ELECTRONIC SUPPLEMENTARY INFORMATION (ESI)

Synthesis, biological and physico-chemical characterization of glycodendrimers and oligopeptides for the treatment of systemic lupus erythematosus

Sarah Tassinari*[a], Silvia Moreno[b] , Hartmut Komber[b], Riccardo Carloni[c], Michela Cangiotti[c], Maria Francesca Ottaviani[c], Dietmar Appelhans*[b]

[a] *Department of Medical Sciences, University of Turin, 10126 Turin, Italy,*
[b] *Leibniz Institute of Polymer Research Dresden, D-01069 Dresden, Germany.*
[c] *Department of Pure and Applied Sciences, Università degli studi di Urbino "Carlo Bo", Urbino 61029, Italy*
*Corresponding authors: sarah.tassinari.1994@gmail.com - sarah.tassinari@unito.it; applhans@ipfdd.de

1. *Methods of characterization of dendrimers and oligopeptides*.

The NMR measurements were carried out on a Bruker DRX 500 NMR spectrometer operating at 500.13 MHz for ^{1}H NMR and at 125.75 MHz for ^{13}C NMR using D_2O and DMSO-$_{d6}$ as solvent. Sodium 3-(trimethylsilyl)-3,3,2,2-tetradeuteropropionate was added for internal calibration (δ (^{13}C) = 0 ppm; δ (^{1}H) = 0 ppm). The signal assignments were done by combination of 1D and 2D NMR experiments using the standard pulse sequences provided by Bruker. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was used to characterize the synthesized oligopeptides, showing high yield of the expected structures. A Bruker Reflex III instrument (Bruker Daltonik GmbH, Bremen, Germany), equipped with a nitrogen laser (337 nm), working in linear mode at an acceleration voltage of 20 KV was used. 10 mg of dihydroxybenzoic acid dissolved in 1 mL dimethylsufoxide/water (1:1) was used as matrix. The oligopeptide concentration was 2 mg/mL (in H_2O). Both solutions were mixed in a ratio of 50/20 (v/v) prior deposition (1 µL) on the MALDI target. Typically, 200 shots were accumulated for one spectrum. The instrument was calibrated with biopolymer standards.

2. *Synthesis of glycodendrimers*

For the synthesis of PAMAM and PPI glycodendrimers, the maltose-function was attached to the dendrimer surface following the reductive amination method (***Figure S1***). The protocol foreseen the dissolving of PAMAM and PPI dendrimers in Na-Borate Buffer (oil bath, 50°C, agitation, 1h) and after the addition of maltose units to the solution (oil bath, 50°C, agitation, 30min). Then the Borane-Pyridine complex (8M, 1 eq. for 1:1 ratio and 0.25 eq. for 1:0.25 ratio) was slowly added to the solution (oil bath, 50°C, agitation, 1 week) to mediate the reduction of the terminal amino groups. The sample was then left at 50°C for a week at reflux. Once the reaction was completed, the product was purified using a dialysis membrane (MWCO 2,000 Dalton); the dialysis process was carried out for 4 days in 5 L of deionized water, with four water changes per day. Lyophilisation led to a solid product. The reagents and used amounts are shown in ***Table S1*** for PAMAM glycodendrimer and in ***Table S2*** for PPI glycodendrimers. The yields of glycodendrimers synthesis are reported in ***Table S3***.

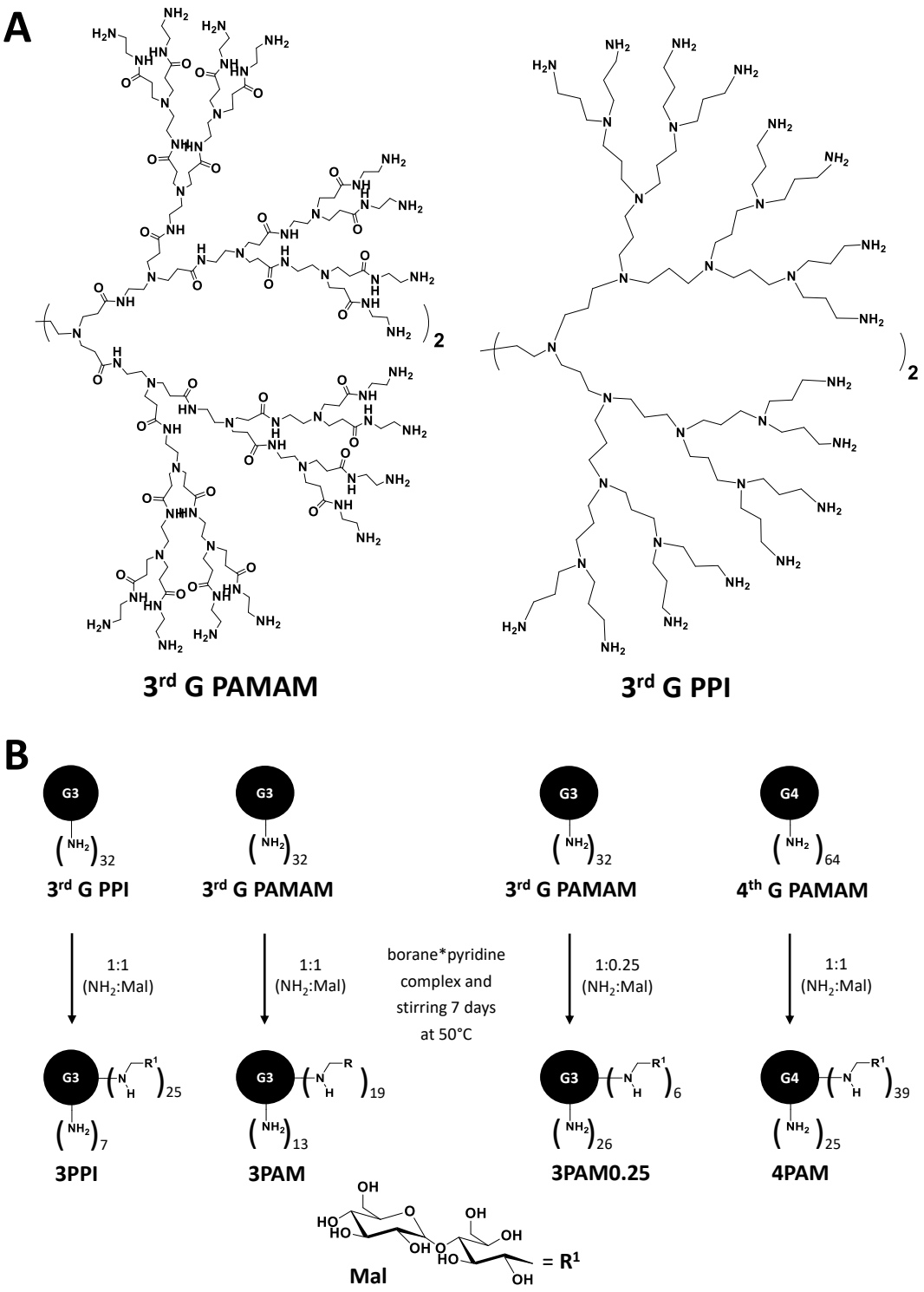

Figure S1 *– (A) Molecular structures of 3rd generation (G) PAMAM and PPI dendrimers with 32 terminal primary amino groups. (B) Synthetic scheme for open-shell maltose decorated PPI and PAMAM glycodendrimers obtained by reductive amination of 3rd generation polyamine dendrimers using borane-pyridine complex, maltose (Mal) and sodium borate buffer. Degree of chemically coupled Mal units at terminal primary amino groups was validated by an ^{1}H NMR approach, explained in ESI below.*

Table S1: Reagents used in the synthesis of PAMAM glycodendrimers.

3PAM (1:1)

Reagents	**Äq**	**n (mol)**	**M (g/mol)**	**m (g)**	**V (ml)**
PAMAM G3	1	$(7.64 * 10^{-5})*32= 2.445*10^{-3}$	6937	0.530	-
Maltose Monohydrate	1	$2.445*10^{-3}$	360.32	0.880	-
*Borane*Pyridine Complex 8M*	1	$2.445*10^{-3}$	-	-	0.31
Na-Borate-Buffer	-	-	-	-	25

4PAM (1:1)

Reagents	**Äq**	**n (mol)**	**M (g/mol)**	**m (g)**	**V (ml)**
PAMAM G4	1	(3.658 * 10-5)*32= 2.341*10-3	14215	0.520	-
Maltose Monohydrate	1	$2.341*10^{-3}$	360.32	0.844	-
*Borane*Pyridine Complex 8M*	1	$2.341*10^{-3}$	-	-	0.29
Na-Borate-Buffer	-	-	-	-	25

3PAM0.25 (1:0.25)

Reagents	**Äq**	**n (mol)**	**M (g/mol)**	**m (g)**	**V (ml)**
PAMAM G3	1	$(6.775 * 10\text{-}5)*8 = 5.420*10^{-4}$	6937	0.47	-
Maltose Monohydrate	0.25	$5.420*10^{-4}$	360.32	0.195	-
*Borane*Pyridine Complex 8M*	0.25	$5.420*10^{-4}$	-	-	0.07
Na-Borate-Buffer	-	-	-	-	20

Table S2: Reagents used in the synthesis of PPI glycodendrimers.

3PPI (1:1)

Reagents	**Äq**	**n (mol)**	**M (g/mol)**	**m (g)**	**V (ml)**
PPI G3	1	$(9.278 * 10\text{-}5)*32= 2.969 *10^{-3}$	3513.84	0.323	-
Maltose monohydrate	1	$2.969 *10^{-3}$	360.32	1.07	-
*Borane*Pyridine complex 8M*	1	$2.969 *10^{-3}$	-	-	0.37
Na-Borate-Buffer	-	-	-	-	20

Table S3 Yields of glycodendrimers after reductive amination

Dendrimer	*Reaction*	*Grams (g)*	*Yield*
3PAM	*PAMAM G3 : MALTOSE (1:1)*	0.696	49%
4PAM	*PAMAM G4 : MALTOSE (1:1)*	0.735	56%
3PAM0.25	*PAMAM G3 : MALTOSE (1:0.25)*	0.376	57%
3PPI	*PPI G3 : MALTOSE (1:1)*	1.019	73%

3. Characterization of open-shell glycodendrimers 3PPI, 3PAM, 3PAM0.25 and 4PAM by NMR

The yield of open-shell PAMAM glycodendrimers was a bit lower as observed for the open-shell PPI glycodendrimer (***Table S3***). ^{1}H NMR characterization of open-shell glycodendrimers enabled us to determine the degree of coupled maltose units at both kind of glycodendrimers (***Table S4***), using an established NMR approach.[1] Thus, after the reductive amination of PAMAM and PPI dendrimers, residual primary and secondary amino groups are present in all open-shell glycodendrimers synthesized (***Figure S2 + S3***), including the designation for proton assignments in each glycodendrimer. Furthermore, the molecular weights of new PAMAM glycodendrimers could not be determined by MALDI-TOF-MS. Undefined degraded part structures of PAMAM glycodendrimers, **3PAM**, **3PAM0.25**, and **4PAM** were observed.

The comparison of ^{1}H NMR spectra (***Figure S4***) reveals the increasing content of reduced maltose units in **3PAM0.25** and **3PAM**. The highest content is reached for an almost **completely maltosylated PAMAM G3** (***Figure S4d***), depicted as reference. The degree of chemically coupled maltose units on dendritic PAMAM scaffolds can be determined from the normalized signal intensity of all H_b protons and the intensity of H_1 of coupled maltose units (***Figure S5***). This approach is also applicable for the determination of chemically coupled maltose units at **4PAM**. In case of **3PPI** (***Figure S6***), selected protons (a,d,g,j,m) of dendritic PPI scaffold was used as reference to determine chemically coupled maltose units (H_1). ***Table S4*** summarizes the determined numbers of attached maltose units at open-shell glycodendrimers used in this study.

While the ^{1}H NMR spectra of maltosylated PAMAM dendrimers (***Figure S4***) show a strong signal overlap, the ^{13}C NMR spectra (***Figure S7***) allow additional conclusions. Again, the spectra of non-maltosylated (***Figure S7a***) and almost **completely maltosylated** (***Figure S7d***) **PAMAM G3** undoubtedly give reference points. A comparison with the spectra of **3PAM0.25** (***Figure S7b***) and **3PAM** (***Figure S7c***) with low and intermediate degree of maltosylation mainly reveals significant differences in the 50–40 ppm region. These additional ^{13}C NMR signals should have their cause in the dominating mono-maltosylated ethylamino groups which are not present in the reference dendrimers. This cannot fully explain the large number of additional signals compared to almost **completely maltosylated PAMAM G3** (***Figure S7d***). We argue that the neighbourhood of different substitution patterns (-NH_2 / -NHR^1 / -$N(R^1)_2$) in open-shell maltosylated PAMAM glycodendrimers, **3PAM0.25** and **3PAM**, result in additional chemical shift effects. In case of almost **completely maltosylated PAMAM G3** all terminal groups have the -$N(R^1)_2$ pattern (***Figure S2***).

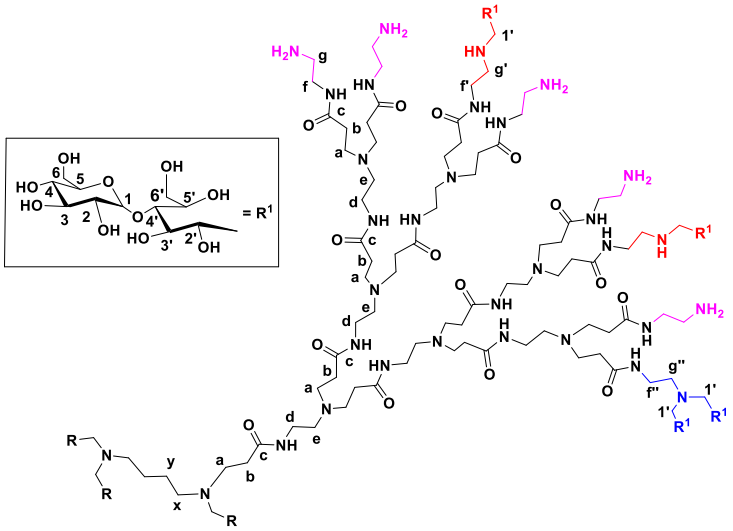

Figure S2. *Generalized structure of a maltose-modified PAMAM G3 containing unreacted terminal ethylamino groups (pink), mono-maltosylated ethylamino groups (red) and double (fully)-maltosylated ethylamino groups (blue). The designation of the different positions is applied for the assignment of* ^{1}H *and* ^{13}C *NMR signals. R = PAMAM dendron with* R^1

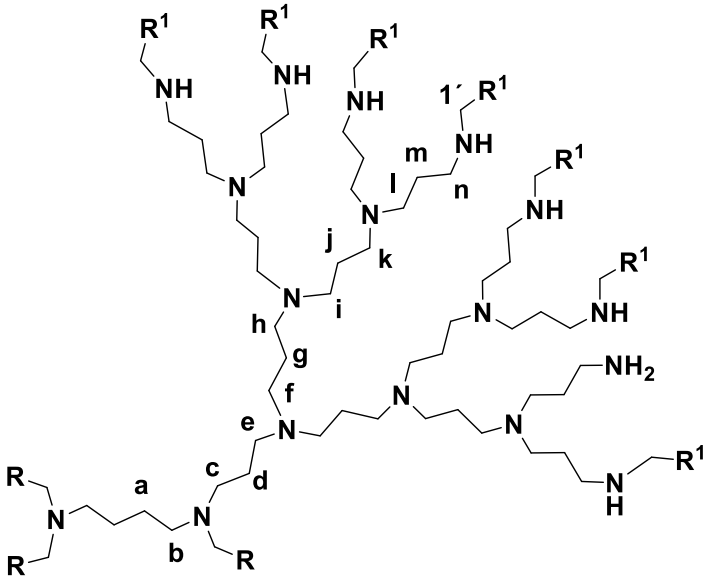

Figure S3. *Generalized structure of 3PPI containing unreacted terminal amino groups and terminal mono-maltosylated amino groups. The designation of the different positions is applied for the assignment of* ^{1}H *NMR signals.* R^1 *= coupled reduced maltose as shown in Figure S2. R = PPI dendron with* R^1*. Various open-shell maltosylated PPI glycodendrimers was used in several biological studies slightly varying in the degree of maltosylation as open-shell PPI glycodendrimer.*[2-4]

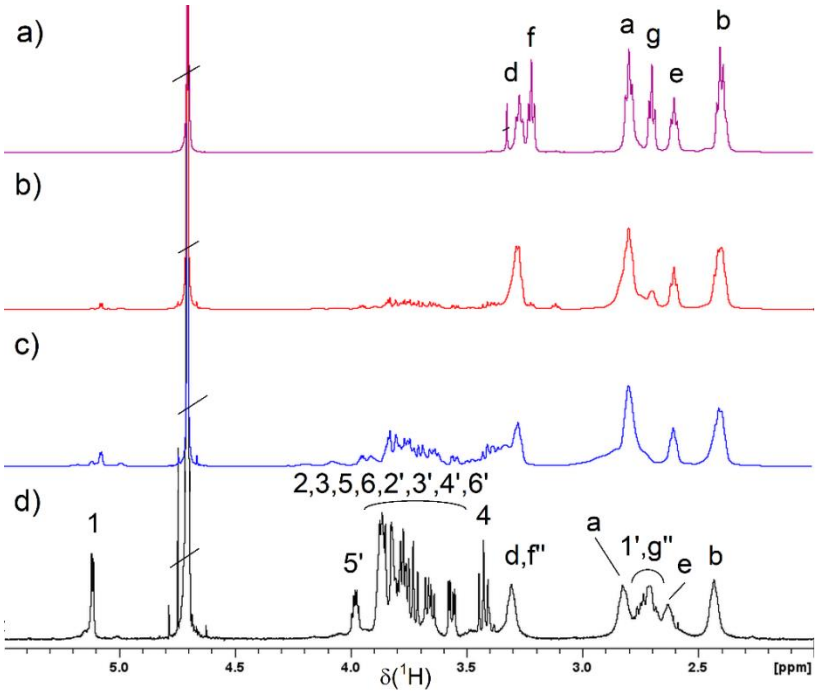

Figure S4. *^{1}H NMR spectra of (a) pure, non-maltosylated **PAMAM G3** as reference (b) **3PAM0.25** (1:0.25 for NH_2:maltose), (c) **3PAM** (1:1 for NH_2:maltose) and (d) almost **completely maltosylated PAMAM G3** as a reference (solvent: D_2O). The signal region of H_b has the same intensity in all spectra (intensity reference).*

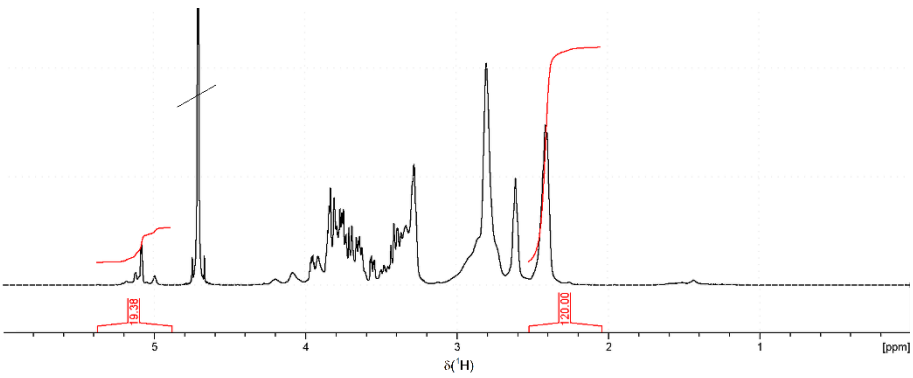

Figure S5. *^{1}H NMR spectrum of **3PAM** (1:1 for NH_2:maltose) with integral regions used to calculate the percentage of reduced maltose units The integral value of H_b protons was set to the number of these hydrogens (120) in **3PAM** (**Figure S2**). Thus, the maximal integral of H_1 is 32 for 1:1 conversion. Here: 19.38 / 32 = 60.5 % (+- 2.5%)).*

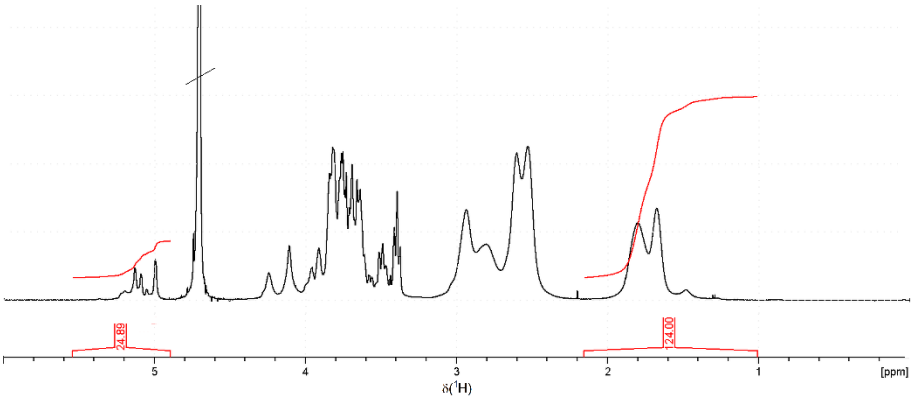

Figure S6. *^{1}H NMR spectrum of **3PPI** (1:1 for NH_2:maltose) with integral regions used to calculate the percentage of reduced maltose units The integral value of H_b protons was set to the number of these hydrogens (120) in **3PPI** (**Figure S3**).* Four additional protons result from the butyl moiety of the core. *Thus, the maximal integral of H_1 is 32 for 1:1 conversion. Here: 24.89 / 32 = 77.8 % (+- 2.5%)).*

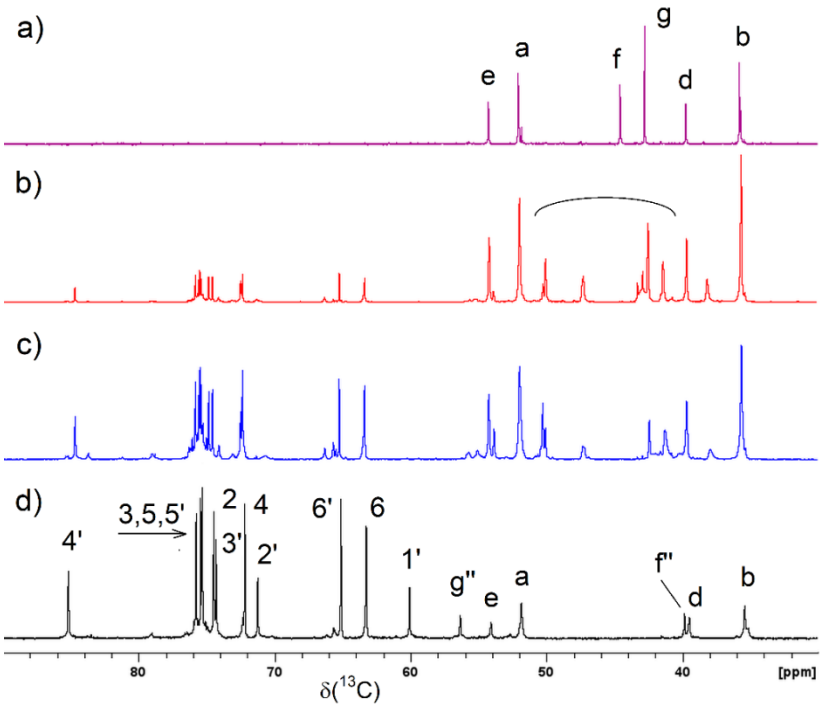

Figure S7. *^{13}C NMR spectra of (a) pure, non-maltosylated **PAMAM G3** as reference (b) **3PAM0.25** (1:0.25 for NH_2:maltose), (c) **3PAM** (1:1 for NH_2:maltose) and (d) almost **completely maltosylated PAMAM G3** as a reference (solvent: D_2O).*

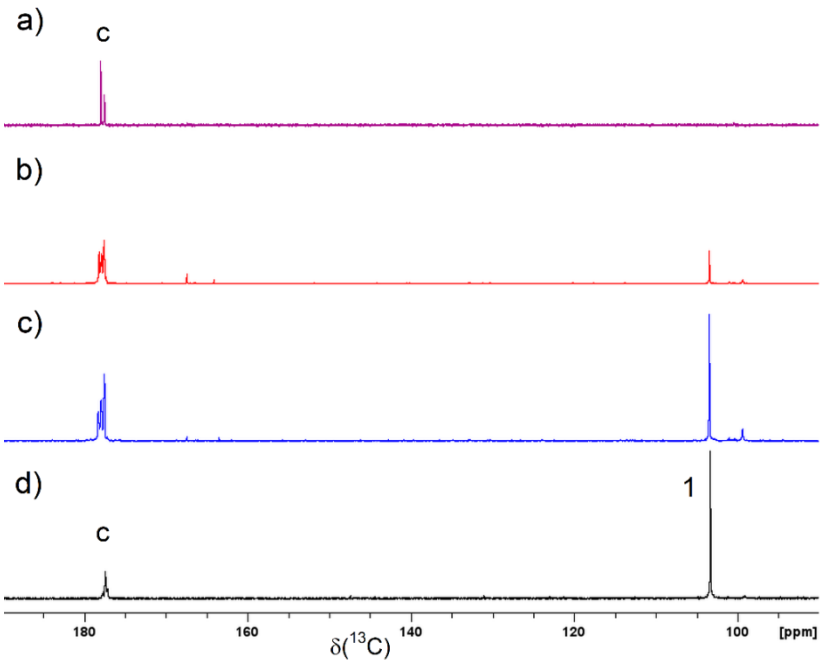

Figure S8. *^{13}C NMR spectra of (a) pure, non-maltosylated* ***PAMAM G3*** *as reference (b)* ***3PAM0.25*** *(1:0.25 for NH_2:maltose), (c)* ***3PAM*** *(1:1 for NH_2:maltose) and (d) almost* ***completely maltosylated PAMAM G3*** *as a reference (solvent: D_2O), showing the region above 90 ppm.*

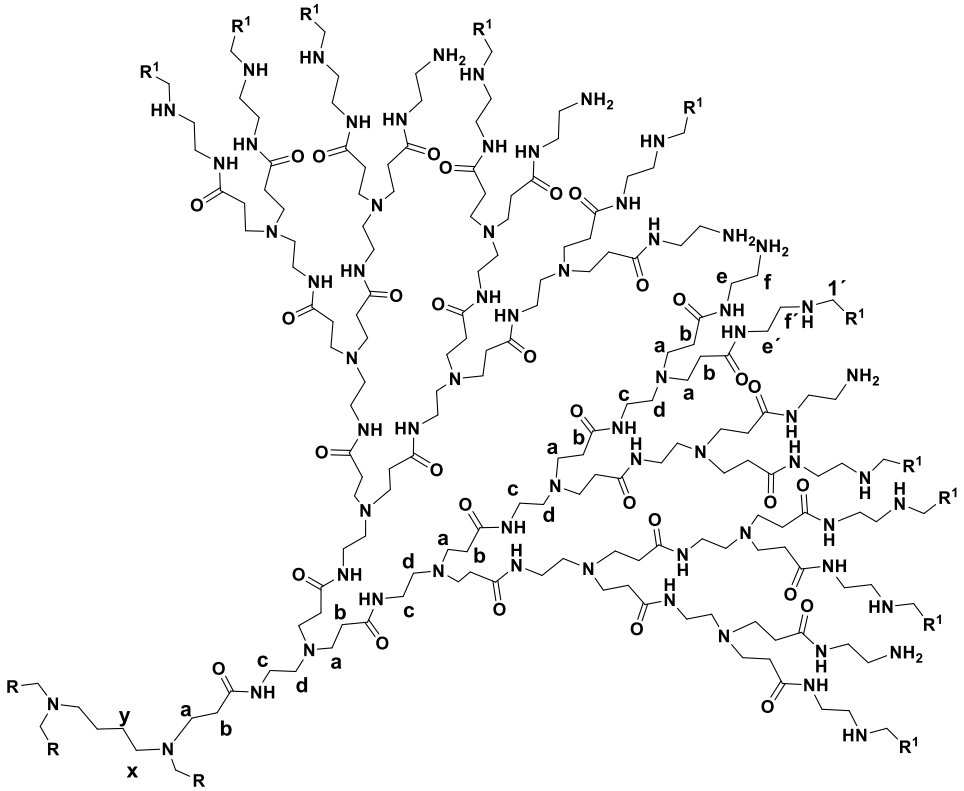

Figure S9. *Designation of simplified 4PAM scaffold for ^{1}H NMR assignment. R^1 = coupled reduced maltose unit as shown in* ***Figure S2****. R = PAMAM dendron with R^1.*

Table S4 *Molecular weights (MW) of open-shell glycodendrimers determined by ^{1}H NMR spectra*

Dendrimer	NH_2 : Mal[a]	Dendrimer[b] (g/mol)	Mal[a] (g/mol)	Theoretical MW (g/mol)	Number of Mal[c] (100%, theoretical)	Experimental MW[d] (g/mol)	Number of Mal[d] (%, experimental)
3PAM	1 : 1	6937 (32 NH_2)	324.28	17314.12	32	13200	19.4 (60.5)
3PAM0.25	1 : 0.25	6937 (32 NH_2)	324.28	9531.28	8	8900	6 (75.0)
4PAM	1 : 1	14215 (64 NH_2)	324.28	34969.24	64	27000	39.4 (61.5)
3PPI	1 : 1	3514 (32 NH_2)	324.28	13891.02	32	12100	25 (77.8)

[a] Mal = maltose; [b] M_w = molecular weight, number in parentheses presents amount of primary amino groups in dendrimer; [c] theoretically attached Mal units on corresponding dendrimer; [d] experimentally determined number of Mal on dendrimer by ^{1}H NMR approach (estimated error: +- 1.5 units). MW round up and down by 100 numbers.

3PAM - ^{1}H NMR (D_2O): δ = 5.4-4.9 (1), 4.3-3,4 (2-6, 2´-6´), 3.4-3.2 (d,f,f´), 3.05-2.69 (a,1´,g,g´), 2.66-2.56 (e), 2.5-2.3 ppm (b).

3PAM0.25 - ^{1}H NMR (D_2O): δ = 5.25-4.9 (1), 4.3-3,4 (2-6, 2´-6´), 3.4-3.2 (d,f,f´), 3.05-2.65 (a,1´,g,g´), 2.65-2.55 (e), 2.5-2.3 ppm (b).

4PAM - ^{1}H NMR (D_2O): δ = 5.4-4.9 (1), 4.3-3,4 (2-6, 2´-6´), 3.4-3.2 (c,e,e´), 3.05-2.69 (a,1´,f,f´), 2.66-2.56 (d), 2.5-2.3 ppm (b).

3PPI - ^{1}H NMR (D_2O): δ = 5.5-4.9 (1), 4.3-3,3 (2-6, 2´-6´), 3.05-2.69 (b,c,e,f,h,i,k,l,n,1´), 2.18-1.00 ppm (a,d,g,j,m).

4. Synthesis of TEMPO-labelled Glycodendrimers

Since the EPR technique allows the study of paramagnetic substances only, the glycodendrimers (**3PAM**, **4PAM** and **3PPI**) were spin-labelled with TEMPO (2,2,6,6-tetramethylpiperidine-N-oxyl) nitroxide radical groups, to monitor their performance. Glycodendrimer labelling was carried out by dissolving glycodendrimers in water and TEMPO-4-isothiocyanate in DMSO. The two solutions were then joined stirring, under controlled atmosphere. The reaction lasts for four days, in a dark environment to protect the radicals under argon atmosphere. After reaction completion, the product was purified with a dialysis membrane with a MWCO of 2 kDa. The dialysis was carried out for 4 days in 5 L of deionized water under light protection, with four water changes per day. Lyophilization led to a solid product. The labelling procedure is described in **Figure S10**.

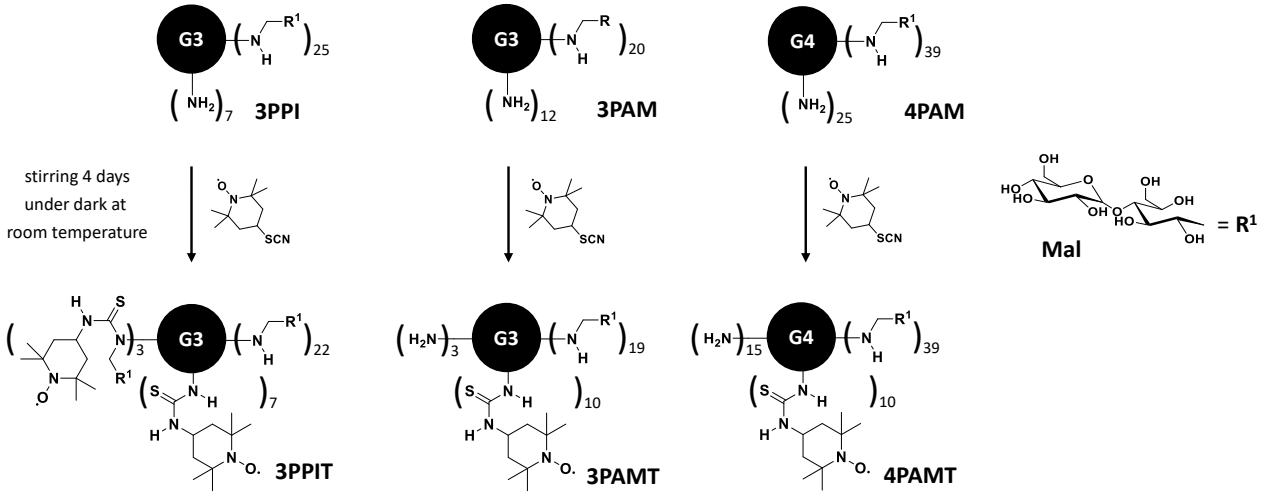

Figure S10 *– TEMPO labeling reactions of 3PPI, 3PAM and 4PAM. The reaction was performed in DMSO/water under argon to allow the displacement of the oxygen from inside, to avoid collateral reactions with the TEMPO group. All reactions were conducted in absence of light, to avoid the degradation of the radicals.*

Table S5 lists the reagents and their amounts used in the synthesis of TEMPO-labelled glycodendrimers, while Table S6 shows the yield of TEMPO-labelled glycodendrimers after addition reaction.

Table S5 Reagents used in the synthesis of TEMPO modified glycodendrimers.

3PAMT

Reagents	**Äq**	**n (mol)**	**M (g/mol)**	**m (g)**	**V (ml)**
PAMAM G3 + Maltose	1	$(1.446*10^{-5})*12= 1.736*10^{-4}$	17286.12	0.25	-
4-Isothiocyanato-TEMPO	12	$1.736*10^{-4}$	360.32	0.037	-
DMSO	-	-	-	-	22.5
H_2O	-	-	-	-	22.5

4PAMT

Reagents	**Äq**	**n (mol)**	**M (g/mol)**	**m (g)**	**V (ml)**
PAMAM G4 + Maltose	1	$(7.150*10^{-6})*12=8.580*10^{-5}$	34969.24	0.25	-
4-Isothiocyanato-TEMPO	12	$8.580*10^{-5}$	213.32	0.018	-
DMSO	-	-	-	-	10

H_2O	-	-	-	-	10

3PPIT

Reagents	**Äq**	**n (mol)**	**M (g/mol)**	**m (g)**	**V (ml)**
PPI G3 + Maltose	1	$2.16*10^{-5}$	13890.96	0.3	-
4-Isothiocyanato-TEMPO	10	$2.16*10^{-4}$	213.32	0.046	-
DMSO	-	-	-	-	10
H_2O	-	-	-	-	10

Table S6 Yields of TEMPO-labelled glycodendrimers through addition reactions

Reaction	***Amount (g)***	***Yield***
3PAM *with TEMPO-SCN*	0.222	77%
4PAM *with TEMPO-SCN*	0.178	66%
3PPI *with TEMPO-SCNTEMPO*	0.316	92%

5. Synthesis of oligopeptides

The oligopeptides chosen for the interaction with the antibodies are made by units of biotin, PEG, cyclooctyne and glutamate. The number of PEG units in the two compounds is different: 59 units for OLI1 and 22 units for OLI2 (***Figure 1***). ***Figure S11*** shows the synthetic approach for producing the oligopeptides OLI1 and OLI2. Biotin-PEG-NH_2 (0.069 mmol for OLI1, and 0.055 mmol for OLI2), BCN-NHS (0.276 mmol for OLI1 and 0.14 mmol for OLI2), and trimethylamine (NEt_3), 0.711 mmol for OLI1 and 0.142 mmol for OLI2, were left equilibrating in 3 mL anhydrous DMSO under argon atmosphere and stirred for 3 days at 35 °C. The crude Biotin-PEG-BCN product was purified by dialysis against 5 L deionized water for 1 day using a regenerated cellulose membrane with a MWCO of 1 kDa (water changed 10x). Lyophilization led to the yellowish, solid Biotin-PEG-BCN (Biotin-PEG_{22}-cyclooctyne: 1500 g/mol (MALDI-TOF MS); Biotin-PEG_{59}-cyclooctyne: 3500 g/mol (MALDI-TOF MS)). This last product (0.007 mmol for OLI1 and 0.008 mmol for OLI2) and N_3-PEG_4-EEE (0.013 mmol for both OLI1 and OLI2; 620.56 g/mol; MS [mz] = 621.3 $[M+H]^+$ by JPT Peptide Technology) was taken up in 4 mL ultrapure water and stirred for 3 days at 40 °C. Non-reacted excess N_3-PEG-EEE was removed by dialysis against 5 L deionized water for 1 day using a regenerated cellulose membrane with a MWCO of 1 kDa (water changed 10x). Lyophilization led to quantitative yellowish, solid products (94 % for OLI1 and 100 % for OLI2). Characterization was carried out with MALDI-TOF MS (***Figure S12***), which confirmed the desired synthesis of OLI1 and OLI2 with their molecular weights of 4000 g/mol for OLI1 and 2100 g/mol for OLI2.

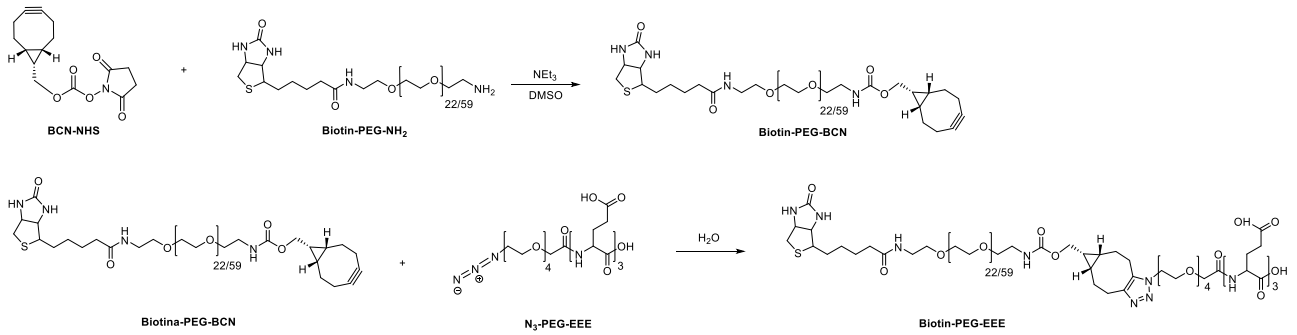

Figure S11. *Synthesis of the oligopeptides.*

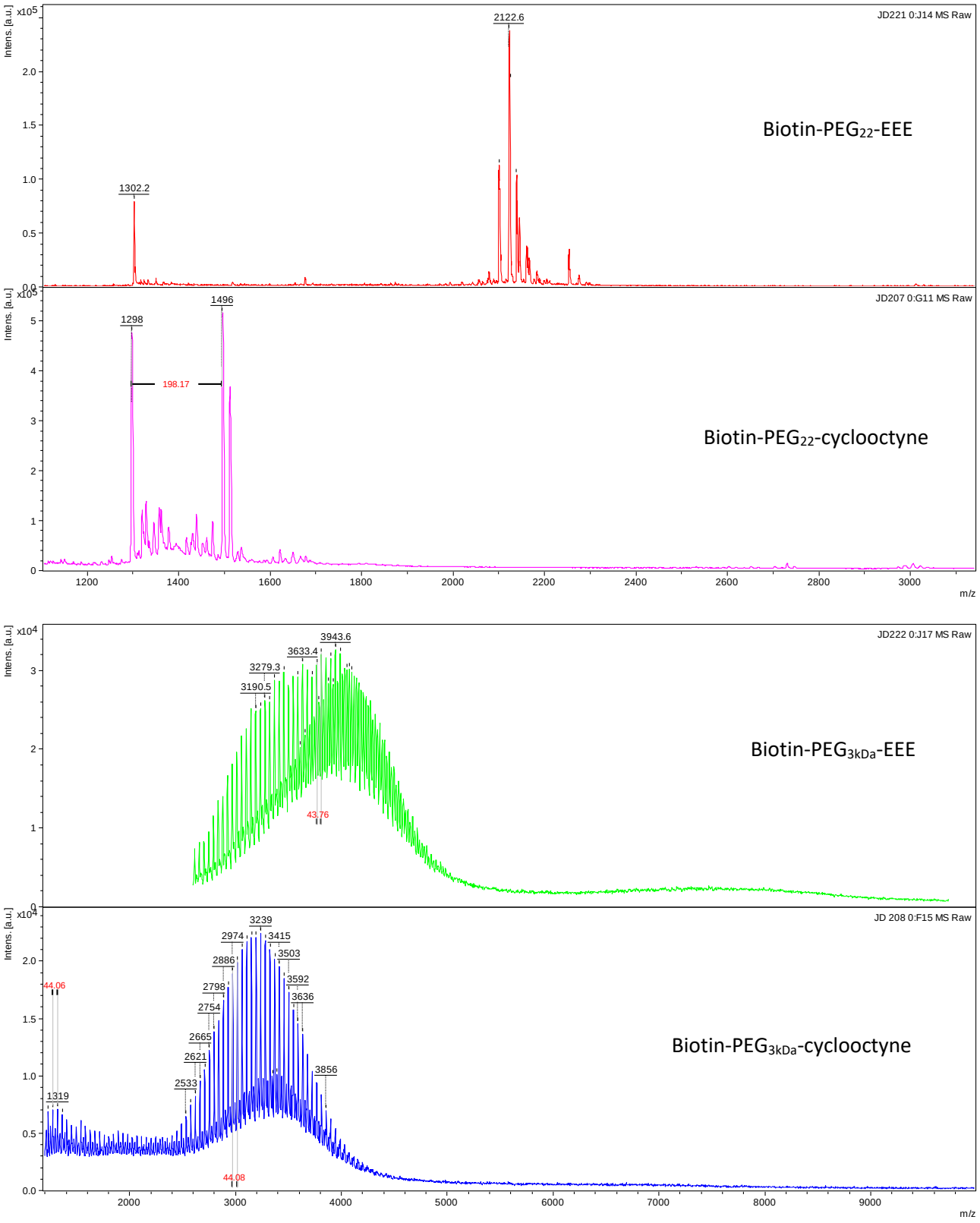

Figure S12. *MALDI-TOF MS spectra of the oligopeptides, OLI1 (Biotin-PEG$_{3kDa}$-EEE) and OLI2 (Biotin-PEG$_{22}$-EEE), including their precursors Biotin-PEG$_{3kDa}$-cyclooctyne and Biotin-PEG$_{22}$-cyclooctyne.*

Precursor of OLI1 (**Biotin-PEG$_{3kDA}$-cyclooctyne)**- ^{1}H NMR (DMSO-d$_6$): δ = 7.8 (13), 7.0 (6), 6.4-6.2 (20, 21), 4.3 (22), 4.1 (19), 4.0 (5), 3.7-3.0 (7,8,9,10,11,12), 2.85-2.75 (23), 2.58 (18), 2.3-1.75 (1,2,14,15), 2.05-0.7 ppm (3,4,16,17).

Precursor of OLI2 (Biotin-PEG$_{22}$-cyclooctyne) - ^{1}H NMR (DMSO-d$_6$): 7.8 (13), 7.3-7.0 (6), 6.4-6.2 (20, 21), 4.3 (22), 4.1 (19), 4.0 (5), 3.7-3.0 (7,8,9,10,11,12), 2.85-2.75 (23), 2.58 (18), 2.3-1.75 (1,2,14,15), 2.05-0.7 ppm (3,4,16,17).

OLI1 (**Biotin-PEG$_{3kDA}$-EEE)**- ^{1}H NMR (DMSO-d$_6$): δ = 7.8 (13), 7.0 (6), 6.4-6.2 (20, 21), 4.4 (g), 4.3 (22), 4.1 (19), 4.0 (5), 3.9-2.9 (7,8,9,10,11,12,a-e), 2.85-2.75 (23), 2.58 (18), 2.3-1.5 (1,2,14,15,h,i), 2.05-0.7 ppm (3,4,16,17).

OLI2 (Biotin-PEG$_{22}$-EEE) - ^{1}H NMR (DMSO-d$_6$): 7.8 (13), 7.3-7.0 (6), 6.4-6.2 (20, 21), 4.4 (g) 4.3 (22), 4.1 (19), 4.0 (5), 3.9-2.9 (7,8,9,10,11,12, a-e), 2.85-2.75 (23), 2.58 (18), 2.3-1.5 (1,2,14,15,h,i), 2.05-0.7 ppm (3,4,16,17).

^{1}H NMR characterization of both precursors (***Figure S13*** + ***S14***) and oligopeptides, OLI1 and OLI2, (***Figure S15*** + ***S16***) further confirmed the realization of desired oligopeptide structures, OLI1 and OLI2, in **Figure 1**. Characteristic ^{1}H NMR signal patterns of both precursors could be identified: amide protons (20, 21) of urea group in biotin unit; protons of CH-groups (19, 22) next to amide protons in biotin unit; CH_2O-group (5) of the bicycle with yne unit; and the different aliphatic protons of bicycle (1-4) and aliphatic protons (14-17) of the biotin unit between 0.7 and 2.3 ppm besides (i) the different CH_2-groups of PEG chain between 3.0 and 3.7 ppm and (ii) CH_2- and CH-group next to the sulfur atom in the biotin unit between 2.9 and 2.5 ppm. The assigned ^{1}H NMR signal patterns correspond to previously published ^{1}H NMR data for Biotin-PEG$_{3kDA}$-cyclooctyne.[5] Analysis of ^{1}H NMR spectra of OLI1 and OLI2 revealed the appearance of additional protons: CH-group in glutamate units (g, 4.4 ppm) and ethylene unit (a, b, 3.9-3.5 ppm) between triazole ring and short PEG chain with terminal EEE unit. The other aliphatic protons of glutamate (h and i) are in typical ppm range (1.5-2.3 ppm) known from literature.[6] In case of OLI1 with longer PEG$_{3kDA}$ broadening/suppressing of various aliphatic protons between 0.7 and 2.3 ppm is given. Generally, acidic protons (j, k) and amide proton (f) of EEE unit are not assignable due to different reasons (e.g. H/D exchange and high enlargement of ^{1}H NMR signals triggered by longer PEG chain in OLI1.)

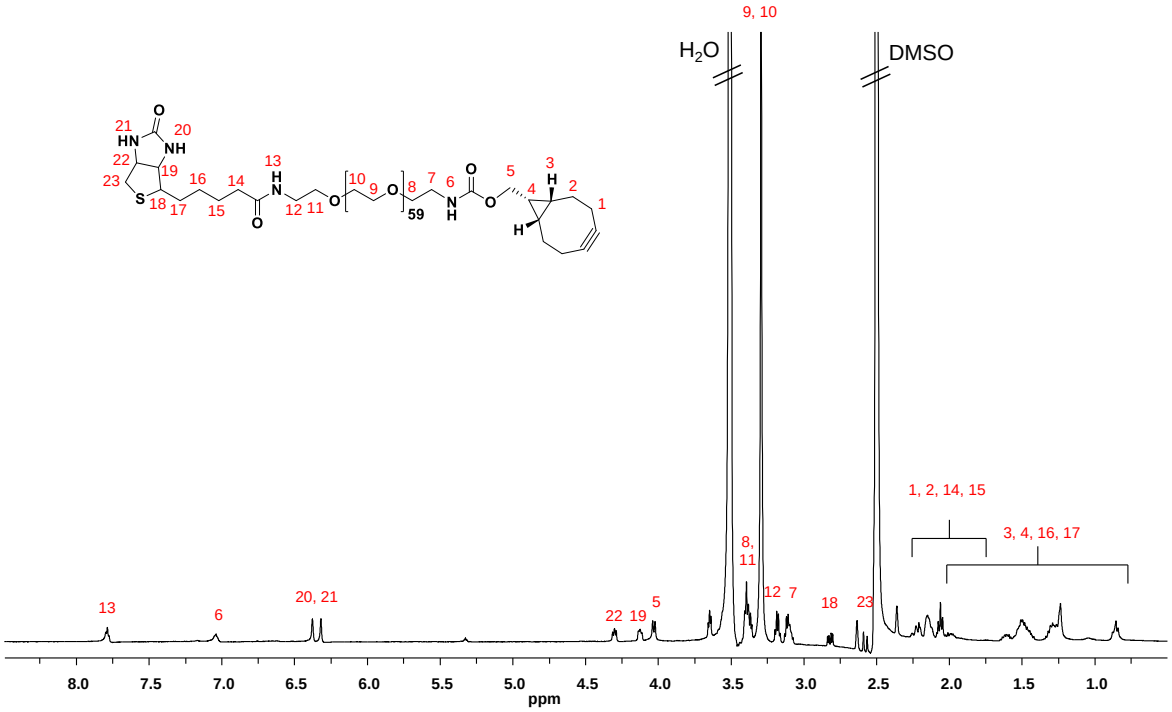

Figure S13. *^{1}H NMR spectrum of Biotin-PEG-BCN with PEG$_{3kDA}$ as precursor for the synthesis of OLI1 (DMSO-$_{d6}$).*

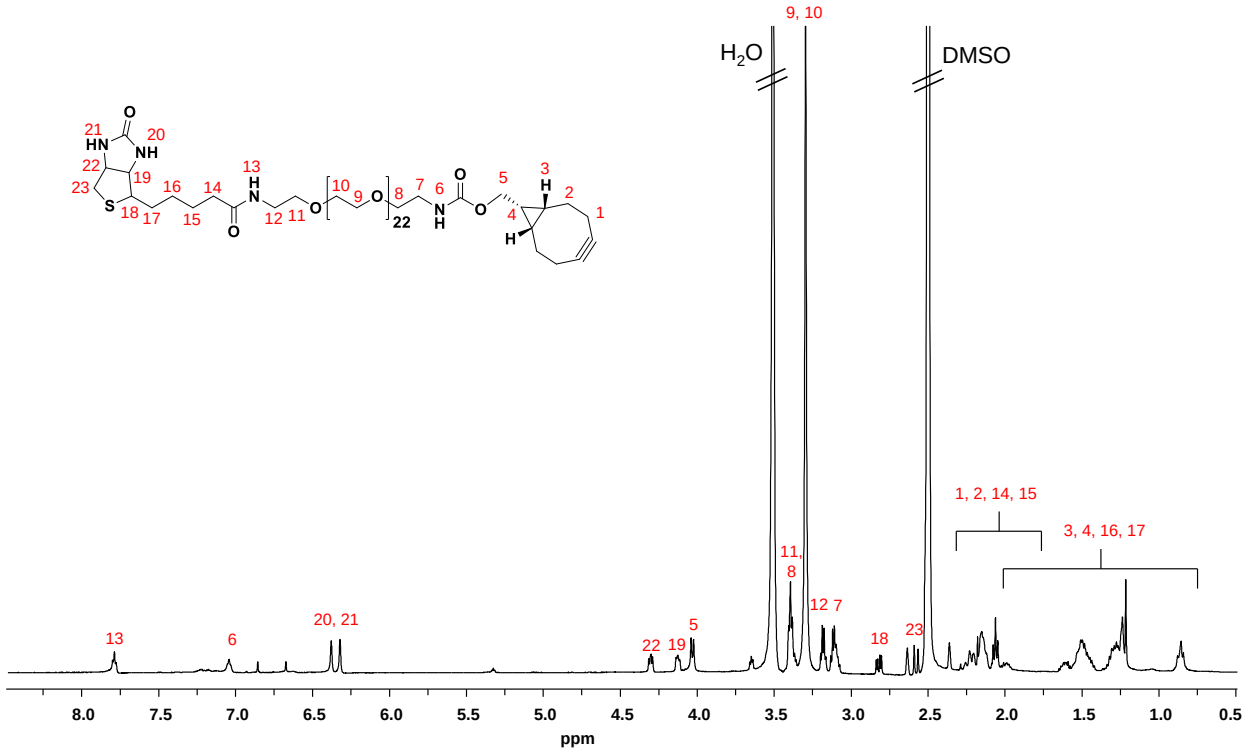

Figure S14. *^{1}H NMR spectrum of Biotin-PEG-BCN with PEG$_{22}$ as precursor for the synthesis of OLI2 (DMSO-$_{d6}$).*

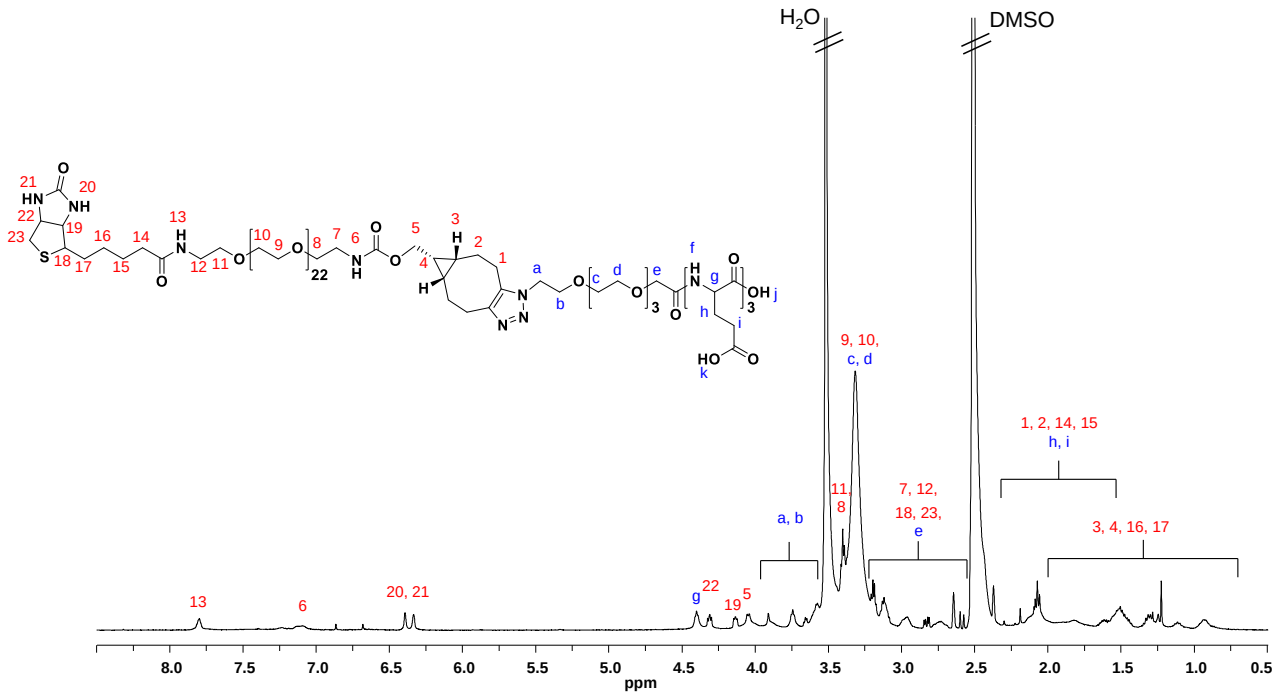

Figure S15. *^{1}H NMR spectrum of OLI2 with shorter PEG chain PEG$_{22}$ (DMSO-$_{d6}$).*

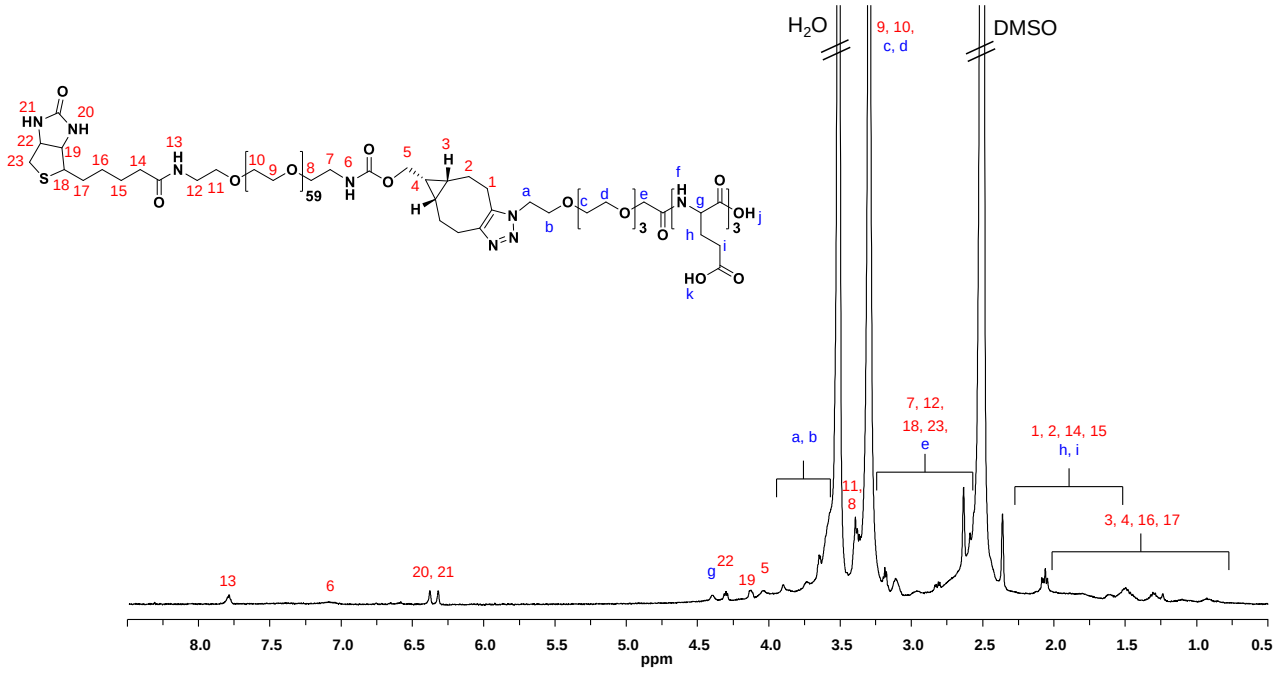

Figure S16. *^{1}H NMR spectrum of OLI1 with longer PEG chain PEG$_{3kDA}$ (DMSO-$_{d6}$).*

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