymer) dissolved in DMSO were added via syringe. The solution was protected from light and stirred for 3 d at 40 °C under dry nitrogen atmosphere. After that, the labeled polymer was directly subjected to dialysis against water for 3 d. Subsequent lyophilization yielded 41 mg (yield: 95.6%) of labeled polymer as fluffy, yellow solid.

2.4.7. Selective Deprotection of the Mannose Group of Labeled Man-PSar$_n$-*b*-PGlu(OBn)$_m$

30.3 mg of Oregon Green 488-labeled polymer were suspended in 1 mL NaOH$_{(aq)}$ solution adjusted to pH 9. The suspension was stirred for 18 h at room temperature and subsequently subjected to dialysis against Millipore water for 3 d. To remove traces of free dye remaining from the labeling reaction the lyophilized polymer was further purified by reverse phase HPLC. Subsequent lyophilization yielded 19.04 mg (yield: 63.2%) of selectively deprotected polymer as fluffy, yellow solid.

^{1}H NMR of Man$_{depr}$-PSar$_n$-*b*-PGlu(OBn)$_m$ (400 MHz, DMSO-D$_6$): δ (ppm) = 7.56–7.05 (5mH, br, –C$_6$H$_5$), 6.91 (d, $^3J_{H\text{-}H}$ = 7.64 Hz, OG 488), 6.59 (dd, $^3J_{H\text{-}H}$ = 2.37 Hz, $^3J_{H\text{-}H}$ = 11.30 Hz, OG 488), 5.62 (1H, man. ini), 5.23–4.84 (2mH, br,–CH$_2$–C$_6$H$_5$), 4.65–3.67 ((2n + 1m + 3)H, br, –NCH$_3$–CH$_2$–CO– and –CO–CH–NH– and man.ini), 3.54–3.47 (14H, br, man.ini), 3.10–2.62 ((3n + 2)H, br, –NCH3– and man.ini), 2.41–1.60 ((4m)H, br, –CH$_2$–CH$_2$–COOBn), 1.61–0.98 (br, OG 488), 0.91–0.74 (br, OG 488).

2.4.8. Full Deprotection of Man-PSar$_n$-*b*-PLys(TFA)$_m$

In a glass vial, 30 mg of diblock copolymer were dissolved in 1 mL of Methanol. 10 eq. of hydrazine hydrate (with respect to acetyl and TFA groups) were added and the solution was stirred for 3 d at room temperature. After that, the deprotected polymer was directly subjected to dialysis, first against NaHCO$_{3(aq)}$, then against millipore water. Subsequent lyophilization yielded the labeled polymer as fluffy, colorless solid. Yield: 81.3%.

^{1}H NMR of Man$_{depr}$-PSar$_n$-*b*-PLys$_m$ (400 MHz, D$_2$O): δ(ppm) = 5.50 (1H, d, $^3J_{H\text{-}H}$ = 1.89 Hz, man.ini), 4.58–3.88 ((2n + 1m)H, br, –NCH$_3$–CH$_2$–CO– and –CO–CH–NH–), 3.73–3.46 (14H, br, man.ini), 3.24–2.50 ((3n + 2m + 2)H, br, –NCH3– and –CH$_2$–NH$_2$ and man.ini), 1.96–1.00 (6mH, br, –CH–CH$_2$–CH$_2$–CH$_2$–CH$_2$–NH$_2$).

2.4.9. Preparation of Micelles

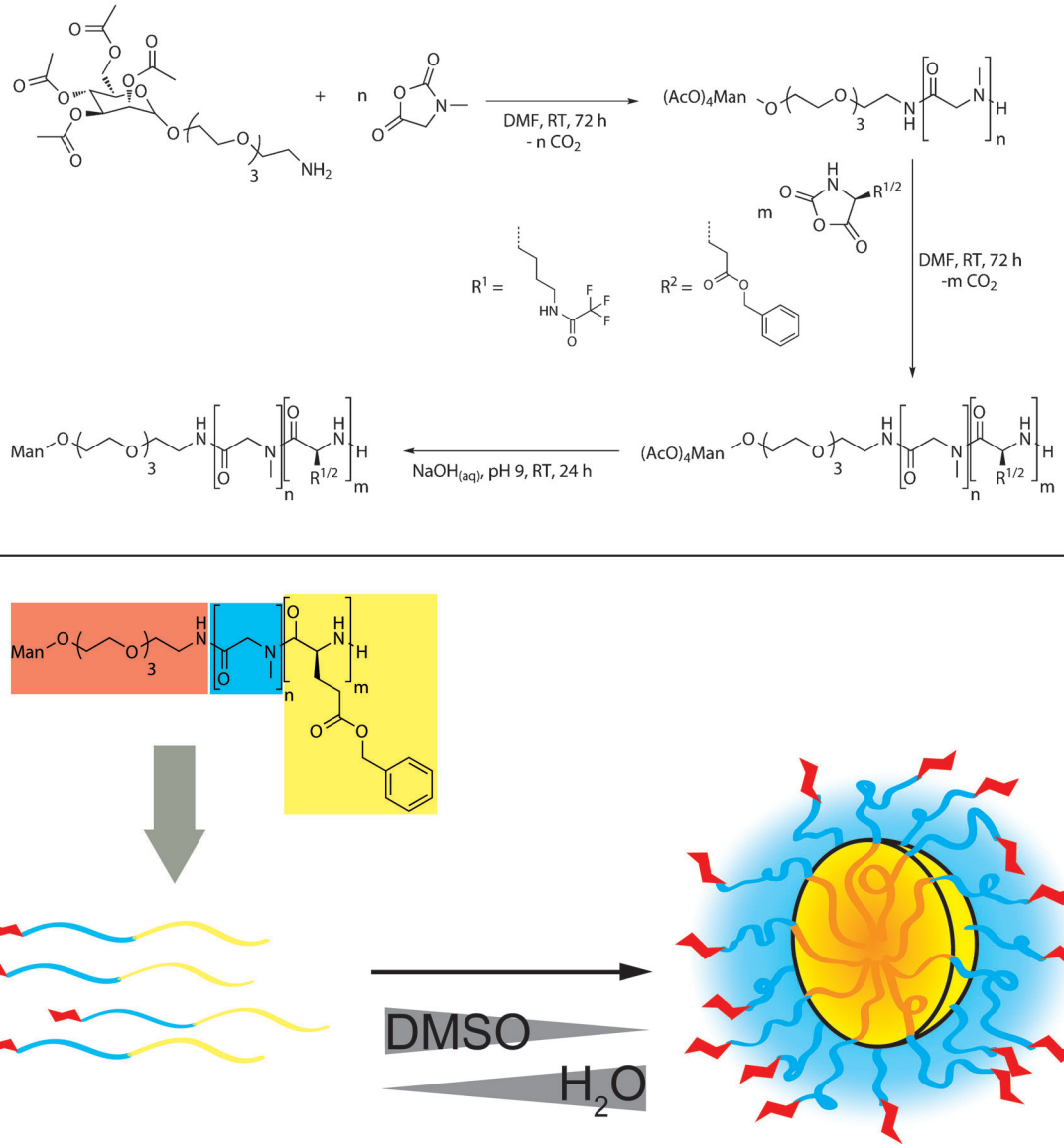
8.54 mg of (Man)-PSar$_n$-*b*-PGlu(OBn)$_m$ were dissolved in 2.2 mL of DMSO. Under constant stirring 7.8 mL of Millipore water were added slowly via a syringe pump (0.5 mL · min^{-1}). The micelle solution was dialyzed against Millipore water for 3 d. After lyophilization micelle solutions with a final concentration of 2 mg · mL^{-1} were prepared by dissolving the micelles in the appropriate amount of PBS buffer.

2.5.1. Cellular Uptake of Micelles

On day one, 150.000 DC 2.4 cells or BMDCs in 500 μL of medium were seeded onto a 24 well plate, respectively. The next day, 20 μg of micelles per well were added. After 1 h, cells were harvested with PBS EDTA buffer (DC 2.4) or by frequent pipetting (BMDCs). Subsequently, cells were washed two times with PBS EDTA buffer, fixed with PBS buffer containing 0.7% PFA and subjected to FACS analysis.

2.5.2. Determination of Cellular Toxicity of PeptoMicelles in DC 2.4 Cells by MTT Assay

Cytotoxicity of PeptoMicelles was assessed by MTT assay using the "CellTiter 96® Non-Radioactive Cell Proliferation Assay" kit (Promega, Madison, USA) according to manufacturer's protocol. DC 2.4 cells were seeded onto 96-well culture plates at a concentration of 5×10^4 cells per well. Cells were incubated with 10/50 μg micelles of P2 in a final volume of 100 μL



Scheme 1. Synthesis of mannose-functionalized PSar-*block*-PGlu(OBn) or PSar-*block*-PLys(TFA) block copolypept(o) ides, selective deprotection of mannose end groups and self-assembly of Man-PSar-*block*-PGlu(OBn) copolymers into PeptoMicelles targeting mannose-binding receptors.

(hexaplicates) for 24 h. The next day, 30 μL of MTT substrate solution were added to each well and cells were cultured for another 4 h. Subsequently, 100 μL of "solubilization solution/stop mix" were added to each well. After 1 h, the absorbance at 570 nm was measured.

2.5.3. Blocking Studies with Mannan and Dynasore-OH

On day one, DC2.4 cells were seeded out as indicated above. The next day, cells were incubated with mannan (0.2/2 mg · mL^{-1}) for 2 h or with dynasore-OH (20 μM) for 30 min. Cells were washed four times with medium and micelles (50 μg per well) or ovalbumine (20 μg per well) were added. After one hour cells were harvested as indicated above, fixed with PBS buffer containing 0.7% PFA and subjected to FACS analysis.

2.5.4. Cell Culture

DC 2.4 cells were cultivated at 37 °C, 5% CO_2 atmosphere in RPMI medium supplemented with 10% FCS (PAA, Cölbe, Germany), 2 mM L-glutamine (Biochrom AG, Berlin, Germany), 100 U · mL^{-1} penicillin, 100 μg · mL^{-1} streptomycin (Gibco, Paisly, UK) and 50 μM β-mercaptoethanol (Roth, Karlsruhe, Germany). Medium was changed regularly.

Bone marrow-derived DCs (BMDCs) were differentiated from bone marrow progenitors of C57BL/6 mice as first described by

Table 1. Characteristics of synthesized block copolymers and micelles.

	Polymer	Calculated block ratio	Block ratio[a)]	$\overline{M}n$[b)]	Đ[b)]	R_h [nm][c)]	μ_2[c)]
P1	Man-PSar-*b*-PGlu(OBn)	300:30	277:28	46.2	1.13	63.7	0.13
P2	PSar-*b*-PGlu(OBn)	300:30	309:25	40.7	1.12	75.8	0.15
P3	Man-PSar-*b*-PLys(TFA)	300:30	319:29	49.4	1.17	nd	nd

[a)]As determined by ^{1}H NMR spectroscopy in DMSO-D_6; [b)]as determined by GPC with HFIP as solvent and PMMA standards; [c)]as determined by DLS in PBS buffer, $c = 0.0667\ g \cdot L^{-1}$.

Scheicher et al.[55] and modified by Gisch et al.[56] DC culture medium (IMDM with 5 vol.-% FCS (PAA, Cölbe, Germany), 2 mM L-glutamine (Biochrom AG, Berlin, Germany), 100 U · mL^{-1} penicillin, 100 µg · mL^{-1} streptomycin (Gibco, Paisly, UK)) and 50 µM β-mercaptoethanol (Roth, Karlsruhe, Germany), supplemented with 5% of mGM-CSF containing cell culture supernatant derived from X63. Ag8-653 myeloma cells stably transfected with a murine GM-CSF expression construct;[57] a kind gift by Dr. B. Stockinger, National Institute for Medical Research, London) was replenished on days 3 and 6 of culture. BM cells were seeded on 100 mm^2 bacterial dishes (2×10^6 cells/10 mL) and cultivated at 37 °C, 10% CO_2 atmosphere. Aliquots of non-adherent and loosely adherent immature BM-DCs were harvested on days 6 or 7 of culture and were reseeded (10^6 cells · mL^{-1}) on wells of six well tissue-culture plates (StarLab, Hamburg, Germany).

3. Results and Discussion

As mentioned above, PSar as well as various other polypept(o)ides are accessible by nucleophilic ring-opening polymerization of α-amino acid *N*-carboxyanhydrides (NCAs) as well as *N*-thiocarboxyanhydrides (NTAs).[48] In this approach functional amines can be employed as long as they do not interfere with the polymerization itself. Therefore, we have synthesized an acetylated mannose derivative, in which a primary amine is linked with a glycol spacer to the cyclic hexose moiety by an α-glycosidic bond. The α-glycosidic linkage is known to be of major importance for any biorecognition process.[58] During synthesis, the neigboring group effect ensures the formation of the α-glycosidic linkage, which can be clearly seen in the coupled HSQC measurement (see Supporting Information, Section 3.8). In the next step, the primary amine of the mannose derivative was used to initiate the sequential polymerization of sarcosine (Sar), lysine(TFA) (Lys(TFA)), and glutamic acid(OBn) (Glu(OBn)) NCAs (see Scheme 1).

The polymerizations proceeded in a well-controlled manner yielding block copolypept(o)ides with narrow monomodal molecular weight distributions and low dispersities between 1.13 and 1.17 (see Table 1).

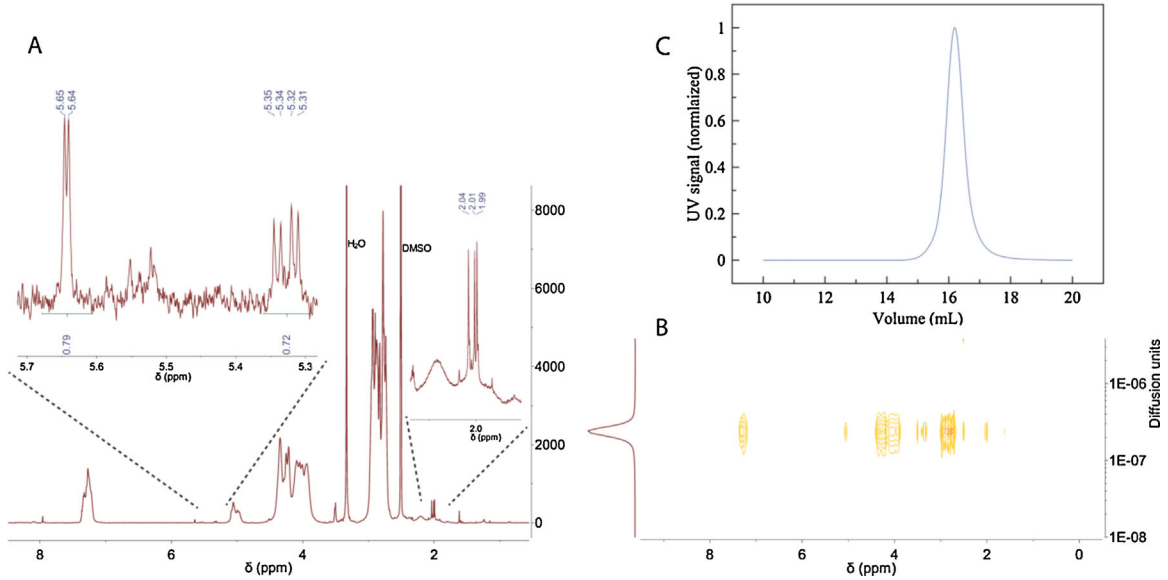


Figure 1. (A) 400 MHz ^{1}H NMR of Man-PSar$_{277}$-*block*-PGlu(OBn)$_{28}$ (P1) showing mannose end group-derived signals, (B) DOSY NMR of Man-PSar$_{277}$-*block*-PGlu(OBn)$_{28}$ proving the linkage between mannose end group and polymer, (C) GPC plot of Man-PSar$_{277}$-*block*-PGlu(OBn)$_{28}$ showing unimodal weight distribution.

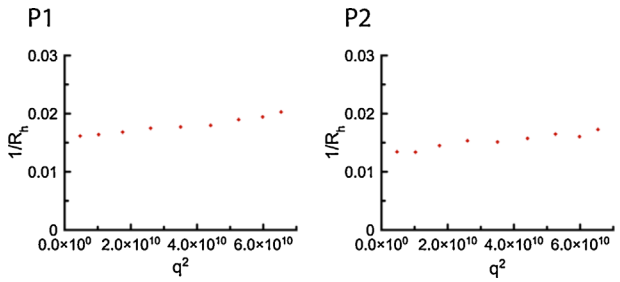


Figure 2. Dynamic light scattering of PeptoMicelles P1 ($R_h = 63.7$ nm) and P2 ($R_h = 75.8$ nm).

The ^{1}H NMR signal of the acetyl protecting groups can be easily integrated and related to the polysarcosine backbone protons enabling precise determination of the degree of polymerization.

Diffusion-ordered spectroscopy (DOSY) NMR experiments displayed only one diffusing species, which reflects the absence of free initiators or low molecular weight polymers (see Figure 1).

Polymer-analogous reactions such as labeling of polymers with dyes (see experimental part Section 2.4.6) are easy to conduct by using the terminal amino groups of the protected polypeptide blocks. In case of Man-PSar-*b*-PGlu (OBn), the chosen protecting groups enable the selective deprotection of the glycoside using $NaOH_{(aq)}$, pH 9. Specificity of these deprotection conditions was verified by relating the benzyl ester-derived ^{1}H NMR signals before and after deprotection of the mannose end group (see Supporting Information Section 4.1 and 4.3). In case of Man-PSar-*b*-PLys(TFA) the simultaneous deprotection of glycoside and polymer is feasible by using hydrazine hydrate (see Supporting Information Section 4.2 and 4.4). Therefore, this versatile synthetic pathway can be used for the generation of labeled mannose-functionalized PeptoMicelles as well as of PeptoPlexes.

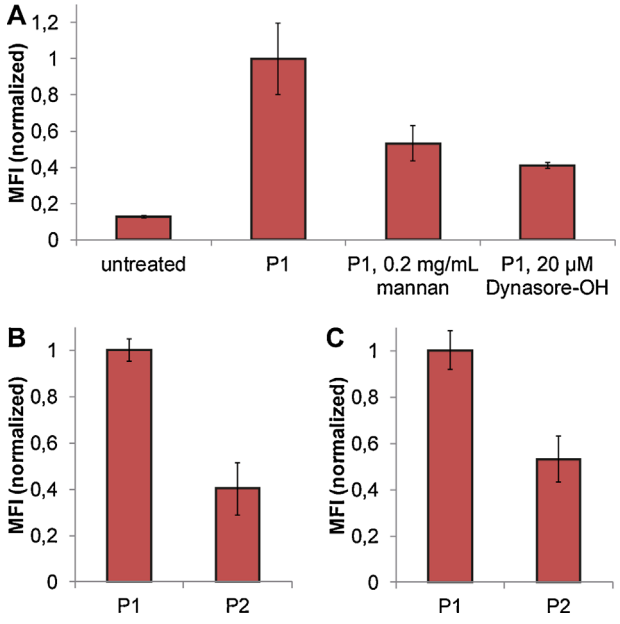


Figure 3. Uptake studies with P1 and P2. (A) Blocking experiment with DC 2.4 cells. (B) Comparison of uptake efficiency with DC 2.4 cells. (C) Comparison of uptake efficiency with bone marrow-derived dendritic cells (BMDCs). ($n = 3$).

For preparation of PeptoMicelles labeled block copolymers P1 or P2 were diluted in DMSO and the solvent was gradually switched to H_2O (see Scheme 1). Characterization of micelles by dynamic light scattering in both cases revealed a minor angle-dependency which is most likely related to dispersity of the micelles (see Figure 2). Hydrodynamic radii were 63.7 nm for P1 and 75.8 nm for P2. These particle sizes are still likely to allow entering cells by clathrin-mediated endocytosis.[59]

After DLS measurements PeptoMicelles were subjected to biological evaluation. Initially, the cellular uptake of mannose-functionalized PeptoMicelles was investigated using DC 2.4 cells. This cell line is well established as model for DCs and is known to express mannose-binding receptors (MRs).[60] Consequently, it has been utilized for uptake studies with mannose-modified nanoparticles in many publications.[60–64]

MTT assay performed with DC 2.4 cells did not display any decrease in cell viability related to the presence of PeptoMicelles at concentrations up to 0.5 mg · mL^{-1} (see Supporting Information Section 4.8), which appears well above suitable concentrations for modulation of DC-function in vivo. MR binding of mannose-functionalized PeptoMicelles and subsequent MR-mediated endocytosis were verified with a dual strategy. DC 2.4 cells were preincubated with mannan to saturate MRs and therefore intercept binding and endocytosis of functionalized PeptoMicelles on receptor level. To interrupt endocytosis on a more downstream level, cells were preincubated with dynasore-OH which does not interfere with MR binding of PeptoMicelles but prevents scission of endocytic vesicles by targeting dynamin-1 and dynamin-2.[65] Experiments for cells pretreated in this manner were conducted with mannosylated PeptoMicelles (P1) and with ovalbumin (OVA) as model protein for MR-mediated uptake[66] (see Figure 3A and Supporting Information Section 4.7). Blocking with mannan led to a slight decrease of binding/uptake for OVA (approx. 25%), while binding/uptake of P1 was lowered to around 50% of starting level. The still existing binding/uptake of OVA and P1 may be related to incomplete saturation of MRs, mannan-triggered recruitment/recycling[67,68] of additional MRs to the cell surface and action of alternative uptake pathways like macropinocytosis and phagocytosis. In line, binding/uptake of OVA and P1 was not reduced any further by higher mannan concentrations but increased again (data not shown), also suggesting recruitment of additional MRs. After

preincubation of cells with dynasore-OH, the uptake of OVA and P1 was diminished by 80% and 60%, respectively. This finding confirms that P1—like OVA—is mainly taken up via clathrin-mediated endocytosis which is known to be the endocytic mechanism behind MR-driven uptake.[67] As in case of mannan, the still existing uptake of OVA and P1 after pretreatment of cells with dynasore-OH may be attributable to alternative uptake routes.

After confirmation of MR-governed uptake of P1 we investigated its performance in relation to non-mannosylated PeptoMicelles (P2). In addition to DC 2.4 cells, bone marrow-derived dendritic cells (BMDCs) were used as representative of MR-expressing DCs derived from primary progenitors. As expected, P1 showed a significantly higher cellular interaction compared to P2 in case of DC 2.4 cells (Figure 3C) and BMDCs (Figure 3D). In both cell types the binding/uptake of P1 exceeded the binding/uptake of P2 by approximately 70%. Furthermore, the uptake ratio between P1 and P2 was comparable to that between P1 with and P1 without mannan/dynasore-OH preincubation.

All these findings point to an enhancement of uptake of P1 by interaction with MRs in comparison with P2. Unspecific mechanisms such as macropinocytosis contribute considerably to the uptake of PeptoMicelles, irrespective of their mannosylation. These results are in line to reports of other groups who describe mannosylation-related increase in uptake of—in this case—PLGA or dextran particles yet also observing unspecific uptake.[62,69] Though, mannosylation of PeptoMicelles induces a substantial increase in cellular uptake, which may enable targeting of MR-expressing cells like DCs or macrophages.

4. Conclusion

In this work we demonstrated a versatile synthetic pathway to block copolypept(o)ides with bioactive end groups. The derived block copolymers possess low dispersity indices around 1.1 ensuring well-controlled polymerization. The reported pathway enables the synthesis of amphiphilic block copolymers or block ionomers with mannose end groups using different protecting groups (orthogonally or simultaneously cleavable). These polymers can self-assemble into polymeric core-shell structures, e.g. micelles, which were successfully used in uptake experiments with DC 2.4 cells as well as BMDCs. Our findings provide first evidence that mannosylated PeptoMicelles possess the potential to target MRs on cells of the innate immune system.

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