Electronic Supplementary Material (ESI) for Chemical Communications

Supporting information for

Dual-purpose PEG scaffolds for the preparation soft and biofunctional hydrogels: the convergence between CuAAC and Thiol-Ene reactions

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Supporting figures and tables

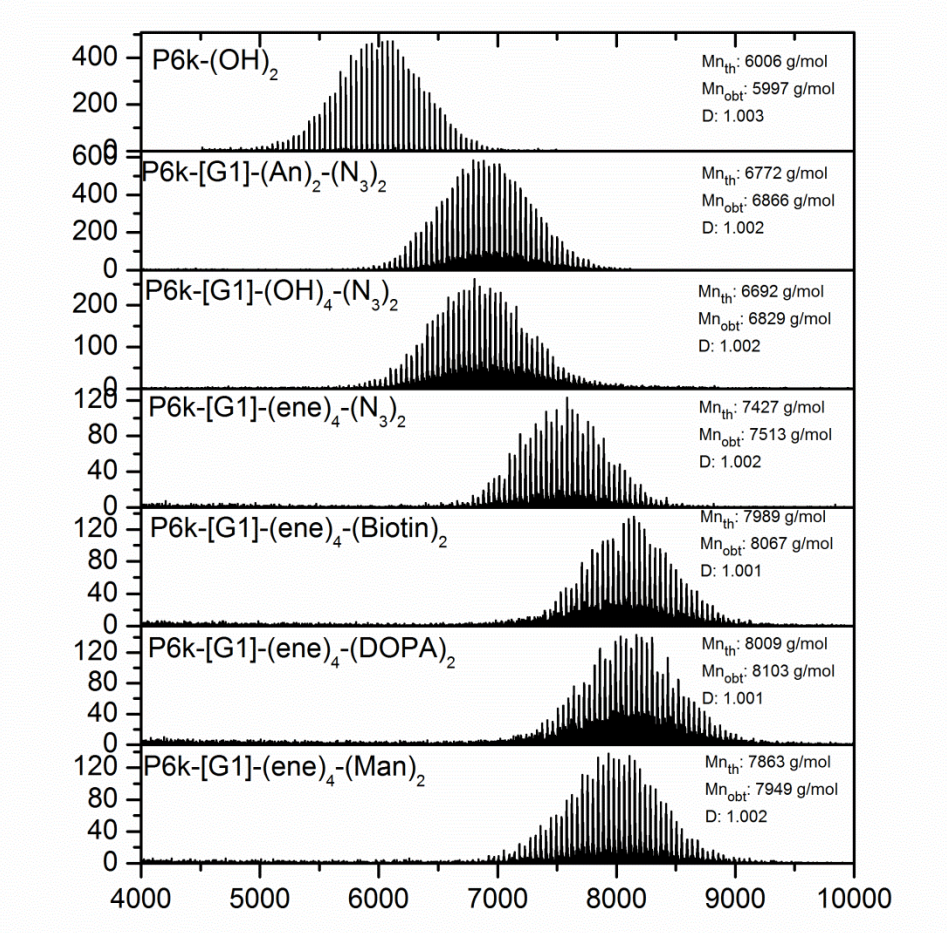


Figure S1. MALDI-ToF following the synthesis of the P6k-G1 series. Inset molecular weights (Mn) and dispersities (D) were obtained using Polytools v. 1.0.

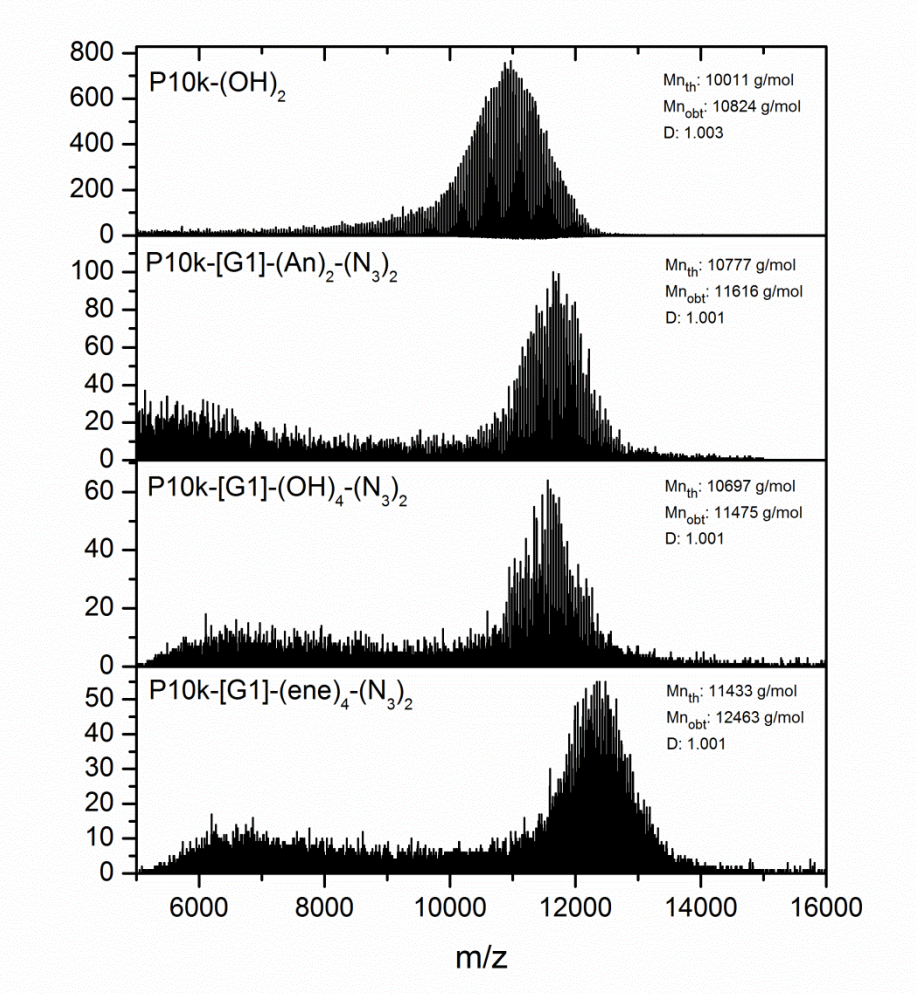


Figure S2. MALDI-ToF following the synthesis of the P10k-G1 series. Inset molecular weights (Mn) and dispersities (D) were obtained using Polytools v. 1.0.

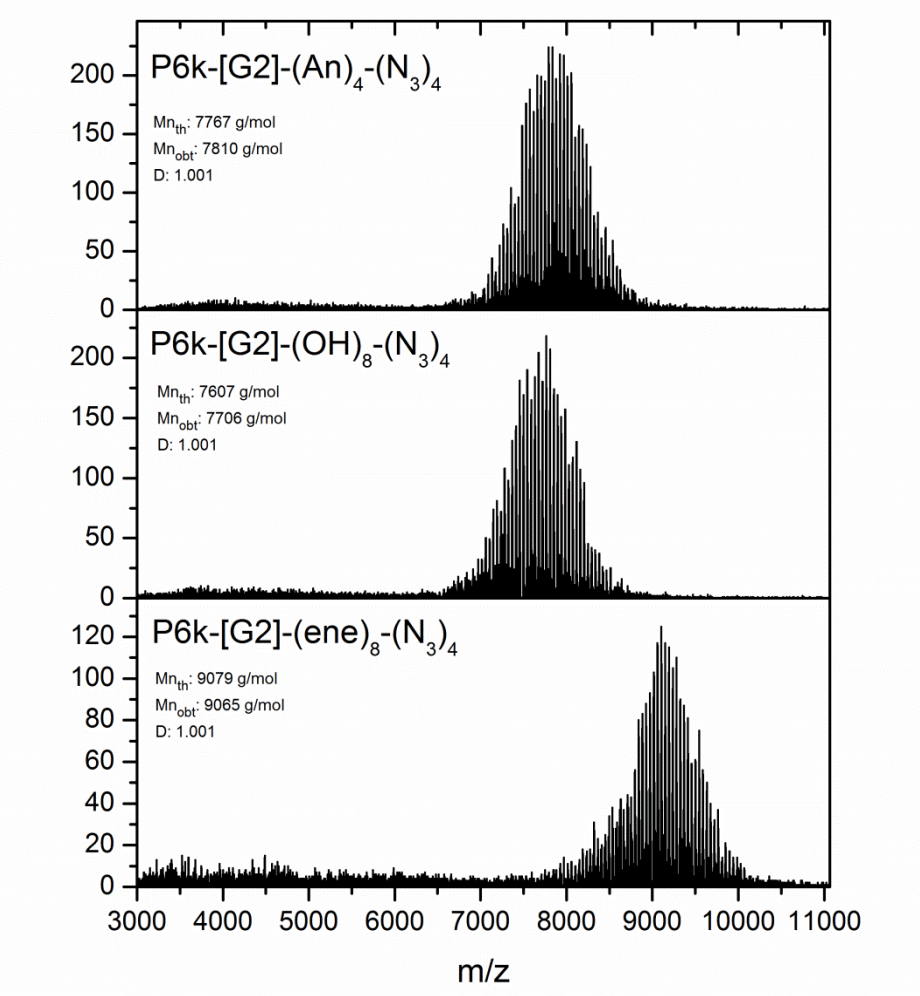


Figure S3. MALDI-ToF following the synthesis of the P6k-G2 series. Inset molecular weights (Mn) and dispersities (D) were obtained using Polytools v. 1.0. MALDI-ToF spectra for P6k-[G2]-$(ene)_8$-$(Man)_4$ **16** could not be obtained.

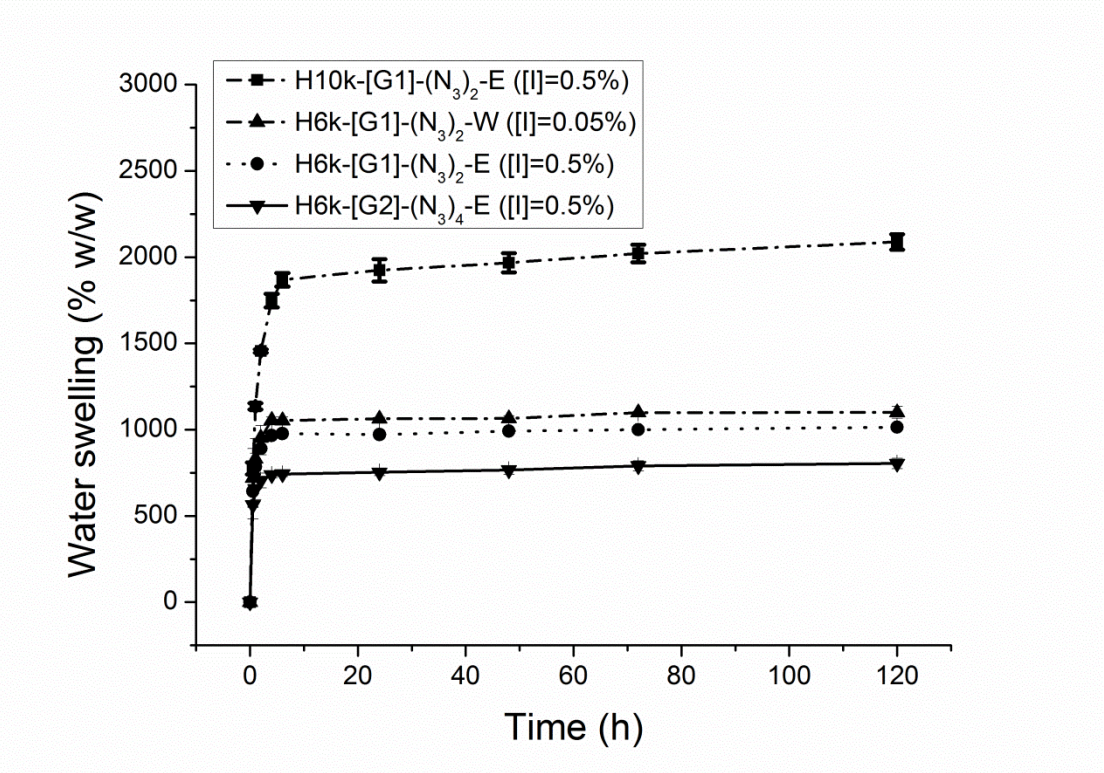


Figure S4. Water swelling profiles of azide functional hydrogels.

Table S1. GF-AAS measurements of residual copper concentrations at different stages of purification (treatment).

Material	Treatment	GF-AAS [Cu] (ppb (µg/L))	Sample concentration (mg/mL)	Cu level in dry material(ppm)	Effective hydrogel conc.[a] (ppm (mg/L))
P6k-[G1]-[ene]$_4$-(Man)$_2$	CuAAC conc (theoretical max)	-	-	34760[b]	1931.11
P6k-[G1]-(ene)$_4$-(Man)$_2$	Al_2O_3 column	13910 ± 90	5.5	2529.09 ± 16.36	140.51
P6k-[G1]-[ene]4-(Man)$_2$	Al_2O_3 column +Dialysis with 0.5 wt% EDTA	25.36 ± 1.35	5.19	4.89 ± 0.26	0.27
Hydrogel: P6k-[G1]-(ene)$_4$-(Man)$_2$ + P2k-(SH)$_2$	Al_2O_3 column + Dialysis of gel with 0.5 wt% EDTA	20.76 ± 2.86	5.87	3.54 ± 0.49	0.20
P6k-[G1]-(ene)$_4$-(DOPA)$_2$	Al_2O_3 column +Dialysis with 0.5 wt% EDTA	61.59 ± 3.76	5.2	11.84 ± 0.72	0.66
P6k-[G2]-(ene)$_8$-(Man)$_4$	Al_2O_3 column +Dialysis with 0.5 wt% EDTA	35.03 ± 2.31	5.1	6.87 ± 0.45	0.38
Blank		0.15 ± 0.123			
Detection limit: 3 × 0.123 = 0.369 µg/L					

[a] Calculated assuming an average swelling degree of 1000% for all P6k-G1 gels and 800% for P6k-G2 gels.

[b] Theoretical maximum values based on CuAAC reaction conditions used.

Experimental

Materials

All chemicals were purchased and used as received from Sigma-Aldrich unless otherwise noted. Flash chromatography was performed using 32-64 D 60 Å silica gel from ICN SiliTech (ICN Biomedicals GmbH, Eschwege, Germany). Irgacure 2959 was purchased from Ciba. 2,2-bis(methylol)propionic acid (bis-MPA) was kindly donated from Perstorp AB.

Nomenclature

Acet = Acetylene

An = Acetonide

Bis-MPA = 2,2-bis(methylol)propionic acid

DMAP = 4-Dimethylaminopyridine

DOPA = L-Dopamine

ene = Alkene

EtOAc = Ethyl acetate

EtOH = Ethanol

[G#]= Dendron generation, # = 1, 2

Hep =Heptane

Man = α-D-Mannose

MeOH = Methanol

N_3 = Azide

OH = Hydroxyl

PXk = PEG or Poly(ethylene glycol) followed by molecular weight in kDa (e.g. P6k = PEG 6 kDa)

TEA = Triethylamine

Methods

MALDI-TOF. Matrix-assisted laser desorption ionization time-of flight mass spectroscopy (MALDI-ToF MS) was conducted on a Bruker UltraFlex MALDI-TOF MS with a SCOUT-MTP Ion Source (Bruker Daltonics) equipped with a N_2-laser (337 nm), a gridless ion source and a reflector. All spectra were acquired using a Linear-positive method with an acceleration of 25 kV. The laser intensity was set to the lowest value possible to acquire high-resolution spectra. The instrument was calibrated using SpheriCal^{TM} calibrants purchased from Polymer Factory Sweden AB. A THF solution of HABA or DHB (10 mg/mL) doped with sodium trifluoroacetate was used as matrix. The obtained spectra were analyzed with FlexAnalysis Bruker Daltonics version 2.2 and Polytools version 1.0.

^{1}H NMR and ^{13}C NMR. NMR experiments were performed on a Bruker Avance 400 MHz instrument. ^{1}H-NMR spectra were acquired with a spectral window of 20 ppm, an acquisition time of 4 seconds, and a relaxation delay of 1 second. ^{13}C NMR spectra were acquired with a spectral window of 240 ppm, an acquisition time of 0.7 seconds, and a relaxation delay of 2 seconds.

General procedure for hydrogel formation. All hydrogels were prepared by dissolving PEG-dendritic hybrid materials decorated with ene functionality along with P2k-$(SH)_2$ (equimolar rates of ene:thiol) in either MQ water or EtOH at 30 wt% (w/w) of dry material. To the PEG-precursor solution photoinitiator Irgacure 2959 (I) was added (dissolved in a small amount of EtOH, final [I] = 0.5 wt% or 0.05 wt% and in water based hydrogels [EtOH] find < 0.5 wt%) after which the solution was thoroughly mixed by vortexing. The mixture was then pippeted into an appropriate mold (e.g. Teflon disc mold, cover glass slip, cell culturing well) and cured by shining UV-light for 10 min (100W Blak Ray B-100AP Hg UV lamp (365 nm) at 9 mW/cm^2 intensity as determined with an Uvicure Plus High Energy UV Integrating Radiometer (EIT, USA), measuring UVA at 320–390 nm). All hydrogels used for the below described analysis were made as triplicate samples (n=3).

GF-AAS. The copper levels were analyzed by graphite furnace atomic absorption spectroscopy (GF-AAS) (Perkin Elmer AAnalyst 800). Measured results were based on three replicate readings of each

sample and quality control samples of known concentrations analyzed consecutively. All results are presented with the metal concentrations of the blank reference solutions subtracted. The limit of detection (based on three times the standard deviation of blank reference samples) was 0.37 µg/L. Calibration was done with ultrapure water and with standards of known concentrations; 10, 50, and 100 µg/L. Lyophilized samples (either hydrogel or precursor polymer) were prepared by dissolving in nitric acid (pH 2.0) at 5-7 mg/mL and leaving to degrade the polymer/hydrogel structures for a period of 48h prior to analysis.

Swelling. Hydrogels were dried at 50°C under vaccum for 12 h before swelling in deionized water and the degree of swelling (time points: 0.5, 1, 2, 4, 6, 24, 48, 72, 120 h) was calculated according to:

$$\%\ swelling = \frac{m_{swollen} - m_{dry}}{m_{dry}} \times 100$$

Were $m_{swollen}$ and m_{dry} are the weights in the swollen and dry state respectively.

Rheology. Hydrogel rheological measurements were performed using an ARES RDA-III rheometer, TA with ϒ = 5% and ω= 10 rad/s for the linear viscoelastic regime. Thin hydrogel films, which had been cured between two glass cover-slips separated by 0.5 mm spacers, swelled for 72 h in aqueous media and punched out as Ø 25 mm circular discs, were placed between the Ø 25 mm parallel plates of the instrument and the hydrogel storage modulus (G') was recorded (for these type of hydrogel networks G ≈ G' since G' > G''). The storage modulus was furthermore converted to Young's modulus (*E*) according to rubber elasticity theory, where G = *E*/2(1+*v*), assuming a Poisson's ratio (*v*) of 0.5 for bulk measurements of elastic hydrogel polymer networks.

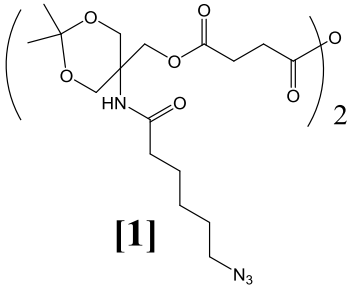
Cell culture conditions. Normal human dermal fibroblasts (NHDF; Karocell Tissue Engineering AB, Stockholm, Sweden) were cultured at 37°C in humidified 5% CO_2 in complete medium composed of Dulbecco's modified Eagle's medium with GlutaMAX™ supplemented with 5% FCS. Culture medium was changed three times weekly. At confluence, cells were washed and split at a ratio of 1:3. NHDF were used for the experiments between passages 5 and 7.

Cell study. The cured hydrogels were washed twice with 1 mL culture medium and the NHDF cells were seeded over the hydrogels at 6000 cells/well in complete medium in triplicate samples. The culture plates were incubated at 37°C in humidified 5% CO_2 for 24 h, 72 h and 7 days. Then, the conditioned medium was aspirated and sample sent for cell viability determination. Thereafter, the medium was centrifuged at 400 g for 5 min at 4°C, and supernatants frozen at -80°C until further analysis. The cells were quantified using the NucleoCounter® system (ChemoMetec, Allerød, Denmark). This included the cells first being lyzed and lysates stabilized with disaggregation buffer. The cell nuclei were stained with propidium iodide in the NucleoCassette™ and the nuclei counted by the NucleoCounter® system according to the manufacturer's protocol. The hydrogels remaining were dissolved by increasing the pH to 8 by addition of 1% NaOH just prior to cell counting.

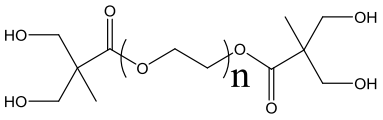
Cell viability. Cell viability was determined by analysis of the lactate dehydrogenase (LDH) content in the supernatants. LDH, a marker of cell membrane integrity was measured using a spectrophotometric evaluation of LDH-mediated conversion of pyruvic acid to lactic acid performed by C-Laboratory at Sahlgrenska University Hospital, Göteborg, Sweden.

Synthesis

Synthesis of compounds 1-2, 17, 19, 22: The synthesis of compounds **1-2, 17, 19, 22** was done according to earlier described procedures: Compound **1** and **22** as described in [1]; compound **2** as described in [2]; compound **17** as described in [3]; compound **19** as described in [4].



[1]



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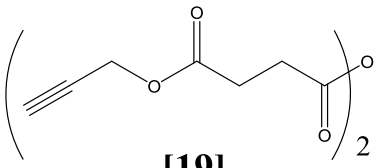
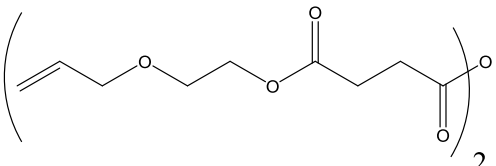
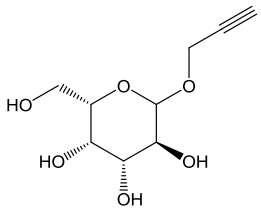


[19]

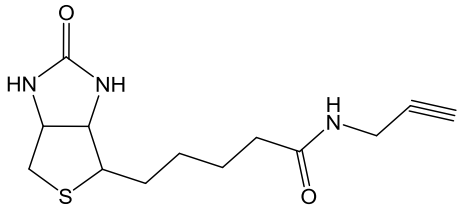


[2]



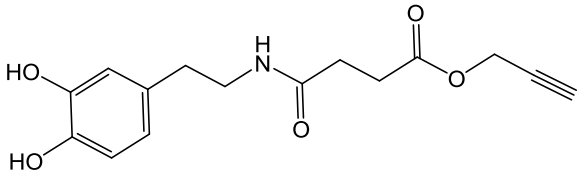
[22]

Synthesis of Biotin-Acetylene (20): D-Biotin (1.1 g , 4.5 mmol), propargyl amine, EDC hydrochloride, and DMAP were reacted in DMF (20 mL) and CH_2Cl_2 (10 mL) over night. The crude product was concentrated and purified by flash chromatography eluting the product at 90:10 CH_2Cl_2:MeOH. The product was obtained as a white solid after solvent evaporation. Yield: 74.6 % (0.947 g). **^{1}H NMR** (400 MHz, DMSO): δ_{ppm} 1.32-1.30 (m, 2H), 1.40-1.65 (m, 4H), 2.07 (t, *J* = 7.4 Hz, 2H), 2.56 (d, *J* = 12.4 Hz, 1H), 2.79-2.84 (m, 1H) , 3.07-3.11 (m, 2H), 3.82-3.84 (m, 2H), 4.11-4.14 (m, 1H), 4.28-4.32 (m, 1H), 6.36 (s, 1H), 6.42 (s, 1H), 8.23 (t, *J* = 5.2 Hz, 1H).**^{13}C NMR** (100 MHz, DMSO): δ_{ppm} 25.16, 27.74, 28.05, 28.21, 34.89, 55.42, 59.22, 61.06, 72.83, 81.38, 162.75, 171.86.



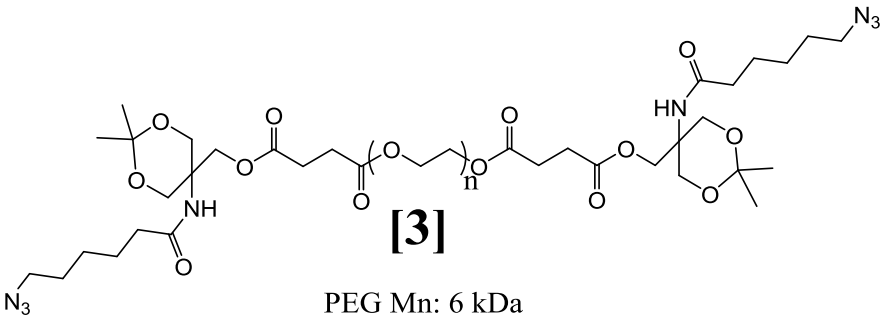
[20]

Synthesis of DOPA-Acetylene (21): Dopamine hydrochloride (20 g, 105.5 mmol) was dissolved in TEA (12.81 g, 126.6 mmol) and DMSO (100 mL). 4-oxo-4-(prop-2-yn-1-yloxy)butanoic anhydride **19** (27.9 g, 94.92 mmol) was slowly added under cooling and the reaction was left to reach room temperature overnight. The reaction mixture was extracted with diethyl ether (2L) and washed with $NaHSO_4$ (10%) four times (4x200 mL). The organic extracts was dried over $MgSO_4$, filtered and concentrated. The product was purified by flash chromatography eluting the product in 80:20 EtOAc:Hep. The product was obtained as pale oil after removal of solvent. Yield: 58 % (18.3 g). **^{1}H NMR** (400 MHz, MeOD): δ_{ppm} 2.45 (t, *J* = 6.9 Hz, 2H), 2.61 (t, *J* = 7.2 Hz, 4H), 2.88 (t, *J* = 2.5 Hz, 1H), 3.29-3.33 (m, 2H), 4.67 (d, *J* = 2.5 Hz, 2H), 6.50-6.68 (m, 3H), 7.93 (s, 1H). **^{13}C NMR** (100 MHz, MeOD): δ_{ppm} 30.32, 31.42, 36.03, 42.51, 53.03, 76.32, 78.87, 116.48, 116.96, 132.18, 144.85, 146.33, 173.52, 174.10.



[21]

Synthesis of P6k-[G1]-$(An)_2$-$(N_3)_2$ (3): PEG-6k-$(OH)_2$ was first freeze-dried to remove the water content. PEG-6k-$(OH)_2$ (20 g, 3.3 mmol), AB_2C anhydride **1** (10.4 g, 13.3 mmol) and DMAP (81.4 mg, 0.67 mmol) were then reacted in pyridine (4 mL) and CH_2Cl_2 overnight. ^{13}C NMR was used to confirm the presence of unconsumed anhydride at 168.14 ppm. The reaction was concentrated and re-dissolved in THF followed by precipitation in diethyl ether and filtration. The filtrate was dried and a second precipitation in diethyl ether was performed. The product was collected as a white powder. Yield: 90 % (21.31 g). **^{1}H NMR** (400 MHz, $CDCl_3$): δ_{ppm} 1.34-1.43 (m, 16H), 1.51-1.61 (m, 8H), 2.12 (t, J=7.5 Hz, 4H), 2.55-2.64 (m, 8H), 3.21 (t, *J* = 6.8 Hz, 4H), 3.50-3.75 (m, 546H) , 4.17 (t, *J* = 4.8 Hz, 4H), 4.20 (d, *J* = 11.9 Hz, 2H), 4.44 (s, 4H), 5.85 (s, 2H). **^{13}C NMR** (100 MHz, $CDCl_3$): δ_{ppm} 23.24, 24.15, 24.97, 26.29, 28.70, 29.14, 51.29, 53.13, 62.30, 63.81, 64.11, 69.06, 70.53, 98.75, 172.50, 172.52, 173.49.

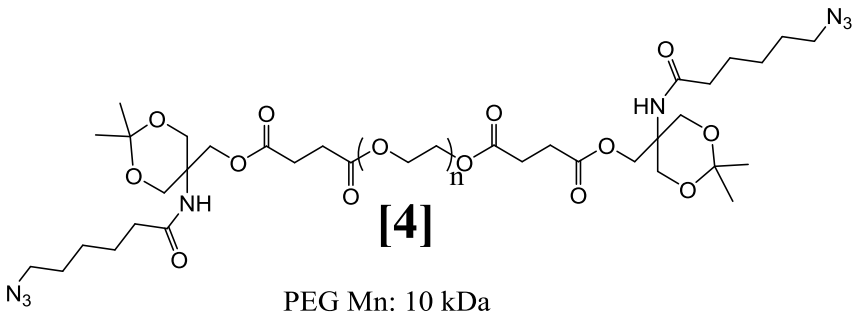


[3]

PEG Mn: 6 kDa

Synthesis of P10k-[G1]-$(An)_2$-$(N_3)_2$ (4): PEG-10k-$(OH)_2$ was first freeze-dried to remove the water content. PEG-10k-$(OH)_2$ (1.36 g, 0.14 mmol), AB_2C anhydride **1** (0.64 g, 0.82 mmol) and DMAP (3.3 mg, 0.027 mmol) were then reacted in pyridine (1 mL) and CH_2Cl_2 overnight. ^{13}C NMR was used to confirm the presence of unconsumed anhydride at 168.14 ppm. The reaction was concentrated and re-dissolved in THF followed by precipitation in diethyl ether and filtration. The filtrate was dried and a second precipitation in diethyl ether was performed. The product was collected as a white powder. Yield: 82.6 % (1.21 g). **^{1}H NMR** (400 MHz, $CDCl_3$): δ_{ppm} 1.34-1.43 (m, 16H), 1.51-1.61 (m, 8H), 2.12 (t, J=7.5 Hz, 4H), 2.55-2.64 (m, 8H), 3.21 (t, *J* = 6.8 Hz, 4H), 3.50-3.75 (m, 910H) , 4.17 (t, *J* = 4.8 Hz, 4H),

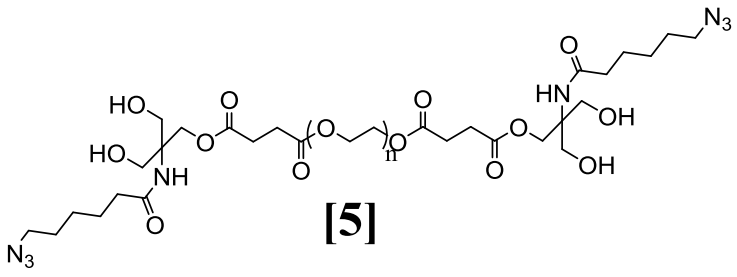
4.20 (d, *J* = 11.9 Hz, 2H), 4.44 (s, 4H), 5.85 (s, 2H). **^{13}C NMR** (100 MHz, $CDCl_3$): δ_{ppm} 23.24, 24.15, 24.97, 26.29, 28.70, 29.14, 51.29, 53.13, 62.30, 63.81, 64.11, 69.06, 70.53, 98.75, 172.50, 172.52, 173.49.



[4]

PEG Mn: 10 kDa

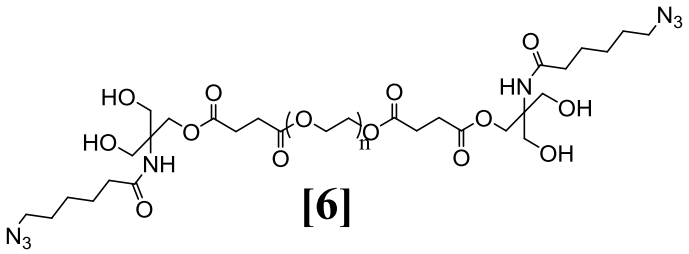
Synthesis of P6k-[G1]-(OH)$_4$-(N$_3$)$_2$ (5): P6k-[G1]-(An)$_2$-(N$_3$)$_2$ **3** (9.12 g, 1.3 mmol) was dissolved in H_2O (200 mL). pTSA (1.13 g, 5.9 mmol, 2.2 eq/acetonide group) was added and the reaction was monitored by ^{1}H NMR until the complete deprotection of peripheral hydroxyls occurred. The reaction mixture was diluted with brine (200 mL) and extracted with CH_2Cl_2. The organic phase was then washed three times with 50:50 solution of brine:H_2O followed by drying with $MgSO_4$ and filtration. The product was dried by lyophilization and obtained as a white powder. Yield: 95 % (8.52 g). **^{1}H NMR** (400 MHz, $CDCl_3$): δ_{ppm} 1.33-1.41 (m, 4H), 1.55-1.66 (m, 8H), 2.23 (t, *J* = 7.5 Hz, 4H), 2.59-2.69 (m, 8H), 3.25 (t, *J* = 6.8 Hz, 4H), 3.50-3.78 (m, 544H), 4.20-4.26 (m, 12H), 4.30 (s, 4H), 6.35 (s, 2H).**^{13}C NMR** (100 MHz, $CDCl_3$): δ_{ppm} 25.22, 26.34, 28.72, 29.28, 29.34, 36.73, 51.30, 61.40, 62.52, 63.04, 64.21, 69.07, 70.69, 172.76, 172.86, 174.68.



[5]

PEG Mn: 6 kDa

Synthesis of P10k-[G1]-$(OH)_4$-$(N_3)_2$ (6): P10k-[G1]-$(An)_2$-$(N_3)_2$ **4** (1.22 g, 0.11 mmol) was dissolved in H_2O (50 mL). pTSA (95 mg, 0.50 mmol, 2.2 eq/acetonide group) was added and the reaction was monitored by ^{1}H NMR until the complete deprotection of peripheral hydroxyls occurred. The reaction mixture was diluted with brine (200 mL) and extracted with CH_2Cl_2. The organic phase was then washed three times with 50:50 solution of brine:H_2O followed by drying with $MgSO_4$ and filtration. The product was dried by lyophilization and obtained as a white powder. Yield: 99 % (1.2 g). **^{1}H NMR** (400 MHz, $CDCl_3$): δ_{ppm} 1.33-1.41 (m, 4H), 1.55-1.66 (m, 8H), 2.23 (t, *J* = 7.5 Hz, 4H), 2.59-2.69 (m, 8H), 3.25 (t, *J* = 6.8 Hz, 4H), 3.50-3.78 (m, 908H), 4.20-4.26 (m, 12H), 4.30 (s, 4H), 6.35 (s, 2H).**^{13}C NMR** (100 MHz, $CDCl_3$): δ_{ppm} 25.22, 26.34, 28.72, 29.28, 29.34, 36.73, 51.30, 61.40, 62.52, 63.04, 64.21, 69.07, 70.69, 172.76, 172.86, 174.68.

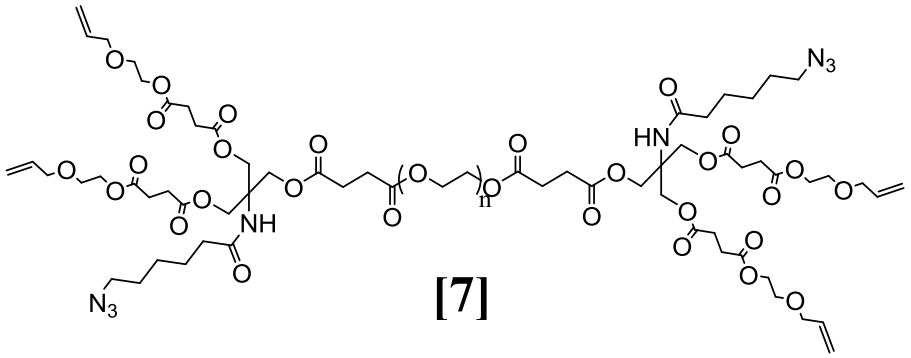


[6]

PEG Mn: 10 kDa

Synthesis of P6k-[G1]-(ene)$_4$-$(N_3)_2$ (7): P6k-[G1]-$(OH)_4$-$(N_3)_2$ **5** (7.85 g, 1.2 mmol) and DMAP (57.3 mg, 0.47 mmol) were dissolved in pyridine (4 mL) and CH_2Cl_2. 4-(2-(allyloxy)ethoxy)-4-oxobutanoic anhydride **2** (3.63g, 9.4 mmol) was added and the reaction was left over night. ^{13}C NMR was used to confirm the presence of unconsumed anhydride at 167.92 ppm. The reaction was concentrated and re-dissolved in THF followed by precipitation in diethyl ether and filtration. The filtrate was dried and a second precipitation in diethyl ether was performed. The product was collected as a white powder. Yield: 88 % (7.66 g). **^{1}H NMR** (400 MHz, $CDCl_3$): δ_{ppm} 1.31-1.39 (m, 4H), 1.53-1.62 (m, 8H), 2.15 (t, *J* = 7.5 Hz, 4H), 2.57-2.67 (m, 24H), 3.24 (t, *J* = 6.8 Hz, 4H), 3.52-3.79 (m, 548H), 3.99 (d, *J* = 5.7 Hz, 8H), 4.19-4.22 (m, 12H), 4.42 (s, 4H), 5.16-5.28 (m, 8H), 5.82-5.92 (m, 4H), 6.00 (s, 2H). **^{13}C NMR** (100 MHz,

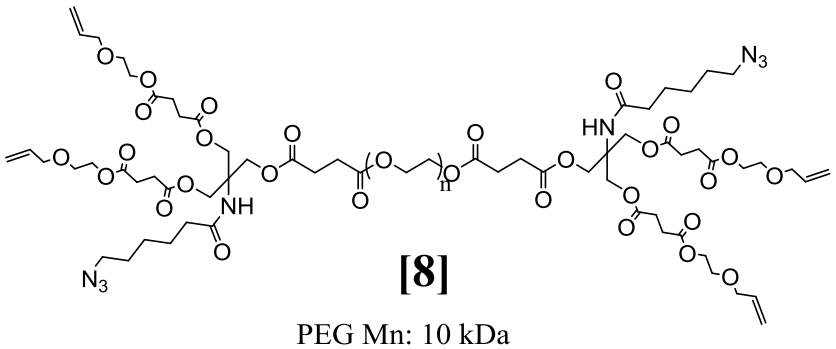
$CDCl_3$): δ_{ppm} 24.90, 25.68, 26.29, 28.67, 29.05, 36.59, 51.27, 58.11, 62.76, 64.05, 64.09, 65.91, 67.79, 68.02, 69.06, 70.69, 117.60, 134.38, 171.85, 172.31, 173.30.



[7]

PEG Mn: 6 kDa

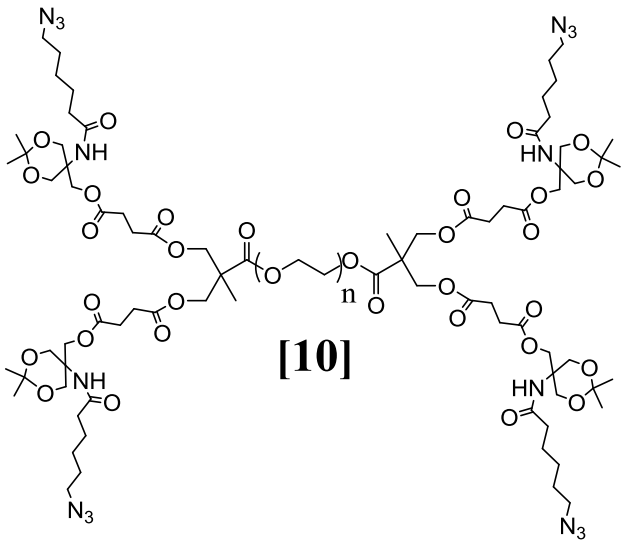
Synthesis of P10k-[G1]-(ene)$_4$-(N3)$_2$ (8): P10k-[G1]-$(OH)_4$-$(N_3)_2$ **6** (1.2 g, 0.11 mmol) and DMAP (6 mg, 0.045 mmol) were dissolved in pyridine (1 mL) and CH_2Cl_2. 4-(2-(allyloxy)ethoxy)-4-oxobutanoic anhydride **2** (260 mg, 0.67 mmol) was added and the reaction was left over night. ^{13}C NMR was used to confirm the presence of unconsumed anhydride at 167.92 ppm. The reaction was concentrated and re-dissolved in THF followed by precipitation in diethyl ether and filtration. The filtrate was dried and a second precipitation in diethyl ether was performed. The product was collected as a white powder. Yield of final product: 93 % (1.19 g). **^{1}H NMR** (400 MHz, $CDCl_3$): δ_{ppm} 1.36-1.43 (m, 4H), 1.57-1.66 (m, 8H), 2.18 (t, *J* = 8 Hz, 4H), 2.60-2.70 (m, 24H), 3.26 (t, *J* = 8 Hz, 4H), 3.58-3.76 (m, 916H), 3.79 (t, *J* = 4 Hz, 4H), 4.02 (d, *J* = 8 Hz, 8H), 4.22-4.26 (m, 12H), 4.46 (s, 4H), 5.16-5.28 (m, 8H), 5.85-5.95 (m, 4H), 6.00 (s, 2Hz). **^{13}C NMR** (100 MHz, $CDCl_3$): δ_{ppm} 24.90, 25.68, 26.29, 28.67, 29.05, 36.59, 51.27, 58.11, 62.76, 64.05, 64.09, 65.91, 67.79, 68.02, 69.06, 70.69, 117.60, 134.38, 171.85, 172.31, 173.30.



[8]

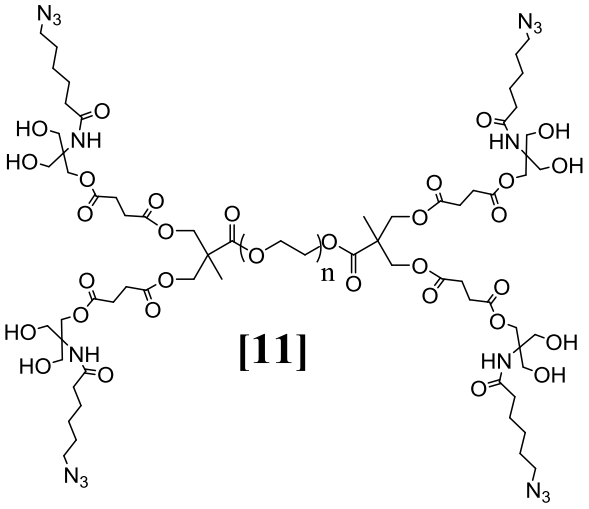
PEG Mn: 10 kDa

Synthesis of P6k-[G2]-$(An)_4$-$(N_3)_4$ (10): Starting from P6k-[G1]-$(OH)_4$ **9** (12.44 g, 2.0 mmol) the synthesis was performed according to the equivalent procedure as for P6k-[G1]-$(An)_2$-$(N_3)_2$ **3**. Yield of product: 91 % (14.08 g). **^{1}H NMR** (400 MHz, $CDCl_3$): δ_{ppm} 1.20 (s, 6H), 1.29-1.47 (m, 32H), 1.48-1.66 (m, 16H), 2.14 (t, *J* = 7.4 Hz, 8H), 2.55-2.62 (m, 16H), 3.23 (t, *J* = 6.8 Hz, 8H), 3.40-3.65 (m, 546H), 3.66-3.84 (m, 12H), 4.07-4.27 (m, 12H), 4.44 (s, 8H), 5.87 (s, 4H). **^{13}C NMR** (100 MHz, $CDCl_3$): δ_{ppm} 17.95, 23.52, 23.96, 25.06, 26.32, 28.74, 28.99, 36.86, 46.34, 51.34, 53.05, 62.35, 63.96, 64.39, 65.58, 68.92, 70.61, 70.62, 70.68, 98.80, 171.92, 172.39, 172.68, 173.53.



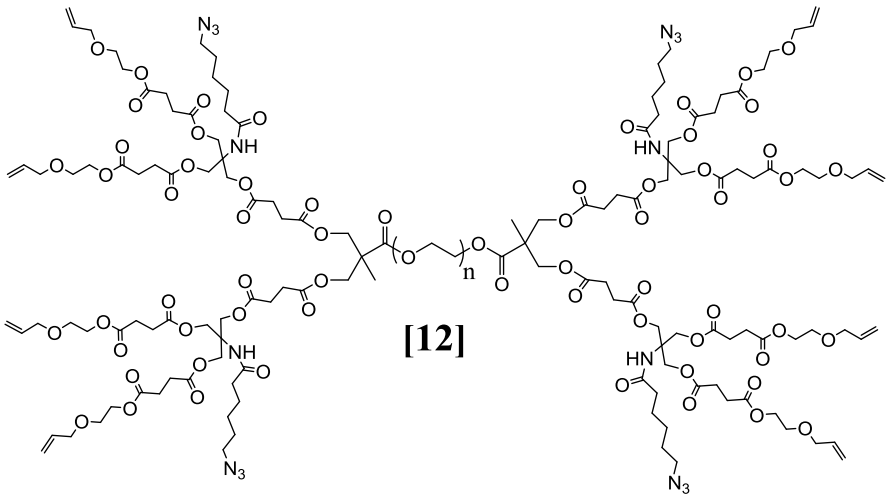
[10]

Synthesis of P6k-G2-OH_8-$(N_3)_4$ (11): Starting from P6k-[G2]-$(An)_4$-$(N_3)_4$ **10** (13.8 g, 1.8 mmol) the synthesis was performed according to the equivalent procedure as for P6k-[G1]-$(OH)_4$-$(N_3)_2$ **5**. Yield of final product: 80 % (10.81 g). **^{1}H NMR** (400 MHz, $CDCl_3$): δ_{ppm} 1.24 (s, 6H), 1.29-1.47 (m, 8H), 1.48-1.66 (m, 16H), 2.26 (t, *J* = 7.4 Hz, 8H), 2.56-2.70 (m, 16H), 3.27 (t, *J* = 6.8 Hz, 8H), 3.40-3.80 (m, 558H), 4.14-4.36 (m, 12H), 4.41-4.52 (m, 16H), 6.50 (s, 4H). **^{13}C NMR** (100 MHz, $CDCl_3$): δ_{ppm} 18.00, 25.20, 26.29, 28.67, 29.08, 36.69, 46.33, 51.27, 61.30, 62.22, 62.94, 65.45, 68.87, 70.59, 70.63, 171.10, 172.62, 172.81, 174.77.



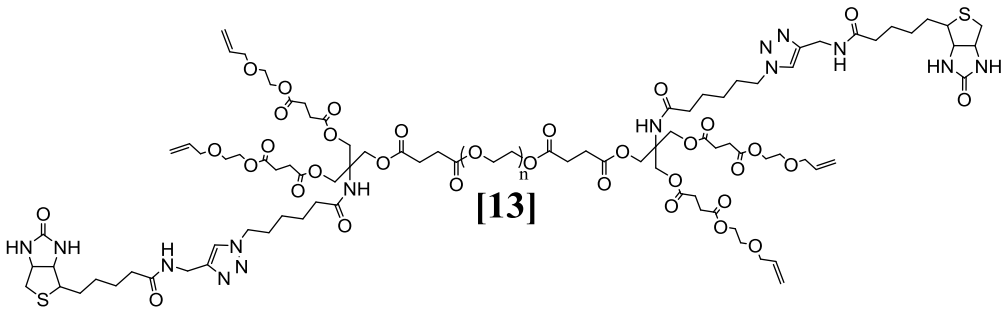
[11]

Synthesis of P6k-G2-ene$_8$-(N$_3$)$_4$ (12): Starting from P6k-G2-OH$_8$-(N$_3$)$_4$ **21** (9.96 g, 1.3 mmol) the synthesis was performed according to the same procedure as for P6k-G1-ene$_4$-(N$_3$)$_2$ **4**. Yield of final product: 87 % (10.29 g). **^{1}H NMR** (400 MHz, $CDCl_3$): δ_{ppm} 1.23 (s, 6H), 1.29-1.47 (m, 8H), 1.48-1.67 (m, 16H), 2.16 (t, *J* = 7.5 Hz, 8H), 2.53-2.71 (m, 48H), 3.26 (t, *J* = 6.9 Hz, 8H), 3.40-3.80 (m, 560H), 4.01 (dt, *J* = 5.5, 1.5 Hz, 40H), 4.14-4.33 (m, 20H), 4.42 (s, 8H), 5.05-5.38 (m, 16H), 5.83-6.07 (m, 8H), 6.05 (s, 4H). **^{13}C NMR** (100 MHz, $CDCl_3$): δ_{ppm} 17.75, 24.85, 26.22, 28.60, 28.75, 28.96, 36.53, 46.22, 51.20, 57.97, 62.70, 62.74, 64.02, 64.20, 65.49, 67.71, 68.79, 70.55, 72.12, 117.53, 134.31, 171.66, 171.68, 171.81, 172.13, 172.26, 173.23.



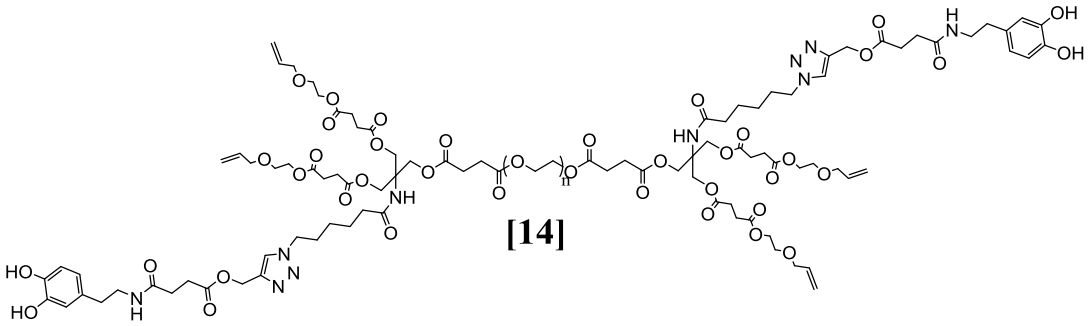
[12]

Synthesis of P6k-[G1]-(ene)$_4$-(Biotin)$_2$ (13): P6k-[G1]-(ene)$_4$-(N_3)$_2$ **7** (3.0 g, 0.4 mmol) was dissolved in THF. An aqueous solution of Biotin-Acetylene **20** (295 mg, 1.1 mmol), $CuSO_4$ (40.3 mg, 0.16 mmol) and sodium ascorbate (64.0 mg, 0.32 mmol) was added (total THF:H_2O-ratio of 5:1). The reaction was left stirring at 35°C overnight and was confirmed through ^{1}H-NMR by the complete disappearance of the peak at 3.24 ppm corresponding to the protons neighboring the azides. The crude reaction mixture was passed through a neutral Al_2O_3 column, concentrated and further purified by dialysis with 0.5 wt% EDTA solution (pH 7.0). Lyophilization afforded the pure product as a white powder. Yield: 83 % (2.68 g). **^{1}H NMR** (400 MHz, $CDCl_3$): δ_{ppm} 1.24-1.33 (m, 4H), 1.40-1.42 (m, 4H), 1.57-1.69 (m, 16H), 2.15-2.24 (m, 8H), 2.57-2.67 (m, 24H), 2.90-2.94 (m, 2H), 3.12-3.16 (m, 2H), 3.52-3.79 (m, 548H), 4.01 (dt, *J* = 5.8, 1.4 Hz, 8H), 4.18-4.25 (m, 12H), 4.33 (t, *J* = 6.8 Hz, 4H), 4.40-4.47 (m, 16H), 4.51-4.54 (m, 4H), 5.17-5.33 (m, 8H), 5.84-5.95 (m, 4H), 7.62 (s, 2H). **^{13}C NMR** (100 MHz, $CDCl_3$): 24.56 25.32, 25.88, 27.87, 27.97, 28.86, 28.94, 29.80, 34.46, 35.57, 36.17, 49.80, 50.01, 51.17, 55.60, 57.95, 60.18, 61.60, 62.59, 63.94, 63.99, 67.69, 68.94, 70.51, 72.08, 117.51, 122.51, 134.27, 144.96, 164.17, 171.78, 172.27, 173.35.



Synthesis of P6k-[G1]-(ene)$_4$-(DOPA)$_2$ (14): P6k-[G1]-(ene)$_4$-(N_3)$_2$ **7** (3.0 g, 0.4 mmol) was dissolved in THF. An aqueous solution of Dopamine-Acetylene **21** (306 mg, 1.1 mmol), $CuSO_4$ (40.3 mg, 0.16 mmol) and sodium ascorbate (64.0 mg, 0.32 mmol) was added (total THF:H_2O-ratio of 5:1). The reaction was left stirring at 35°C overnight and was confirmed through ^{1}H-NMR by the complete disappearance of the peak at 3.24 ppm corresponding to the protons neighboring the azides. The crude reaction mixture was passed through a neutral Al_2O_3 column, concentrated and further purified by dialysis with 0.5 wt% EDTA solution (pH 7.0). Lyophilization afforded the pure product as

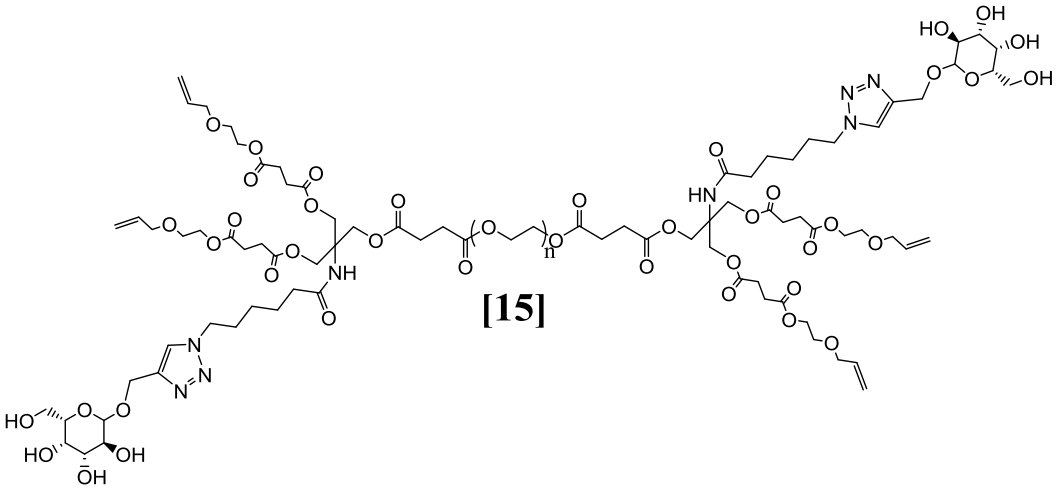
a white powder. Yield: 81 % (2.62 g). **^{1}H NMR** (400 MHz, $CDCl_3$): δ_{ppm} 1.24-1.33 (m, 4H), 1.54-1.64 (m, 8H), 2.12-2.21 (m, 4H), 2.39 (t, *J* = 6.4 Hz, 4H), 2.56-2.72 (m, 32H), 3.37-3.41 (m, 4H), 3.52-3.79 (m, 548H), 4.02 (dt, *J* = 5.7, 1.4 Hz, 8H), 4.20-4.26 (m, 8H), 4.34 (t, *J* = 7.0 Hz, 4H), 4.41-4.49 (m, 12H), 5.16-5.33 (m, 12H), 5.82-5.96 (m, 4H), 6.21 (s, 4H), 6.54-6.81 (m, 6H), 7.66 (s, 2H), 7.86 (s, 4H). **^{13}C NMR** (100 MHz, $CDCl_3$): δ_{ppm} 24.46, 25.75, 28.87, 28.91, 29.47, 29.77, 30.70, 34.65, 36.18, 40.86, 49.80, 50.08, 57.62, 58.03, 62.57, 63.94, 63.99, 67.67, 68.91, 70.50, 72.06, 115.5, 115.85, 117.41, 120.32, 130.89, 134.16, 143.08, 144.49, 171.39, 171.76, 171.80, 172.28, 172.54, 173.40.



[14]

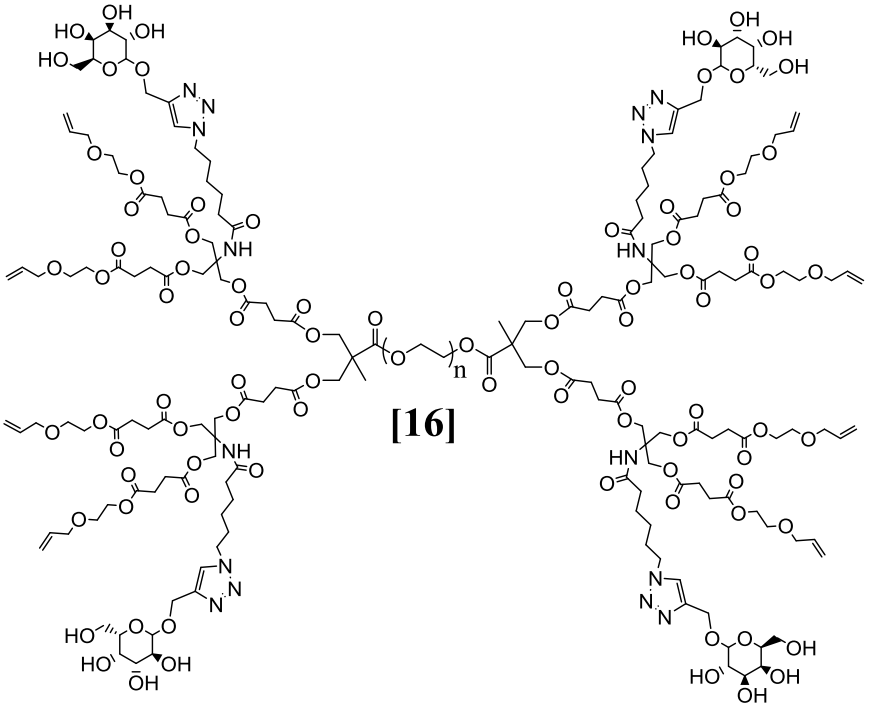
Synthesis of P6k-[G1]-(ene)$_4$-(Man)$_2$ (15): P6k-[G1]-(ene)$_4$-(N_3)$_2$ **7** (3.0 g, 0.4 mmol) was dissolved in THF. An aqueous solution of α-D-Mannopyranoside-2-propyn-1-yl **22** (229 mg, 1.1 mmol), $CuSO_4$ (40.3 mg, 0.16 mmol) and sodium ascorbate (64.0 mg, 0.32 mmol) was added (total THF:H_2O-ratio of 5:1). The reaction was left stirring at 35^{o}C overnight and was confirmed through ^{1}H-NMR by the complete disappearance of the peak at 3.24 ppm corresponding to the protons neighboring the azides. The crude reaction mixture was passed through a neutral Al_2O_3 column, concentrated and further purified by dialysis with 0.5 wt% EDTA solution (pH 7.0). Lyophilization afforded the pure product as a white powder. Yield: 81 % (2.57 g). **^{1}H NMR** (400 MHz, MeOD): δ_{ppm} 1.25-1.43 (m, 4H), 1.60-1.69 (m, 4H), 1.90-2.00 (m, 4H), 2.21 (t, *J* = 7.3 Hz, 4H) 2.65 (s, 24H), 3.44-3.82 (m, 558H), 4.02 (dt, *J* =5.5, 1.5 Hz, 8H), 4.20-4.23 (m, 12H), 4.36-4.44 (m, 12H), 4.57-4.83 (m, 4H), 4.82-4.90 (m, 10H), 5.07-5.41 (m, 8H), 5.80-6.00 (m, 4H), 8.03 (s, 2H). **^{13}C NMR** (100 MHz, MeOD): δ_{ppm} 26.07, 26.92, 29.80, 29.84, 30.98, 36.96, 51.11, 52.90, 58.62, 59.02, 60.69, 62.97, 63.23, 64.99, 68.57, 69.00, 70.02,

71.49, 71.97, 72.48, 72.92, 74.95, 100.73, 117.44, 125.32, 135.94, 145.21, 173.26, 173.91, 174.39, 176.23.



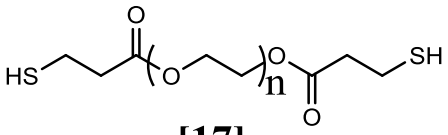
[15]

Synthesis of P6k-G2-ene$_8$-Man$_4$ (16): P6k-[G2]-(ene)$_8$-(N$_3$)$_4$ **12** (2.9 g, 0.32 mmol) was dissolved in THF. An aqueous solution of α-D-Mannopyranoside-2-propyn-1-yl **22** (389 mg, 1.7 mmol), $CuSO_4$ (159 mg, 0.64 mmol) and sodium ascorbate (202 mg, 1.02 mmol) was added (total THF:H_2O-ratio of 5:1). The reaction was left stirring at 35^{o}C overnight and was confirmed through ^{1}H-NMR by the complete disappearance of the peak at 3.26 ppm corresponding to the protons neighboring the azides. The crude reaction mixture was passed through a neutral Al_2O_3 column, concentrated and further purified by dialysis with 0.5 wt% EDTA solution (pH 7.0). Lyophilization afforded the pure product as a white powder. Yield: 73 % (2.31 g). **^{1}H NMR** (400 MHz, $CDCl_3$): δ_{ppm} 1.26 (s, 6H), 1.31-1.39 (m, 8H), 1.61-1.68 (m, 8H), 1.90-2.00 (m, 8H) 2.22 (t, *J* = 7.3 Hz, 8H), 2.59-2.71 (m, 48H), 3.44-3.87 (m, 580H), 4.02 (dt, *J* = 5.4, 1.5 Hz, 40H), 4.14-4.31 (m, 20H), 4.40-4.45 (m, 24H), 4.59-4.81 (m, 8H), 4.85-4.91 (m, 20H), 5.10-5.35 (m, 16H), 5.79-6.08 (m, 8H), 8.04 (s, 4H). **^{13}C NMR** (100 MHz, $CDCl_3$): δ_{ppm} 18.24, 26.13, 26.96, 29.83, 29.88, 31.02, 37.03, 51.16, 52.59, 59.03, 60.71, 62.82, 63.00 63.30, 65.03, 66.73, 68.60, 68.96, 69.03, 71.57, 72.00, 72.50, 72.96, 74.98, 100.76, 117.50, 125.32, 134.31, 144.86, 173.28, 173.32, 173.40, 173.51, 173.96, 176.27.



[16]

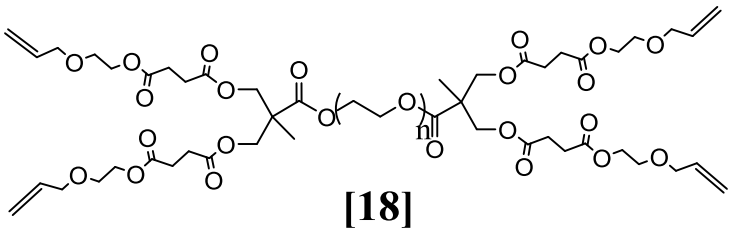
Synthesis of P2k-(SH)$_2$ (17): PEG-2k-(OH)$_2$ (20 g, 10 mmol), pTSA (380 mg, 2 mmol) and 3-mercaptopropionic acid (10.641 g, 100 mmol) were dissolved in 200 mL of toluene. The reaction was refluxed at 130 ^{o}C overnight using a Dean-stark apparatus. The reaction was followed by ^{1}H NMR and upon completion the reaction mixture was concentrated and the crude product was purified by precipitation trice in diethyl ether. Yield of white powder: 78 % (17.2g). **^{1}H NMR** (400 MHz, $CDCl_3$): δ_{ppm} 1.66 (t, *J* = 8.3 Hz, 2H), 2.66 (t, *J* = 6.9 Hz, 4H), 2.68 - 2.83 (m, 4H), 3.57 – 3.69 (m, 182H), 4.24 (t, *J* = 4.4 Hz, 4H). **^{13}C NMR** (100 MHz, $CDCl_3$): δ_{ppm} 19.88, 38.55, 63.90, 69.19, 70.70, 171.70.



[17]

Synthesis of P6k-G1-ene$_4$ (18): P6k-[G1]-(OH)$_4$ **9** (6 g, 0.96 mmol) and DMAP (47 mg, 0.39 mmol) were dissolved in pyridine (2 mL, 24.78 mmol) and CH_2Cl_2. 4-(2-(allyloxy)ethoxy)-4-oxobutanoic

anhydride **2** (2.98 g, 7.7 mmol) was added and the reaction was left over night. ^{13}C NMR was used to confirm the presence of unconsumed anhydride at 167.92 ppm. The reaction was concentrated and re-dissolved in THF followed by precipitation in diethyl ether and filtration. The product was collected as a white powder. Yield: 84 % (5.66 g). **^{1}H NMR** (400 MHz, $CDCl_3$): δ_{ppm} 1.25 (s, 6H), 2.65 (t, *J* = 4.4 Hz, 16H), 3.47-3.82 (m, 548H), **^{13}C NMR** (100 MHz, $CDCl_3$): δ_{ppm}



[18]

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