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Non-Covalent Polyvalent Ligands by Self-Assembly of Small Glycodendrimers: A Novel Concept for the Inhibition of Polyvalent Carbohydrate–Protein Interactions In Vitro and In Vivo

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Abstract: Polyvalent carbohydrate–protein interactions occur frequently in biology, particularly in recognition events on cellular membranes. Collectively, they can be much stronger than corresponding monovalent interactions, rendering it difficult to control them with individual small molecules. Artificial macromolecules have been used as polyvalent ligands to inhibit polyvalent processes; however, both reproducible synthesis and appropriate characterization of such complex entities is demanding. Herein, we present an alternative concept avoiding conventional macromolecules. Small glycodendrimers which fulfill single molecule entity criteria self-assemble to form non-covalent nanoparticles. These particles—not the individual molecules—function as polyvalent ligands, efficiently inhibiting polyvalent processes both in vitro and in vivo. The synthesis and characterization of these glycodendrimers is described in detail. Furthermore, we report on the characterization of the non-covalent nanoparticles formed and on their biological evaluation.

Keywords: carbohydrate–protein interactions · dendrimers · multivalency · polyvalency · self-assembly

Introduction

Polyvalent carbohydrate–protein interactions occur frequently in recognition events on cellular membranes.[1] They are characterized by the simultaneous contact of multiple oligosaccharides (ligands) on one biological entity with multiple proteins (receptors) on another. Their collective properties can be unique and substantially different from properties displayed by monovalent interactions of their constituents.[2] In particular, their affinity can be much greater than in the case of a monovalent interaction between a carbohydrate and a protein (which is generally weak; $K_D = 10^{-3}$–$10^{-4}\,M^{-1}$).[3] As a consequence, the inhibition of disease-relevant polyvalent biological processes with monovalent oligosaccharides has proven difficult. However, approaches utilizing non-natural polyvalent glycopolymers have led to highly potent receptor blockers (Figure 1).[4]

The interaction of an immunoglobulin of class IgM with multiple antigens presented on a cell surface is a typical polyvalent recognition process (Figure 2c).[5] IgMs have ten identical binding sites and consist of five monomeric subunits, joined by disulfide bridges, each of them having a molecular weight of ≈180 kDa (Figure 2b). As an example, the interaction of natural human or non-human primate anti αGal IgMs and the αGal epitope (Galα1–3Galβ1–4GlcNAc; Figure 2a), which is expressed on the cells of mammals but not of humans and Old World monkeys, causes hyperacute rejection, thus hampering pig to primate xenotransplantation.[6] Removal or blockade is a potential prevention therapy. While blocking attempts with the monovalent trisaccharide were unsuccessful, polymers providing multiple copies of the αGal trisaccharide efficiently inhibited anti αGal IgMs

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Supporting information for this article (^{1}H NMR spectra of compounds **11 Gal**, **19 Gal**, **24 Gal**, **29 Gal**, and **30 Gal**) is available on the WWW under http://www.chemeurj.org/ or from the author.

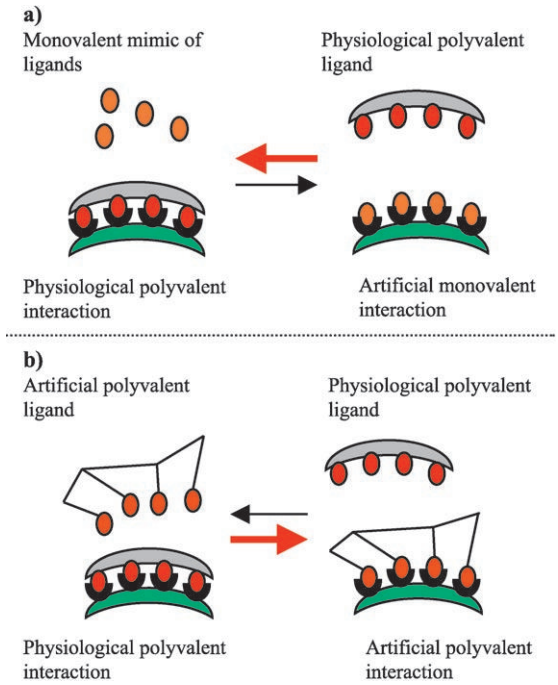


Figure 1. Inhibition of a physiological polyvalent carbohydrate (ligand)–protein (receptor) interaction with a) a monovalent mimic of the ligand and b) an artificial polyvalent ligand. Polyvalent interactions can be orders of magnitude stronger than monovalent interactions. Thus, in case a) inhibition will only occur if the mimic of the physiological ligand is orders of magnitude more potent than a single physiological ligand, or if it is used at very high (millimolar) concentrations. However, in b) a physiological polyvalent interaction competes with an artificial polyvalent interaction. Appropriate design of the artificial polyvalent ligand can lead to inhibition of the physiological process even if the "monovalent" physiological ligand and its "monovalent" mimic show comparable potencies.

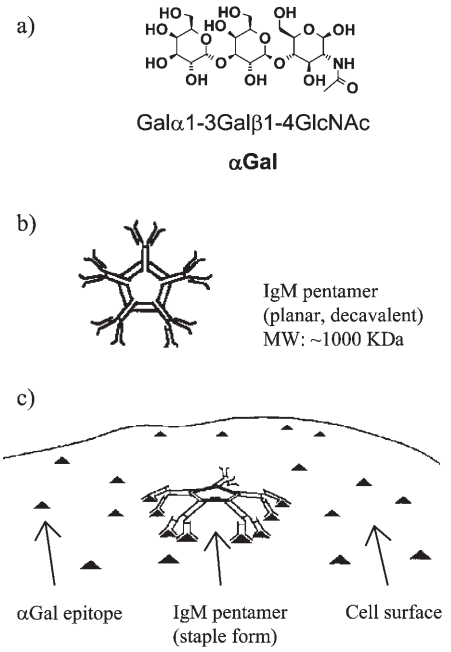


Figure 2. Multivalent interaction between an IgM and the αGal epitope: a) representation of the αGal trisaccharide; b) schematic representation of a circulating, planar IgM directed against the αGal epitope; c) upon polyvalent binding to multiple αGal trisaccharides presented on a cell surface, the IgM adopts a staple form causing complement activation and cell destruction.

in vitro and in vivo. However, macromolecules with a molecular mass comparable to the mass of an IgM ($\approx$1000 kDa) had to be used.[7,8]

As both the reproducible preparation and the appropriate characterization of non-homogeneous macromolecules are very demanding, an alternative concept for the control of polyvalent interactions with well-characterized small molecules is desirable (Figure 3). Such an approach could be based on the supramolecular chemistry[9] of compounds comprising a self-assembling moiety (gray square) and a ligand moiety (red ellipse). Ideally, intermolecular recognition of self-assembling moieties will lead to non-covalent particles presenting multiple ligands. These aggregates—but not the individual molecules (which are far too small)—can function as polyvalent receptor blockers. Interactions of the individual ligands with the polyvalent receptor contribute to the stability of the receptor–ligand complex. The strength of the inhibitory complex is best for a maximal number of both the contacts between individual self-assembling moieties and the individual ligand–receptor interactions. Therefore, in the case of a dynamic, reversible self-assembly process, the polyvalent receptor can be utilized as a template to optimize its own polyvalent inhibitor (Figure 3).

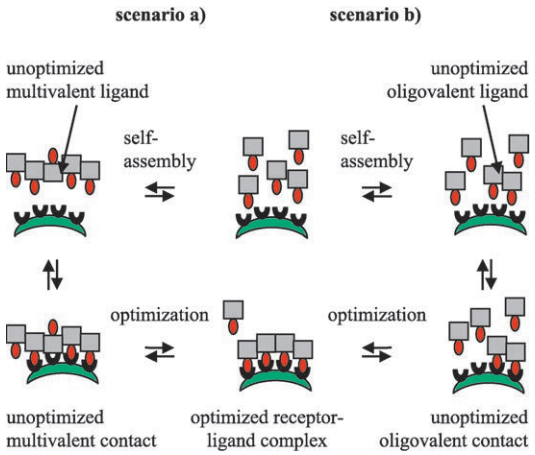
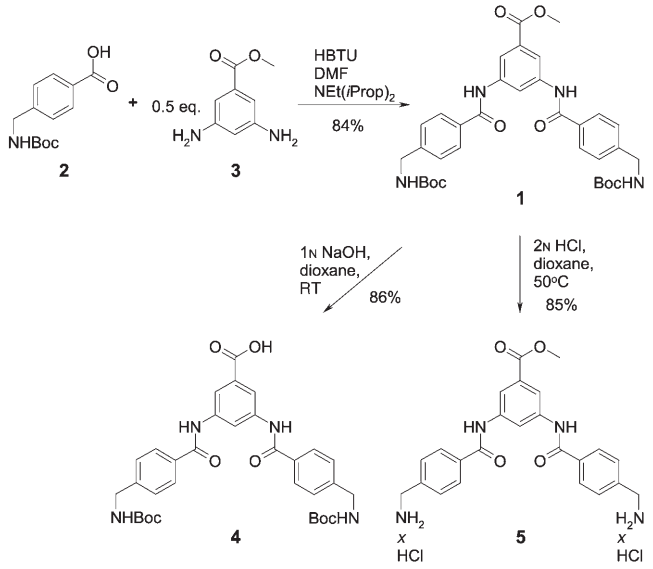


Figure 3. Formation of a tailored polyvalent receptor–ligand complex by dynamic self-assembly of small molecules comprising both a self-assembling moiety (grey square) and a ligand moiety (red ellipse). Scenario a): A preformed polyvalent ligand makes contact with the receptor. Further optimization of the ligand-receptor complex occurs utilizing the polyvalent receptor as a template. Scenario b): A relatively small, oligovalent aggregate binds weakly to the polyvalent receptor. In a second step, the highly potent polyvalent ligand "grows" on the surface of the receptor leading to an optimized receptor–ligand complex.

In a preliminary communication, we recently published our findings on novel glycodendrimers containing endgroups modified with the αGal trisaccharide (ligand).[10,11] We showed that, in aqueous solution, molecules with appropri-

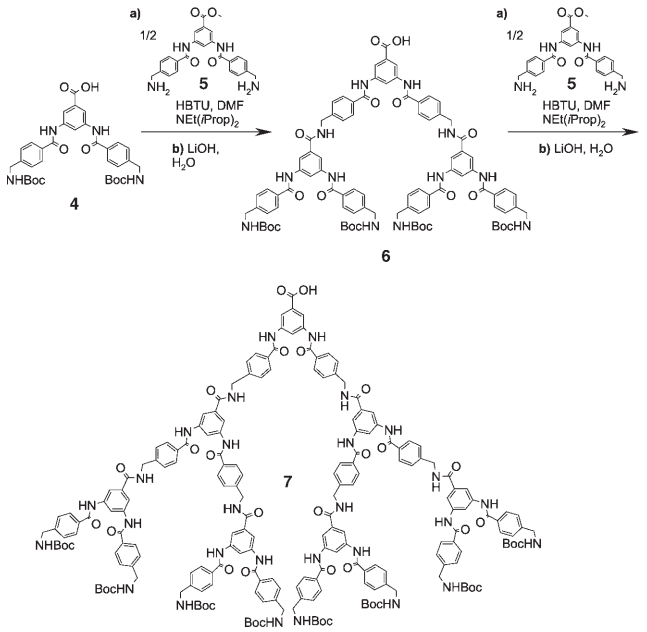
ate core structures (self-assembling moiety) self-assemble to form non-covalent nanoparticles with molecular weights of up to 7000 kDa. These particles — but not the individual molecules — were found to efficiently inhibit the αGal–IgM interaction, both in vitro and in vivo.[10] Here, we discuss the synthesis and characterization of these and additional glycodendrimers and present new evidence that self-assembly strongly depends on the structure and size of the dendrimer core. We show that particle formation is a dynamic process, which is a prerequisite for the formation of an optimized receptor–ligand complex.

Syntheses and characterization of dendrimer core structures: Dendrimer core structures were prepared by a convergent "outside–in" approach[12] using a single, three-directional building block (**1**) to allow for a minimum number of transformations for dendrimer tier construction. The orthogonally protected, rigid wedge **1** consists of three aromatic rings linked via amide bonds. Compound **1** displays a benzoate ester function and two benzylic amines. It was prepared by amide coupling of **2** and **3**. Saponification gave carboxylate **4**, whereas acid treatment furnished diamine **5** (Scheme 1).



Scheme 1. HBTU = *O*-benzotriazol-1-yl-N,N,N′,N′-tetramethyluronium hexafluorophosphate; DMF = dimethylformamide.

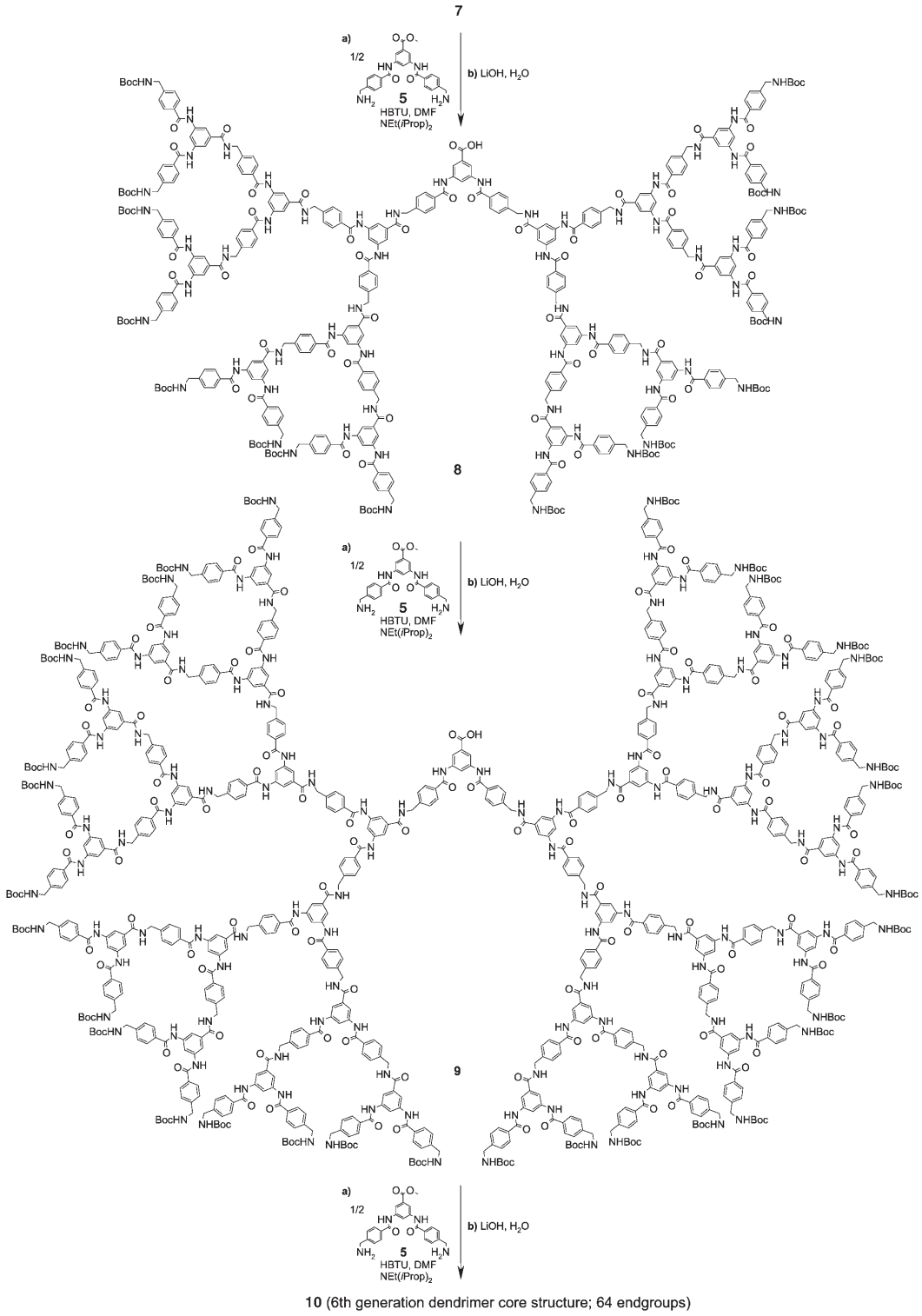
The second-generation core **6** (four endgroups) was assembled from **4** and 0.5 equivalents of **5** in DMF/iPr$_2$NEt using HBTU to promote the amide bond formation (Scheme 2). To ensure complete consumption, it was important to use a large excess of iPr$_2$NEt. After completion of the coupling, water and LiOH were added to the reaction mixture to cleave the methyl ester. The product was isolated by precipitation by adding either water or a less polar organic solvent. Further purification could be achieved by adding a solution of the precipitate in DMF to either water or an organic solvent. The third generation core **7** (eight



Scheme 2.

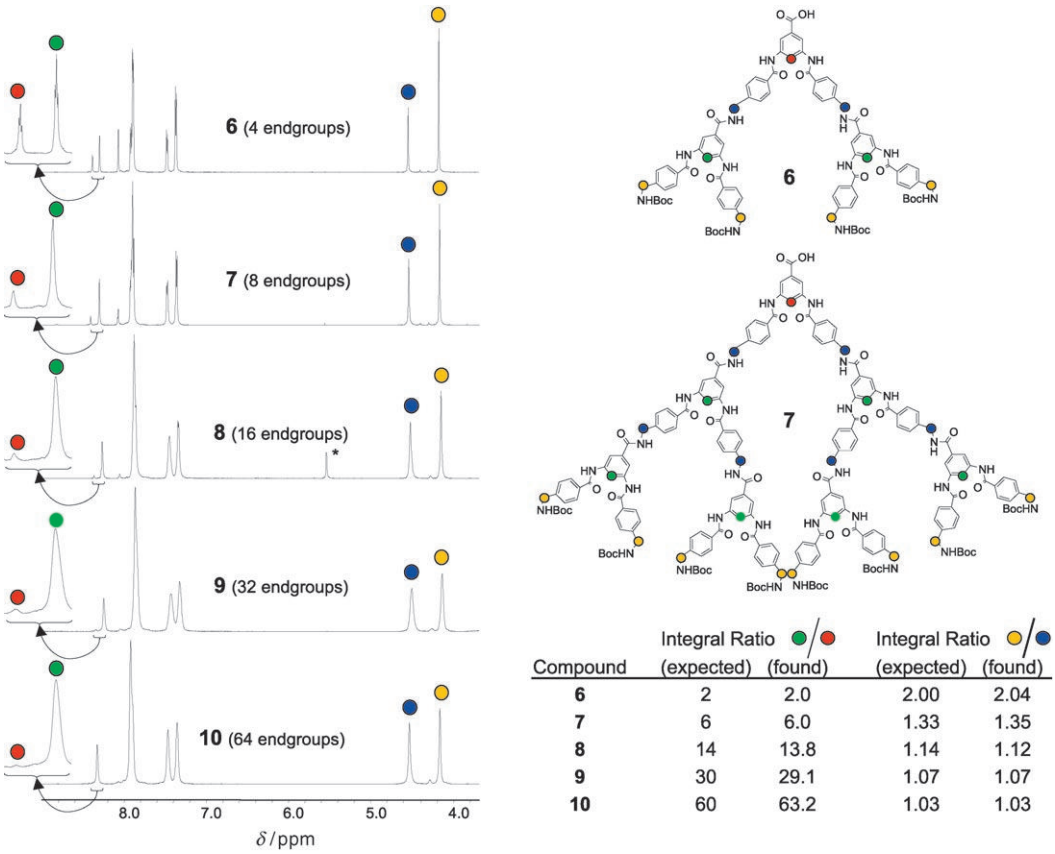
endgroups) was obtained by reacting **6** with 0.5 equivalents of **5** under the same reaction conditions. The fourth-, fifth-, and sixth-generation core structures with 16 endgroups (**8**), 32 endgroups (**9**), and 64 endgroups (**10**), respectively, were prepared following the same procedures (Scheme 3).

The integrity of the dendrimer cores was assessed by ^{1}H NMR spectroscopy using the integral values of indicative protons (Figure 4). All of the compounds contain two types of benzylic protons, giving rise to two distinct signals (see structures in Figure 4). They are either adjacent to a carbamate (yellow) or adjacent to a benzamide (blue). The ratio of these two types of benzylic protons changes characteristically from generation to generation (see table in Figure 4). The changes are considerable only up to the fourth generation core **8** (16 endgroups). For the highest generation cores (**9**, **10**), the ratios of the integrals of "yellow" and "blue" protons are very similar and close to unity, impeding an accurate distinction based on NMR. All measured and expected values were in agreement within the experimental error, but for the reason mentioned above the data can only prove the integrity of the cores up to the fourth generation. The dendrimer core structures contain two types of trisubstituted aromatic rings. These rings bear either a carboxylic acid or a carboxamide moiety. The protons in the *para* positions give rise to two characteristic signals (*para* to carboxylic acid: red; *para* to carboxamide: green). Whereas all of the dendrimers have only one "red" proton, the number of "green" protons increases from generation to generation (see table in Figure 4). The ratios can be determined very accurately up to the fourth generation core **8**. For the highest generation cores, **9** and **10**, the ratios are very high (30:1, 60:1), hampering a highly accurate determination. All measured and expected values were in agreement within the experi-



Scheme 3.

mental error. We conclude that all of the dendrimer core structures are highly homogeneous. Up to the fourth generation core **8** (16 endgroups), their purity is $>95\,\%$. This is remarkable considering that not a single chromatographic purification was required. The convergent synthesis approach allows the preparation of a subsequent dendrimer generation with nearly double the molecular weight by forming just two amide bonds. Thus, side products stemming from incomplete reaction and unconsumed starting materials differ significantly from the product in terms of molecular weight



Compound	Integral Ratio (expected)	Integral Ratio (found)	Integral Ratio (expected)	Integral Ratio (found)
6	2	2.0	2.00	2.04
7	6	6.0	1.33	1.35
8	14	13.8	1.14	1.12
9	30	29.1	1.07	1.07
10	60	63.2	1.03	1.03

Figure 4. ^{1}H NMR spectra ($\delta = 4$–9 ppm) of dendrimer cores **6**–**10** (600 MHz; DMSO/D_2O, 75 °C). The color code for the assignment of selected characteristic signals is illustrated for compounds **6** and **7**. The table shows the expected and the measured ratios of the integrals of the selected signals. *signal stems from CH_2Cl_2.

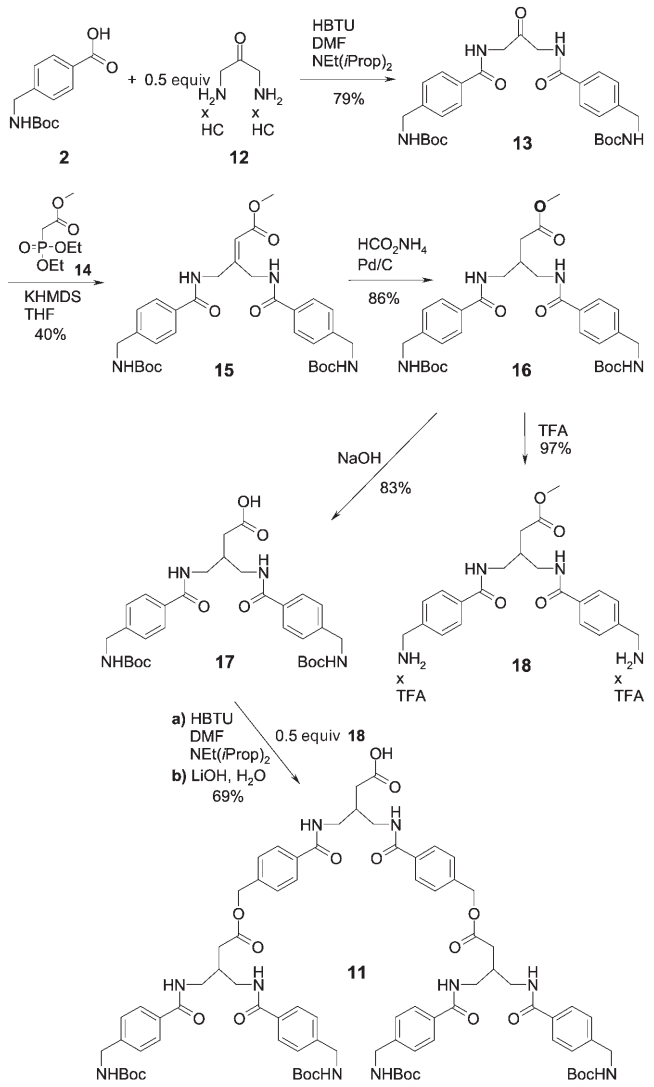
and size, allowing for purification by simple precipitation. We did not attempt to prepare larger dendrimers because of the envisaged difficulties in suitably characterizing these molecules.

Several analogues of **6** with more aliphatic core structures were prepared. Compound **11** contains branched aliphatic elements instead of the three trisubstituted aromatic rings of **6** (Scheme 4). Coupling of **2** with two equivalents of **12** gave ketone **13**, which was reacted with **14** to furnish alkene **15**. In some experiments, an isomer of **15** with the double bond adjacent to a nitrogen atom was isolated. Subsequent hydrogenation of **15** or its isomer gave **16**. Building blocks **17** or **18** were obtained from **16** by selective deprotection. Dendrimer **11** was prepared by condensation of **17** and **18**.

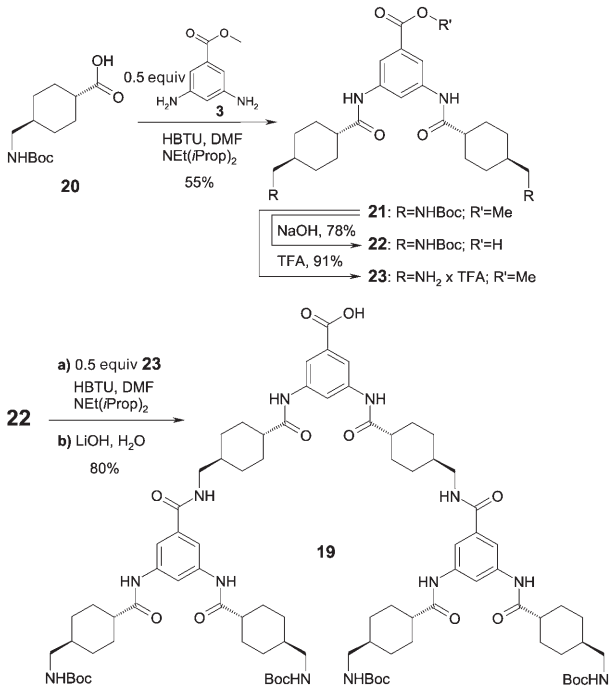
Dendrimer **19** has cyclohexyl rings instead of the six disubstituted aromatic rings of **6**. The core was assembled from cyclohexyl carboxylic acid **20** and benzoate **3** (Scheme 5). Building block **21** was selectively deprotected, and this was followed by amide coupling of the resulting acid **22** and diamine **23**. Finally, the more flexible core structure **24** with linear alkyl chains (C6) instead of the disubstituted aromatic rings of **6** was prepared analogously to **19** using hexanoic acid **25** instead of **20** (Scheme 6).

The second-generation core structures **29** and **30** bear methylated benzamide and methylated anilide functions, respectively. Compound **29** was prepared using building block **31**, which was obtained from **32** and **3** (Scheme 7). Deprotection of **31** gave **33**, which was coupled with **4** to furnish **29**. Compound **30** was prepared from building block **34**, which, in turn, was obtained from benzoate **35** and **2** (Scheme 8). Compound **35** was available from **36**, which was protected to furnish **37.** Subsequent N-methylation yielded **38**. Deesterification and concomitant removal of the N-protecting groups gave access to **35**. Selective deprotection of **34** gave **39** and **40**, which were coupled to furnish **30**.

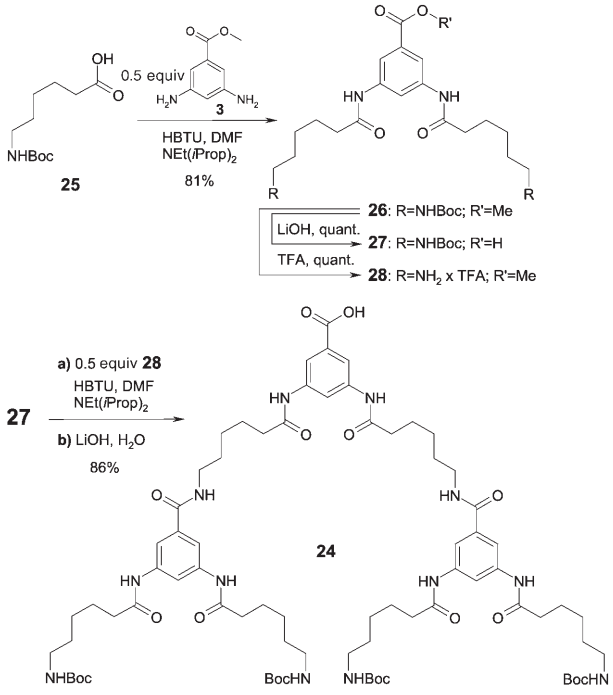
Functionalization of dendrimer core structures and characterization of the glycodendrimers: The convergent synthesis of the core structures requires just two transformations per individual molecule to obtain the next generation dendrimer, which has quite distinct properties compared to the starting materials and potential side products. On the contrary, depending on the size of the core structure, modifications of the endgroups can involve a large number of transformations per individual molecule. Thus, side products stemming from incomplete reactions can have very similar properties compared to the desired product, rendering purification very difficult. To obtain highly homogeneous glycodendrimers, all chemical reactions involved in the functionalization need to proceed in near-quantitative yield. We relied on a



Scheme 4.



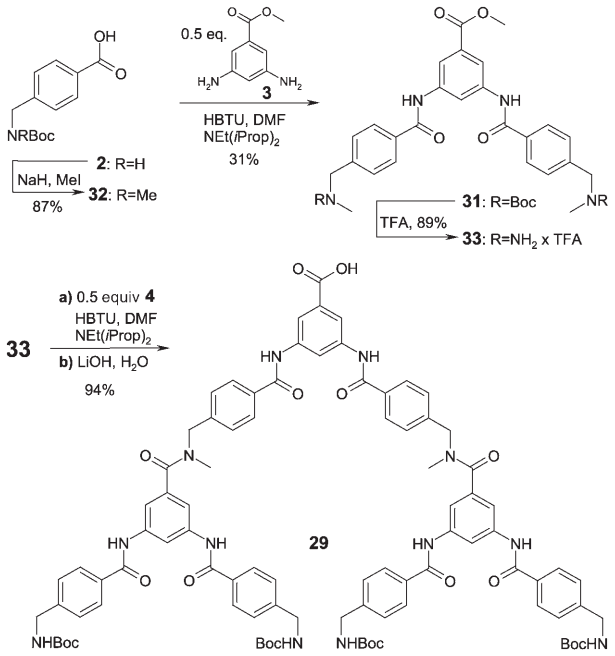
Scheme 5.



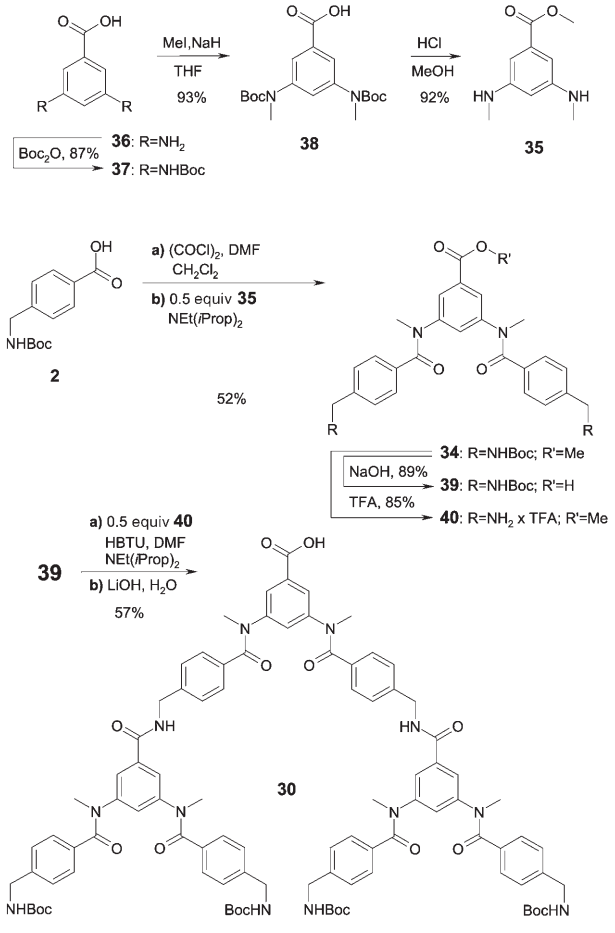
Scheme 6.

strategy which we had previously applied to the highly predictive functionalization of polylysine.[4b] The butoxycarbonyl(Boc)-protected core structures **4**, **6**, **7**, **8**, **9**, **10**, **11**, **19**, **24**, **29**, and **30** were transformed to the free amines using trifluoroacetic acid (TFA)/water in the presence of mercaptoethanol to trap *tert*-butyl cations, thus hindering the formation of *tert*-butylamino groups. The benzylic amines were isolated as TFA salts by precipitation in diethyl ether. The endgroups were further activated by introduction of a chloroacetamide group. Using a large excess of the sterically hindered weak base 2,6-lutidine and chloroacetic anhydride in DMF led to complete conversion. The chloroacetamides were isolated by precipitation in diethyl ether. Glycodendrimers containing the αGal trisaccharide were obtained by reacting the activated core structures with a small excess of αGal-SH[7a] in DMF (Figure 5). Addition of 1,8-diazabicyclo[5.4.0]undecene (DBU) led to complete functionalization within a few minutes. The crude products were precipitated, redissolved in water, and further purified by ultrafiltration. Glycodendrimer **7Lac** with eight lactose residues was obtained using Lac-SH instead of αGal-SH.

All of the glycodendrimers were characterized by NMR spectroscopy. The ^{1}H NMR spectra of **6Gal**, **7Gal**, **8Gal**, **9Gal**, and **10Gal** in DMSO/D_2O (5:1) at 80 °C are shown in Figure 6. The values of the integrals of characteristic signals



Scheme 7.



Scheme 8.

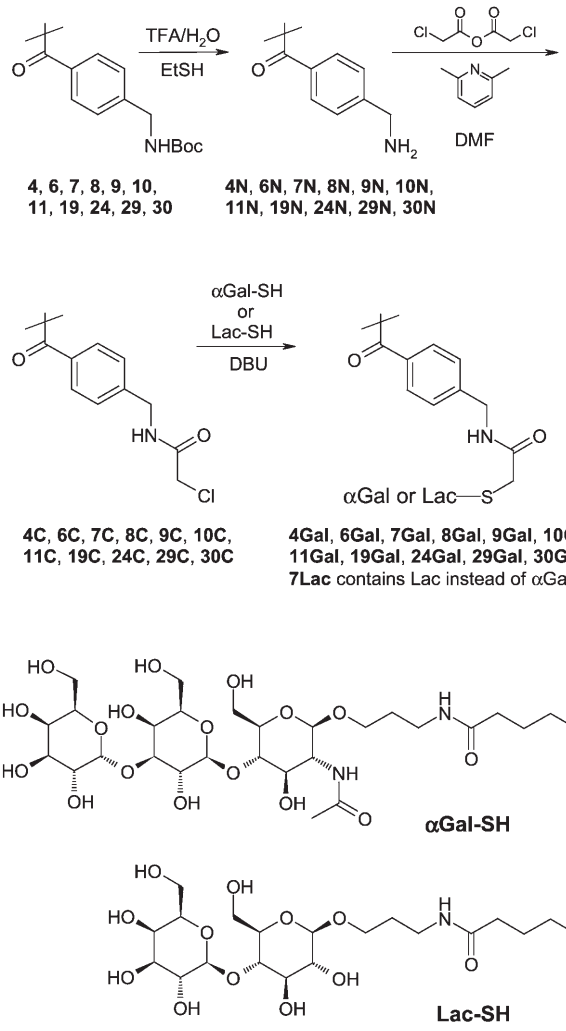


Figure 5. Synthesis of glycodendrimers **4Gal**, **6Gal**, **7Gal**, **8Gal**, **9Gal**, **10Gal**, **11Gal**, **19Gal**, **24Gal**, **29Gal**, **30Gal**, and **7Lac** by functionalization of endgroups of Boc-protected core structures **4**, **6**, **7**, **8**, **9**, **10**, **11**, **19**, **24**, **29**, and **30** (for simplicity only one endgroup is shown).

were used to quantify the degree of endgroup functionalization. The signals of internal benzylic hydrogens of the dendrimer core ($\delta \approx 4.7$ ppm; green) and the 1-hydrogens of the terminal α-linked galactoses ($\delta \approx 5.0$ ppm; red) were always separated at the low field of the aliphatic proton region. Their ratios were characteristic for each dendrimer generation (Figure 7). Expected and measured values were in very good agreement, indicating complete functionalization with the αGal trisaccharide in all cases (see Figure 6). It is important to note that for higher generation glycodendrimers the changes of this ratio are very small as it approaches a value of 2. Thus, in the cases of **9Gal** and **10Gal**, with 32 and 64 endgroups, respectively, the NMR data do not prove complete homogeneity but indicate functionalization of all endgroups. In the cases of **6Gal**, **7Gal**, and **8Gal** with 4, 8, and 16 endgroups, respectively, the data indicate very high purities (>95% for **6Gal** and **7Gal**; >90% for **8Gal**).

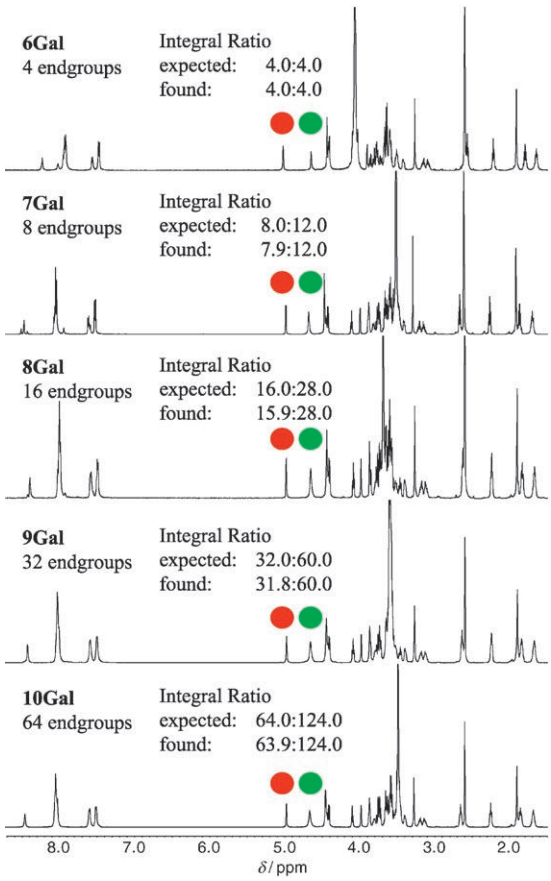


Figure 6. ^{1}H NMR spectra (600 MHz, DMSO/D_2O, 5:1, 80 °C) of **6Gal**, **7Gal**, **8Gal**, **9Gal**, and **10Gal**. The protons giving rise to the signals marked in red and green are shown for glycodendrimer **8Gal** (Figure 7).

Satisfying MALDI mass spectra were obtained for all of the glycodendrimers with the exception of **9Gal** and **10Gal**. The spectrum of **7Gal** with eight endgroups is shown in Figure 8. The expected and measured molecular weights of glycodendrimer **7Gal** are in agreement within the experimental error. Several lower molecular weight signals were detected. According to NMR, the glycodendrimer **7Gal** exhibits very high purity. Therefore, we believe that the additional signals are caused by fragmentation. Fragmentation was also observed for all of the other glycodendrimers, even for the first generation compound **4Gal** which had been purified by chromatography, and could not be avoided when different matrices were used. Because of the aggregation of our glycodendrimers, both HPLC and size-exclusion chromatography proved to be inappropriate for analyzing these molecules.

Characterization of the non-covalent nanoparticles: The self-assembly of our glycodendrimers to form non-covalent nanoparticles was studied by multi-angle light scattering in water (MALS; Table 1). Accordingly, the first generation dendrimer **4Gal** forms small aggregates (50 kDa), whereas **6Gal** forms large particles of 7100 kDa (1500 individual molecules/particle). The particle weights obtained for **7Gal** (2200 kDa) and **8Gal** (1200 kDa) were still very high (250 and 70 individual molecules/particle, respectively). The fifth and sixth generation glycodendrimers **9Gal** and **10Gal** did not aggregate considerably (7 and 3 individual molecules/particle, respectively). Very interestingly, considering the second to the sixth glycodendrimer generations, the molecular weight of the particles formed was seen to decrease with increasing molecular weight of the individual molecules.

The particle weight was not found to depend significantly on the concentration, as shown for dendrimer **6Gal** (7100 kDa at 0.06 mg mL^{-1}, 6000 kDa at 0.03 mg mL^{-1}, 3700 kDa at 0.01 mg mL^{-1}), but is significantly affected by the temperature (Figure 9). It was seen to decrease with increasing temperature (tenfold on going from 30 °C to 70 °C). The ^{1}H NMR spectra in D_2O are also indicative of the formation of particles. As an example, the spectrum of **6Gal** showed very broad signals in the aromatic region (7–8 ppm; dendrimer core structure; Figure 9). With increasing temperature, the signals sharpened. We believe that broad signals are indicative of large particles, and that these fall apart at elevated temperatures leading to sharp signals as typically observed for individual small molecules. In DMSO/D_2O (5:1) mixtures, sharp signals were observed even at low temperatures. It is likely that aggregation is inhibited by organic solvents.

Comparable molecular weights of the particles and very similar NMR spectra were observed at identical temperatures when a hot solution was cooled or when a cold solution was heated. Thus, self-assembly is a dynamic process which rapidly establishes thermodynamic equilibrium (Figure 9).

The particle weight proved to be strongly dependent on the core structure, as revealed by studying the aggregation properties of glycodendrimers with small core modifications compared to **6Gal** and **7Gal**. Glycodendrimer **24Gal**, containing a flexible aliphatic spacer in place of the disubstituted aromatic rings of **6Gal**, did not aggregate at all. Compound **19Gal**, with cyclohexyl rings instead of the disubstituted aromatic rings of **6Gal**, which is more rigid than **24Gal** but less aromatic than **6Gal**, self-assembled but formed aggregates less than a tenth of the size of those with **6Gal**. Glycodendrimer **11Gal**, with branched aliphatic replacements of the trisubstituted aromatic rings, formed very small aggregates (133 kDa). The third generation compounds **7Lac** (8×Lac; 1900 kDa; 250 individual molecules/particle) and **7Gal** (8×αGal; 2200 kDa; 265 individual molecules/particle), with identical backbones but different carbohydrate endgroups, formed aggregates of very similar size. Thus, the particle weight showed no strong dependence on the size of the hydrophilic endgroups.

In aqueous solution, intermolecular hydrogen bonds between individual glycodendrimers seem not to be significantly involved in self-assembly since particle weights were not reduced by the H-bond disrupting reagent guanidinium hydrochloride (1.5 M), as determined for **6Gal** by light scattering (7100 kDa at 0.06 mg mL^{-1}, 6000 kDa at 0.03 mg mL^{-1}, 3700 kDa at 0.01 mg mL^{-1}). In addition, the ^{1}H NMR signals

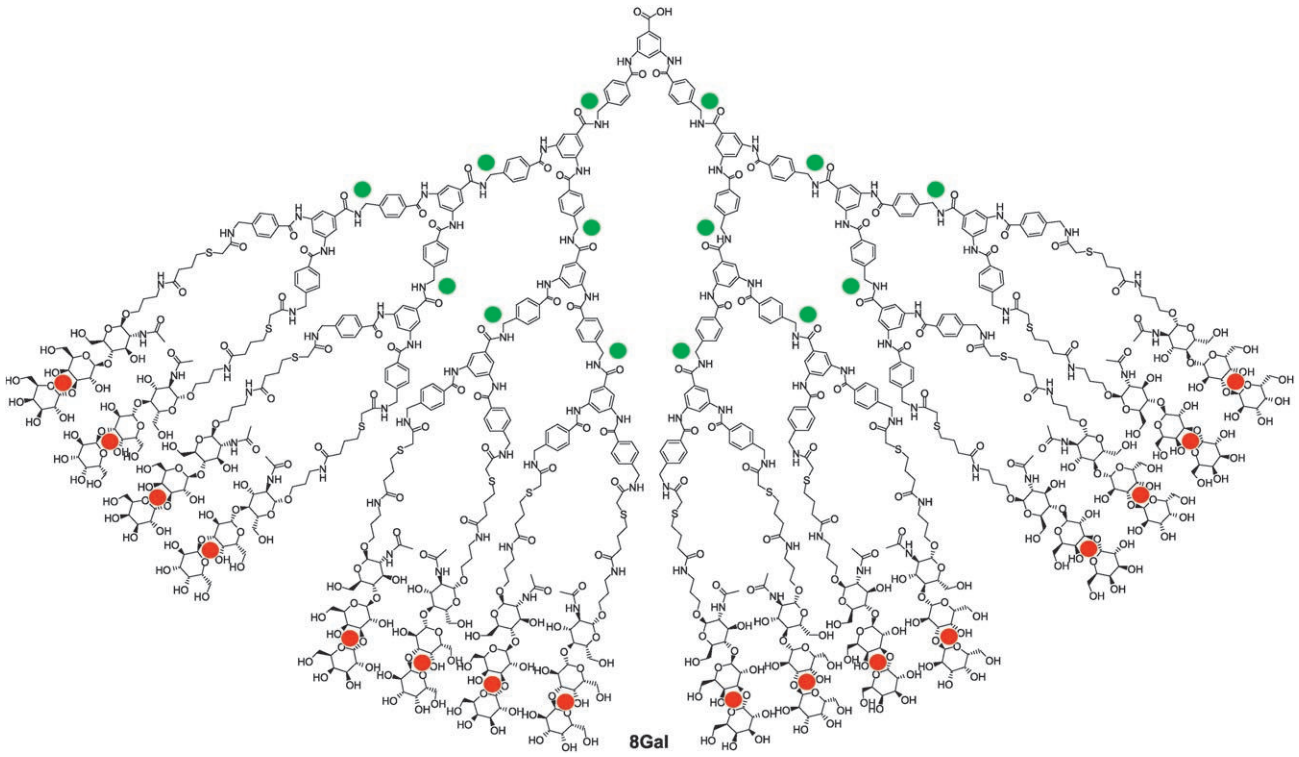


Figure 7. Structure of glycodendrimer **8Gal**. The integrals of the internal benzylic protons (green) and the H-1 protons of the terminal α-linked galactose (red) have been used to quantify the degree of endgroup functionalization (Figure 6).

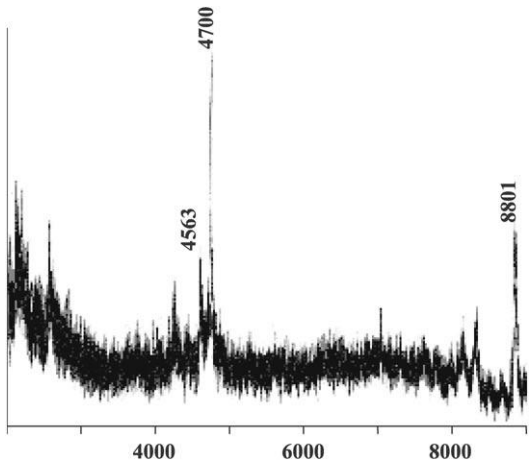


Figure 8. MALDI-MS of glycodendrimer **7Gal** (matrix: 2,5-dihydroxycinnamic acid); calcd. for the Na salt: $C_{393}H_{525}N_{44}NaO_{166}S_8$ 8801.21; found 8801 (broad signal).

of **6Gal** in D_2O at room temperature remained very broad in the presence of guanidinium deuteriumchloride (6 M). Compound **29** with two methylated benzamides self-assembled to still relatively large particles (1100 kDa, 260 individual molecules/particle), whereas compound **30** with six methylated anilides formed only small aggregates (315 kDa, 70 individual molecules/particle; Table 1). It is important to note that the reduced tendency to form aggregates observed for **29** and **30** is not necessarily caused by the breakdown of intramolecular hydrogen bonds formed in **6Gal**, but could also be explained by steric effects of the additional methyl groups on the core conformation.

Rigid, highly aromatic core structures are required for self-assembly. We believe that intramolecular π-stacking leads to a pre-organized core conformation that allows core–core contacts. Self-assembly to form large particles can occur if a glycodendrimer core can be in contact with at least two other core structures at the same time. The core of **4Gal** seems to be too small for efficient core–core interactions, whereas the second generation core of **6Gal** is optimal for self-assembly. The decreasing particle weight obtained for higher generation glycodendrimers (**7Gal**–**10Gal**) could be explained by the increasing size of the dendrimer core and increasing number of large endgroups per individual molecule inducing a more globular shape. As a consequence, the core is more efficiently shielded by carbohydrates, rendering intermolecular core–core contacts more difficult. Increased crowding in the core with each generation number also stabilizes interior binding through π–π interactions and hydrophobic contacts, which further reduce the conformational flexibility of the glycodendrimer.

This hypothesis was supported by molecular modeling. Conformational analyses of the dendrimer core structures of compounds **6Gal** and **7Gal** were carried out by applying a combined MC/LMOD calculation in vacuum. All of the trisaccharide endgroups were removed to reduce the computation time. By visual inspection, low-energy conformations of the core of **6Gal** were selected that allow both re-attach-

Table 1. Properties of the Glycodendrimers.

Compound (endgroups)	*MW* (ind. mol.) [kDa]	MW_{aggr}[a] [kDa]	IgM[b] IC_{50} [μM]	Haemolysis[b] IC_{50} [μM]
4Gal (2)	1.908	50[c]	>10	>100
6Gal (4)	4.198	7100[d]	0.025	0.035
7Gal (8)	8.779	2200[c]	0.010	0.010
7Lac (8)	7.154	1900[c]	>100	n.d.
8Gal (16)	17.941	1200[c]	0.019	0.180
9Gal (32)	36.264	270[c]	>1	0.550
10Gal (64)	72.911	200[c]	>1	2.240
11Gal (4)	4.136	133[d]	>1	n.d.
19Gal (4)	4.234	571[d]	>1	n.d.
24Gal (4)	4.078	7[c]	>1	>100
29Gal (4)	4.226	1100[d]	0.170	n.d.
30Gal (4)	4.282	315[d]	>1	n.d.

[a] The weight-average molar mass (MW_{aggr}) for all of the glycodendrimers was determined by multi-angle light scattering (MALS). Polydispersity values (d*n*/dc) were between 0.18 and 0.22 mL g^{-1}. [b] For active compounds, the average of at least two experiments is quoted (variations within a factor of 2.5). Concentrations refer to equivalent concentration of trisaccharide, not to concentration of oligovalent glycodendrimer. [c] Test concentration: 0.3 mg mL^{-1}. [d] Test concentration: 0.06 mg mL^{-1}.

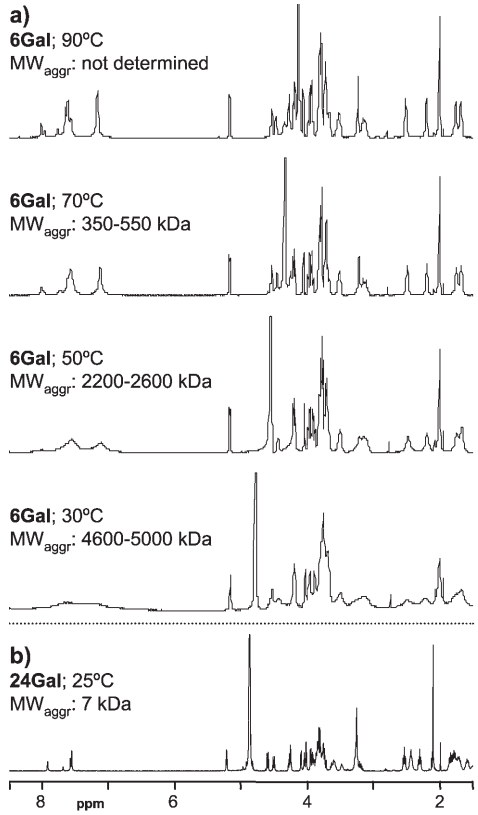


Figure 9. ^{1}H NMR spectra (500 MHz; D_2O) of glycodendrimers **6Gal** and **24Gal**. a) At 30 °C **6Gal** shows very broad signals for the dendrimer core structure ($\delta = 7$–8 ppm), which become sharpened at elevated temperatures. The weight-average molecular mass of the aggregates formed in water (MW_{aggr}) was determined by multi-angle light scattering (MALS). Accordingly, big aggregates self-assemble at low temperatures but dissociate at elevated temperatures. Small aggregates or individual molecules gave rise to sharp NMR signals, whereas big aggregates led to very broad signals. Very similar MW_{aggr} and NMR spectra were obtained upon heating a cold aqueous solution of **6Gal** or upon cooling a hot solution. Thus, self-assembly of glycodendrimer **6Gal** is a dynamic process that rapidly reaches thermodynamic equilibrium. b) **24Gal**, which does not form particles (MW_{aggr} = 7 kDa), showed very sharp signals at 25 °C.

ment of the four trisaccharides without steric interference and solvation of the carbohydrates. Most of these conformations were compact and disk-like, as exemplified by the structure with the lowest energy (Figure 10). Parallel and edge-to-face π–π interactions of the phenyl rings lead to the formation of two hydrophobic surfaces—the front and back side of the disk—which remain accessible even with the trisaccharides attached. Thus, intermolecular core–core contacts are readily possible and aggregation can occur. Although the calculations were performed in vacuo, an aqueous environment with a high dielectric constant would also favor the formation of compact core structures. Low energy conformations of the dendrimer core of compound **7Gal** were also found to be compact. However, due to the larger size of the molecule, they are less disk-like and rather spherical. In addition, it is more difficult to attach the eight trisaccharides without shielding the hydrophobic core surface. Most conformations showed only one hydrophobic surface, which could lead to pair formation but not to aggregation to form large particles. Only a few conformations are suited for aggregation, providing at least two accessible hydrophobic surfaces (ΔE to the absolute minimum >6 kcal). Compared to **6Gal**, the more globular shape of the core of **7Gal** leads to less efficient intermolecular core–core contacts, thereby rationalizing the formation of smaller aggregates. These effects are most probably even more pronounced for the higher generation dendrimers **8Gal**–**10Gal**, resulting in the observed decreasing tendency to aggregate. Interestingly, compared to **6Gal**, low energy conformations of the core of its partially *N*-methylated derivative **30Gal** were less compact and did not provide accessible hydrophobic surfaces for aggregation. This could explain the formation of only small aggregates.

The nanoparticles formed by our glycodendrimers can be deposited on surfaces and then investigated by atom force microscopy (AFM) and transmission electron microscopy. Aqueous solutions of **6Gal**–**10Gal** were applied to hydrophilic mica surfaces and the solvent was evaporated. By atom force microscopy, we observed disk-shaped particles. Their diameters decreased with increasing mass of the individual molecules (61 nm, **6Gal**; 13 nm, **10Gal**), showing the same trend as the molecular weights in solution (Figure 11 a). The size distribution for the individual generations was found to be remarkably homogeneous (within a factor of two). The particles exhibit a pronounced disk shape. Their flatness (1–5 nm) might be caused by interactions with the mica surface. The disk-like morphology might be slightly disrupted by AFM tip convolution artifacts. Particles of

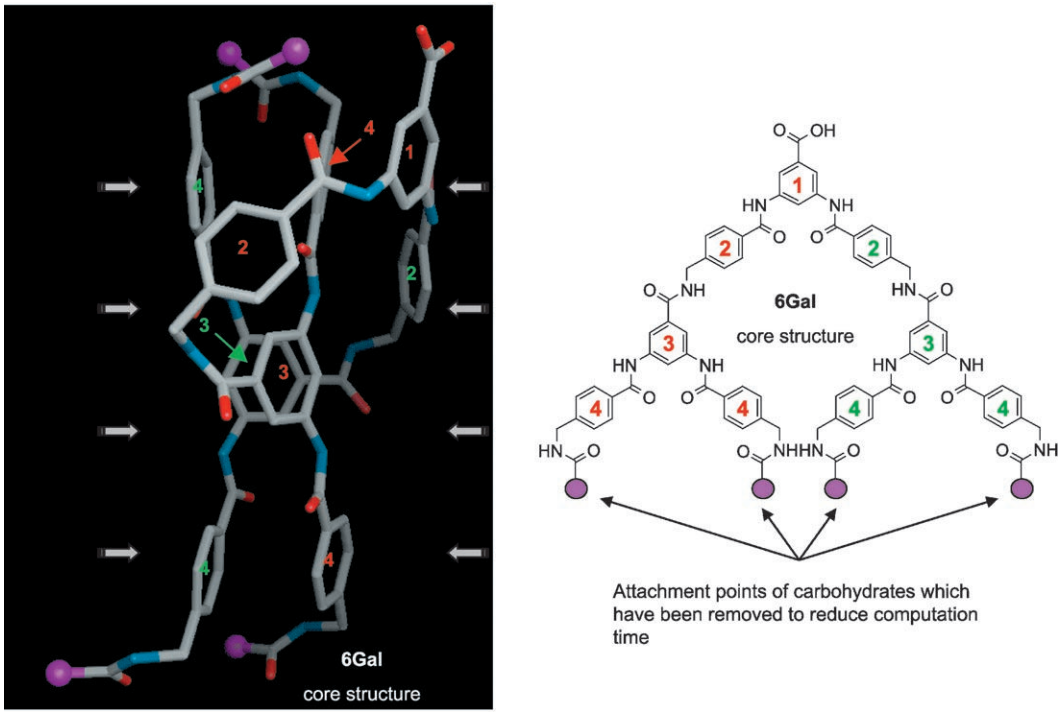


Figure 10. Minimum energy conformation of the core of **6Gal** obtained by MC/LMOD calculations in vacuum. The core adopts a disk-like conformation. The gray arrows indicate the hydrophobic front and back sides of the disk, which are suited for intermolecular core–core contacts allowing aggregation to large particles even in the presence of the trisaccharides.

6Gal were also deposited on a more hydrophobic polyformaldehyde surface and investigated by transmission electron microscopy. The particle diameters were determined to be between 20 and 120 nm, the majority having a diameter of 50 nm (Figure 11b). This is in good agreement with the AFM results on mica. We conclude that the particle size is independent of the properties of the surface on which they are deposited, indicating that the particles are preformed in solution.

Biological evaluation: The potential of our compounds as polyvalent IgM ligands was assessed by employing in vitro assays measuring the inhibition of both the anti-αGal IgM binding to the xenoantigen and the αGal-mediated lysis of pig erythrocytes (Table 1). Concentrations refer to equivalent concentrations of trisaccharide and not to concentrations of glycodendrimers. In both assays, monomeric αGal was inactive at 100 μM, which is in agreement with the finding that individual carbohydrate-protein interactions are often weak.[3] Divalent first generation dendrimer **4Gal**, which forms small aggregates (50 kDa), showed no effect in either assay. The glycodendrimers **6Gal** and **7Gal**, which form large nanoparticles, were highly potent in both assays (0.025, 0.035 μM and 0.010, 0.010 μM, respectively). Compound **8Gal** also showed high potency in the binding assay (0.019 μM), but was significantly less potent in the haemolysis assay (0.18 μM). The potency dropped even more for the larger dendrimers **9Gal** and **10Gal**, which do not aggregate. Thus, especially in the more relevant haemolysis assay, potency clearly correlates with the size of the aggregates, and not with the size of the individual molecules. The inhibition data suggest that optimal particle weight and size for IgM inhibition are obtained with **7Gal**. The particles formed by **6Gal** might be slightly too big, but still showed high potency. The aggregates formed by **4Gal**, **8Gal**, **9Gal**, and **10Gal** are apparently too small to accomplish polyvalent amplification of IgM inhibition. Potency is strongly dependent on the αGal trisaccharide because the aggregating control dendrimer **7Lac** (identical backbone and similar aggregation properties as **7Gal**, but Lac instead of αGal) did not inhibit binding. Thus, antibody inhibition by interaction of the dendrimer backbone can be ruled out. Among the second-generation dendrimers with modified core structures, only compound **29Gal** (which was the only one that formed aggregates of considerable size) showed moderate inhibition in the binding assay. All of the others (**11Gal**, **19Gal**, **24Gal**, and **30Gal**) were found to be inactive. These experiments provide additional evidence that big, non-covalent aggregates and not individual molecules function as polyvalent receptor blockers.

The most potent compound, **7Gal**, was selected for in vivo profiling in cynomolgus monkeys. Three animals were dosed with 1 mg kg^{-1} (i.v.). Assuming 100% bioavailability, the initial blood concentration can be estimated to be ≈10 μM. Within 5 minutes of the injection, the anti-αGal IgMs detected by ELISA were reduced to 20% of the initial value determined prior to the administration and remained at low levels for >4 h. Interestingly, the initial IgM levels in

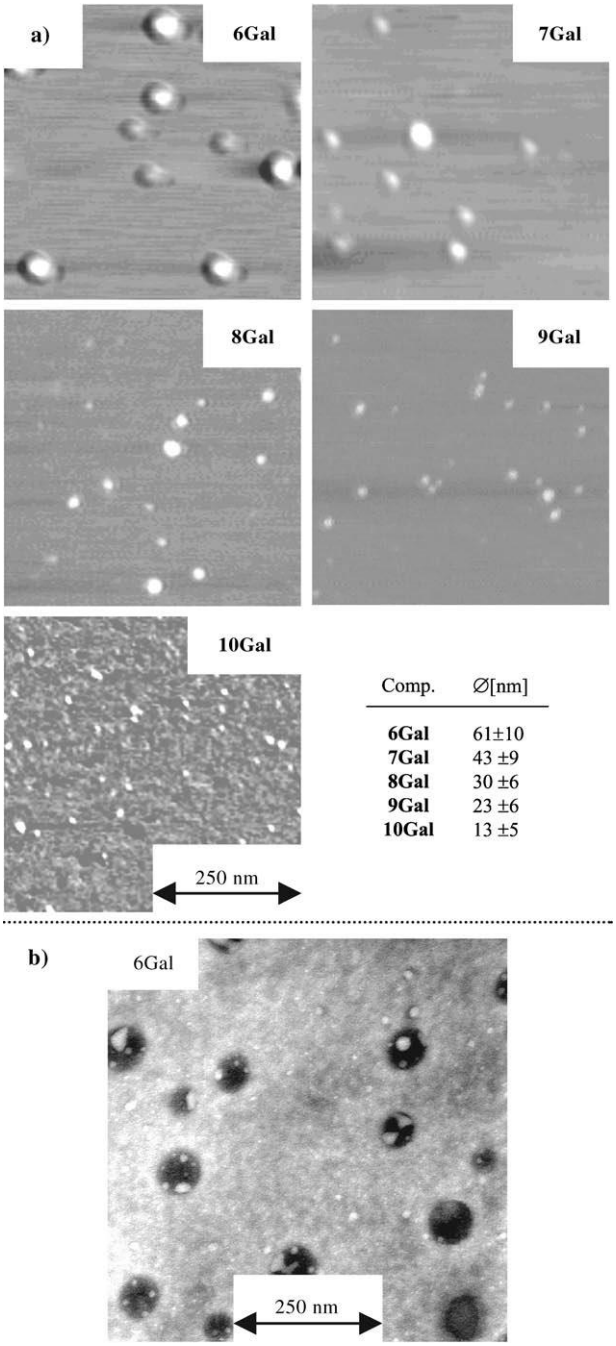


Figure 11. Visualization of particles formed by **6Gal–10Gal**, a) on a mica surface by atomic force microscopy; b) on polyformaldehyde by transmission electron microscopy.

cynomolgus monkeys were found to be 2.3-fold higher than in pooled human sera. Most importantly, anti-αGal antibody-mediated haemolytic activity was completely eliminated.

Conclusion

We have reported on novel, self-assembling small glycodendrimers fulfilling single molecule entity criteria, which form non-covalent nanoparticles in water. The particle sizes are remarkably homogeneous and can be varied over a broad range by appropriate choice of the dendrimer generation. The particles can be deposited on different surfaces by evaporation of the solvent. Their size distribution is rather homogeneous. Self-assembly of our glycodendrimers is a very robust process, which occurs under in vitro assay conditions and even more remarkably in vivo. We have shown that self-assembly of these glycodendrimers is a dynamic equilibrium process. Thus, it is conceivable that non-covalent polyvalent ligands are optimized with respect to size and shape in the presence of natural polyvalent receptors. The control of a broad variety of physiologically relevant polyvalent interactions should be possible.

Experimental Section

General: All reactions were carried out in dry solvents under an argon atmosphere. Reagents were purchased at the highest commercial quality and were used without further purification. NMR spectra were recorded on Bruker DRX-600, DRX-500, AMX-500, AMX-400 or Avance DPX 400 instruments, as well as on Varian Unity 500 and Varian Unity plus 600 instruments. Residual undeuterated solvents were used as an internal reference for the calibration. The following abbreviations are used to denote the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, quint. = quintet, m = multiplet. ESI (electrospray ionization) mass spectra were obtained on a Finnigan MAT 90 mass spectrometer. MALDI (matrix-assisted laser desorption ionization) mass spectra were obtained on a Voyager STR instrument using dihydroxybenzoic acid, α-cyano-4-hydroxycinnamic acid, 2,5-dihydroxycinnamic acid, sinapinic acid or ferulic acid as a matrix. Merck silica gel 60 (particle size 0.040–0.063 μm) was used for flash column chromatography. Ultrafiltrations were performed using Amicon 8010 stirred cells (vol.: 10 mL, diam.: 25 mm) and Amicon YM3 disc membranes (molecular weight cut-off: 3000).

Syntheses of dendrimer core structures

Preparation of 1: Ethyldiisopropylamine (30 mL) was added to a mixture of **2** (10.50 g, 41.84 mmol), **3** (5.00 g, 20.92 mmol), and HBTU (15.86 g, 41.84 mmol) in dry DMF (100 mL). The solution was stirred for 72 h at room temperature. Ethyl acetate (250 mL) was added and the resulting solution was extracted with 1N HCl (2×250 mL), 1N NaOH (2×250 mL), and brine (2×100 mL). The organic phase was treated with decolorizing charcoal (2.5 g), dried with magnesium sulfate, and concentrated to a volume of 40 mL. The solution was then added dropwise to a stirred mixture of diethyl ether (750 mL) and hexane (250 mL). The precipitate formed was filtered off, washed with diethyl ether/hexane, and dried. Compound **1** (10.35 g, 78%) was isolated as a colorless solid. ^{1}H NMR (600 MHz, CD_3OD, room temperature): δ = 1.46 (s, 18H; 2×*tert*-butyl), 3.92 (s, 3H; CO_2CH_3), 4.30 (s, 4H; 2×Ar-CH_2-NH-), 7.41 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 7.90 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-*H*), 8.14 (d, ^3J(H,H) = 2.0 Hz, 2H; Ar-*H*), 8.40 ppm (m, 1H; Ar-*H*); HR-MS: calcd for $C_{34}H_{40}N_4O_8$ $[M+Na]^+$ 655.2738; found 655.2739.

Preparation of 4: A mixture of **1** (5.00 g, 7.91 mmol), 2N NaOH (30 mL), and dioxane (30 mL) was stirred at room temperature for 16 h. Ethyl acetate (150 mL) was added and the resulting mixture was washed with 1N HCl (2×100 mL) and brine (2×100 mL). The organic phase was cooled to 0°C for 16 h. The precipitate that formed was filtered off, washed with cold ethyl acetate, and dried. Compound **4** (4.42 g, 90%) was isolated as a beige solid. ^{1}H NMR (500 MHz, CD_3OD, room temperature): δ = 1.39

(s, 18H; 2× *tert*-butyl), 4.24 (s, 4H; 2× Ar-CH_2-NH-), 7.35 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 7.84 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 8.08 (d, ^3J(H,H) = 2.0 Hz, 2H; Ar-H), 8.35 ppm (m, 1H; Ar-H); HR-MS: calcd for $C_{33}H_{38}N_4O_8$ $[M+Na]^+$ 641.2582; found 641.2584.

Preparation of 5: A mixture of **1** (3.00 g, 4.75 mmol), 2N HCl (12 mL), and dioxane (25 mL) was stirred at 50–60°C for 5 h. Further dioxane (25 mL) was then added and the solution was cooled to 0°C for 4 h. The precipitate that formed was filtered off, washed with cold dioxane, and dried. The dihydrochloride of compound **5** (1.98 g, 83%) was isolated as a beige solid. ^{1}H NMR (500 MHz, D_2O, room temperature): δ = 3.86 (s, 3H; CO_2CH_3), 4.24 (s, 4H; 2× Ar-CH_2-NH-), 7.57 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 7.90 (m, 6H; Ar-*H*), 8.16 ppm (m, 1H; Ar-*H*); HR-MS: calcd. for $C_{24}H_{24}N_4O_4$ $[M+Na]^+$ 455.1690; found 455.1691.

Preparation of 6: Ethyldiisopropylamine (15 mL) was added to a mixture of **4** (3.68 g, 5.96 mmol), **5** (1.50 g, 2.98 mmol), and HBTU (2.26 g, 5.96 mmol) in dry DMF (50 mL). The solution was stirred for 7 h at room temperature. DMF (50 mL), water (25 mL, dropwise), and LiOH monohydrate (3.13 g, 74 mmol) were added and the resulting mixture was stirred for an additional 16 h at room temperature. The solution was then added dropwise to a mixture of acetone and 1N HCl (500 mL, 1:1). The precipitate formed was filtered off and washed with water, acetone, and CH_2Cl_2. Compound **6** (3.26 g, 68%) was isolated as beige solid. ^{1}H NMR (600 MHz, [D$_6$]DMSO, 80°C): δ = 1.41 (s, 36H; 4× *tert*-butyl), 4.22 (d, ^3J(H,H) = 5.5 Hz, 8H; 4× Ar-CH_2-NHBoc), 4.58 (d, ^3J(H,H) = 7.5 Hz, 4H; 2× Ar-CH_2-NH-CO-Ar), 7.11 (br m, 4H; 4× N*H*Boc), 7.40 (d, ^3J(H,H) = 8.0 Hz, 8H; Ar-H), 7.51 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 7.97 (m, 16H; Ar-H), 8.12 (s, 2H; Ar-H), 8.41 (m, 2H; Ar-H), 8.57 (m, 1H; Ar-H), 8.79 (s, 2H; 2× Ar-CH_2-N*H*-CO-Ar), 10.17 (s, 4H; 4× Ar-N*H*-CO-Ar), 10.22 ppm (s, 2H; 2× Ar-N*H*-CO-Ar); HR-MS: calcd for $C_{89}H_{94}N_{12}O_{18}$ $[M+NH_4]^+$ 1636.7147; found 1636.7147.

Preparation of 7: Ethyldiisopropylamine (10 mL) was added to a mixture of **5** (0.47 g, 0.93 mmol), **6** (3.00 g, 1.85 mmol), and HBTU (0.70 g, 0.93 mmol) in dry DMF (35 mL). The solution was stirred for 6 h at room temperature. DMF (15 mL), water (12 mL, dropwise), and LiOH monohydrate (1.95 g, 46.5 mmol) were added and the resulting mixture was stirred for an additional 16 h at room temperature. The solution was added dropwise to a mixture of acetone and 0.2N HCl (750 mL, 1:3). The precipitate formed was filtered off and washed with water, acetone, and CH_2Cl_2. Compound **7** (3.10 g, 92%) was isolated as a beige solid. ^{1}H NMR (600 MHz, [D$_6$]DMSO, 80°C): δ = 1.41 (s, 72H; 8× *tert*-butyl), 4.22 (m, 16H; 8× Ar-CH_2-NHBoc), 4.58 (m, 12H; 6× Ar-CH_2-NH-CO-Ar), 7.29 (br m, 8H; 8× -N*H*Boc), 7.39 (d, ^3J(H,H) = 8.0 Hz, 16H; Ar-*H*), 7.51 (m, 12H; Ar-H), 7.97 (m, 40H; Ar-H), 8.11 (s, 2H; Ar-H), 8.41 (m, 6H; Ar-H), 8.58 (m, 1H; Ar-H), 8.79 (s, 6H; 6× Ar-CH_2-N*H*-CO-Ar), 10.19 ppm (s, 14H; 2× Ar-N*H*-CO-Ar); no MS could be obtained neither by ES nor by MALDI.

Preparation of 8: Ethyldiisopropylamine (10 mL) was added to a mixture of **5** (0.209 g, 0.414 mmol), **7** (3.00 g, 0.828 mmol), and HBTU (0.314 g, 0.828 mmol) in dry DMF (35 mL). The solution was stirred for 6 h at room temperature. DMF (15 mL), water (10 mL, dropwise), and LiOH monohydrate (1.95 g, 46.5 mmol) were added and the mixture was stirred for an additional 16 h at room temperature. The solution was then added dropwise to a mixture of acetone and 0.2N HCl (750 mL, 1:3). The precipitate formed was filtered off and washed with water, acetone, and CH_2Cl_2. Compound **8** (2.85 g, 90%) was isolated as a beige solid. ^{1}H NMR (600 MHz, [D$_6$]DMSO, 80°C): δ = 1.40 (s, 144H; 16× *tert*-butyl), 4.22 (d, ^3J(H,H) = 5.5 Hz, 32H; 16× Ar-CH_2-NHBoc), 4.58 (d, ^3J(H,H) = 7.5 Hz, 28H; 14× Ar-CH_2-NH-CO-Ar), 7.11 (br m, 16H; 16× -NHBoc), 7.39 (d, ^3J(H,H) = 8.0 Hz, 32H; Ar-H), 7.50 (m, ^3J(H,H) = 8.0 Hz, 28H; Ar-H), 7.97 (m, 88H; Ar-H), 8.11 (m, 2H; Ar-H), 8.40 (m, 14H; Ar-H), 8.58 (m, 1H; Ar-H), 8.79 (m, 14H; 14× Ar-CH_2-N*H*-CO-Ar), 10.18 ppm (m, 30H; 30× Ar-N*H*-CO-Ar); no MS could be obtained neither by ES nor by MALDI.

Preparation of 9: Ethyldiisopropylamine (2 mL) was added to a mixture of **5** (59.5 mg, 0.118 mmol), **8** (1800 mg, 0.236 mmol), and HBTU (89.4 mg, 0.236 mmol) in dry DMF (20 mL). The solution was stirred for 6 h at room temperature. Water (3.2 mL, dropwise) and LiOH monohydrate (149 mg, 3.54 mmol) were added and the mixture was stirred for an additional 16 h at room temperature. The solution was added dropwise to a mixture of acetone and 0.2N HCl (750 mL, 1:3). The precipitate formed was filtered off and washed with water, acetone, and CH_2Cl_2. Compound **9** (1760 mg, 95%) was isolated as a beige solid. ^{1}H NMR (600 MHz, [D$_6$]DMSO, 80°C): δ = 1.40 (s, 288H; 32× *tert*-butyl), 4.22 (m, 64H; 32× Ar-CH_2-NHBoc), 4.58 (m, 60H; 30× Ar-CH_2-NH-CO-Ar), 7.10 (br m, 32H; 32× -NHBoc), 7.39 (d, ^3J(H,H) = 8.0 Hz, 64H; Ar-H), 7.50 (d, ^3J(H,H) = 8.0 Hz, 60H; Ar-H), 7.92–8.07 (m, 184H; Ar-H), 8.11 (s, 2H; Ar-H), 8.40 (m, 30H; Ar-H), 8.58 (m, 1H; Ar-H), 8.79 (m, 30H; 30× Ar-CH_2-N*H*-CO-Ar), 10.19 ppm (m, 62H; 62× Ar-N*H*-CO-Ar); no MS could be obtained neither by ES nor by MALDI.

Preparation of 10: Ethyldiisopropylamine (2 mL) was added to a mixture of **5** (12.9 mg, 25.6 μmol), **9** (800 mg, 51.2 μmol), and HBTU (19.4 mg, 51.2 μmol) in dry DMF (10 mL). The solution was stirred for 7 h at room temperature. Water (1.7 mL, dropwise) and LiOH monohydrate (32 mg, 762 μmol) were added and the mixture was stirred for an additional 16 h at room temperature. The solution was added dropwise to a mixture of acetone and 0.2N HCl (450 mL, 1:2). The precipitate formed was filtered off and washed with water, acetone, and CH_2Cl_2. Compound **10** (780 mg, 96%) was isolated as a beige solid. ^{1}H NMR (600 MHz, [D$_6$]DMSO, 80°C): δ = 1.40 (s, 576H; 64× *tert*-butyl), 4.21 (m, 128H; 64× Ar-CH_2-NHBoc), 4.57 (m, 124H; 62× Ar-CH_2-NH-CO-Ar), 7.10 (br m, 64H; 64× -NHBoc), 7.39 (d, ^3J(H,H) = 8.0 Hz, 128H; Ar-H), 7.50 (m, 124H; Ar-H), 7.92–8.10 (m, 378H; Ar-H), 8.40 (m, 62H; Ar-H), the expected signal at δ = 8.58 (m, 1H; Ar-H) could not be detected in the noise, 8.79 (m, 60H; 62× Ar-CH_2-N*H*-CO-Ar), 10.10–10.25 ppm (m, 126H; 126× Ar-N*H*-CO-Ar); no MS could be obtained neither by ES nor by MALDI.

Preparation of 11: Ethyldiisopropylamine (15 mmol) was added to a mixture of **17** (1.40 g, 2.33 mmol), **18** (0.65 g, 1.01 mmol), and HBTU (0.96 g, 2.5 mmol) in dry DMF (30 mL). The solution was stirred overnight at room temperature. Water (5 mL, dropwise) and LiOH monohydrate (3.17 g, 75 mmol) were added and the mixture was stirred for an additional 16 h at room temperature. The solution was then added dropwise to a mixture of acetone and water (500 mL, 1:3). The precipitate formed was filtered off and washed with 1N HCl, water, acetone, and CH_2Cl_2. Compound **11** (800 mg, 69%) was isolated as a beige powder. ^{1}H NMR (400 MHz, [D$_6$]DMSO, 120°C): δ = 1.41 (s, 36H; 4× *tert*-butyl), 2.34 (m, 6H; 3× -CH_2-CH(CH_2-NH-)$_2$), 2.41 (m, 3H; 3× -CH_2-C*H*(CH_2-NH-)$_2$), 3.40 (m, 12H; 3× -CH_2-CH(CH_2-NH-)$_2$), 4.20 (d, ^3J(H,H) = 6.0 Hz, 8H; 2× Ar-CH_2-NHBoc), 4.36 (d, ^3J(H,H) = 6.0 Hz, 4H; 2× Ar-CH_2-NH-), 6.76 (br s, 4H; 4× Ar-CH_2-N*H*Boc), 7.34 (m, 12H; Ar-H), 7.79 (m, 12H; Ar-H), 8.05 ppm (m, 6H; 3× -CH_2-CH(CH_2-N*H*-)$_2$); MALDI-MS: *m*/*z*: 1582 $[M+Na]^+$; HR-MS: calcd. for $C_{83}H_{106}N_{12}O_{18}$ $[M+2Na]^{2+}$ 802.3766; found 802.3764.

Preparation of 13: Compound **12** (3.00 g, 16.75 mmol) was mixed with **2** (8.42 g, 33.5 mmol) in dry DMF (50 mL). HBTU (19.06 g, 50.2 mmol) and ethyldiisopropylamine (29 mL, 167 mmol) were added and the resulting solution was stirred overnight at room temperature. The product was precipitated by slow addition of the crude mixture to well-stirred aqueous 1N HCl (500 mL). The solid was collected by filtration and washed with water, 1N NaOH, 1N HCl, water, and diethyl ether. Compound **13** was isolated as a beige powder (7.30 g, 79%). ^{1}H NMR (400 MHz, [D$_6$]DMSO, room temperature): δ = 1.39 (s, 18H; 2× *tert*-butyl), 4.18 (m, 8H; 2× Ar-CH_2-NH-, -NH-CH_2-CO-CH_2-NH-), 7.32 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 7.46 (t, ^3J(H,H) = 6.0 Hz, 2H; 2× Ar-CH_2-NH-), 7.82 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 8.79 ppm (t, ^3J(H,H) = 5.5 Hz, 2H; -N*H*-CH_2-CO-CH_2-N*H*-); HR-MS: calcd for $C_{29}H_{38}N_4O_7$ $[M+Na]^+$ 593.2372; found 592.2375.

Preparation of 15: Potassium bis(trimethylsilyl)amide (53 mL of a 0.5M solution in toluene) was added at 0°C to a solution of phosphonoacetate **14** (6.9 mL, 37.8 mmol) in THF (75 mL) and the mixture was stirred under argon at room temperature for 15 min. At 0°C, a solution of **13** (7.0 g, 12.6 mmol) in THF (150 mL) was added and stirring was continued for 2.5 h. Saturated aqueous NH_4Cl (150 mL) was added and the resulting mixture was allowed to warm to room temperature. The mixture was extracted with ethyl acetate and the organic phase was washed with 1N HCl (3×150 mL), 1N NaOH (3×150 mL), and brine (3×150 mL).

The solvent was removed and the residue was subjected to flash chromatography on SiO_2 (ethyl acetate/CH_2Cl_2, 2:1→4:1). Compound **15** was isolated as a beige solid (3.1 g, 40%). ^{1}H NMR (400 MHz, [D$_6$]DMSO, 80°C): δ = 1.40 (s, 18H; 2× *tert*-butyl), 3.67 (s, 3H; -COOCH$_3$), 4.09 (d, ^3J(H,H) = 5.5 Hz, 2H; -CH=C(CH_2-NH-)$_2$), 4.57 (d, ^3J(H,H) = 5.5 Hz, 2H; -CH=C(CH_2-NH-)$_2$), 4.19 (d, ^3J(H,H) = 6.0 Hz, 4H; 2× Ar-CH_2-NH-), 5.83 (s, 1H; CH$_3$OOC-CH=C[CH$_2$-NH-]$_2$), 7.04 (brs, 2H; 2× Ar-CH$_2$-NH-), 7.33 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 7.81 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 8.42 (t, ^3J(H,H) = 5.5 Hz, 1H; -CH=C(CH$_2$-NH-)$_2$), 8.46 ppm (t, ^3J(H,H) = 5.5 Hz, 1H; CH$_3$OOC-CH=C(CH$_2$-NH-)$_2$); HR-MS: calcd for $C_{32}H_{42}N_4O_8$ [M+Na]$^+$ 633.2895; found 633.2896.

Isomer of **15** with the double bond adjacent to a nitrogen atom: ^{1}H NMR (400 MHz, [D$_6$]DMSO, 80°C): δ = 1.40 (s, 18H; 2× *tert*-butyl), 3.40 (s, 2H; CH$_3$OOC-CH_2-C(=CH-NH-)(-CH$_2$-NH-)), 3.60 (s, 3H; -COOCH$_3$), 3.98 (d, ^3J(H,H) = 5.5 Hz, 2H; CH$_3$OOC-CH$_2$-C(=CH-NH-)(-CH_2-NH-)), 4.19 (m, 4H; 2× Ar-CH_2-NH-), 7.00 (d, ^3J(H,H) = 10.0 Hz, 1H; CH$_3$OOC-CH$_2$-C(=CH-NH-)(-CH$_2$-NH-)), 7.07 (brs, 2H; 2× Ar-CH$_2$-NH-), 7.34 (m, 4H; Ar-H), 7.81 (m, 4H; Ar-H), 8.31 (t, ^3J(H,H) = 5.5 Hz, 1H; CH$_3$OOC-CH$_2$-C(=CH-NH-)(-CH$_2$-NH-)), 9.39 ppm (d, ^3J-(H,H) = 10.0 Hz, 1H; CH$_3$OOC-CH$_2$-C(=CH-NH-)(-CH$_2$-NH-)); ES-MS: m/z: 633 [M+Na]$^+$.

Preparation of 16: A mixture of **15** (3.00 g, 4.91 mmol), ammonium formate (15.5 g, 245.7 mmol), and Pd (10% on activated charcoal, 160 mg) in methanol (100 mL) was stirred at 20°C for 16 h. Additional ammonium formate (15.5 g, 245.7 mmol) and Pd (10% on activated charcoal, 160 mg) were added and stirring was continued for 24 h. The catalyst was then filtered off, the solvent was removed, and the resulting solid was washed with water. Compound **16** was isolated as a beige powder (2.6 g, 86%). ^{1}H NMR (400 MHz, [D$_6$]DMSO, 80°C): δ = 1.40 (s, 18H; 2× *tert*-butyl), 2.39 (m, 3H; -CH_2-CH(CH$_2$-NH-)$_2$), 3.36 (m, 4H; -CH$_2$-CH-(CH_2-NH-)$_2$), 3.58 (s, 3H; -COOCH$_3$), 4.19 (d, ^3J(H,H) = 6.0 Hz, 4H; 2× Ar-CH_2-NHBoc), 7.05 (brs, 2H; 2× Ar-CH$_2$-NHBoc), 7.33 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 7.79 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 8.22 ppm (m, 2H; -CO-CH$_2$-CH(CH$_2$-NH-)$_2$); HR-MS: calcd for $C_{32}H_{44}N_4O_8$ [M+Na]$^+$ 635.3051; found 635.3052.

Preparation of 17: A mixture of **16** (2.00 g, 3.26 mmol), 2N NaOH (15 mL), and dioxane (15 mL) was stirred at 20°C for 16 h. The mixture was added dropwise to well-stirred 1N HCl (200 mL), and the resulting precipitate was filtered off, washed with diethyl ether, and dried. Compound **17** was isolated as a beige solid (1.62 g, 83%). ^{1}H NMR (400 MHz, [D$_6$]DMSO, 80°C): δ = 1.41 (s, 18H; 2× *tert*-butyl), 2.32 (m, 3H; -CH_2-CH[CH$_2$-NH-]$_2$), 3.37 (m, 4H; -CH$_2$-CH(CH_2-NH-)$_2$), 4.19 (d, ^3J(H,H) = 6.0 Hz, 4H; 2× Ar-CH_2-NHBoc), 7.05 (brs, 2H; 2× Ar-CH$_2$-NHBoc), 7.33 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 7.80 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 8.23 ppm (m, 2H, -CH$_2$-CH(CH$_2$-NH-)$_2$); HR-MS: calcd for $C_{31}H_{42}N_4O_8$ [M+Na]$^+$ 621.2895; found 621.2895.

Preparation of 18: A mixture of **16** (0.75 g, 1.22 mmol), TFA (20 mL), water (1.5 mL), and triethylsilane (0.75 mL) was stirred at 20°C for 3 h. The mixture was added dropwise to diethyl ether (200 mL), and the precipitate formed was filtered off and dried. The TFA salt of compound **18** was isolated as a beige solid (0.76 g, 97%). ^{1}H NMR (400 MHz, [D$_6$]DMSO, 80°C): δ = 2.42 (m, 3H; -CH_2-CH(CH$_2$-NH-)$_2$), 3.38 (m, 4H; -CH$_2$-CH(CH_2-NH-)$_2$), 3.57 (s, 3H; -COOCH$_3$), 4.11 (m, 4H; 2× Ar-CH_2-NH$_2$), 7.54 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 7.89 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 8.19 (brs, 4H; 2× Ar-CH$_2$-NH_2), 8.34 ppm (m, 2H; -CO-CH$_2$-CH(CH$_2$-NH-)$_2$); HR-MS: calcd for $C_{22}H_{28}N_4O_4$ [M+Na]$^+$ 435.2003; found 425.2002.

Preparation of 19: Ethyldiisopropylamine (3.9 mL) was added to a mixture of **22** (0.75 g, 1.12 mmol), **23** (1.41 g, 2.23 mmol), and HBTU (0.85 g, 2.23 mmol) in dry DMF (22 mL). The solution was stirred for 16 h at 20°C. Water (5 mL, dropwise) and LiOH monohydrate (2.33 g, 55 mmol) were added and the mixture was stirred for an additional 16 h at 20°C. The solution was then added dropwise to a mixture of acetone and water (500 mL, 1:3). The precipitate formed was filtered off and washed with 1N HCl, water, acetone, and CH_2Cl_2. Compound **19** was isolated as a beige solid (1.48 g, 80%). ^{1}H NMR (400 MHz, [D$_6$]DMSO, 80°C): δ = 0.97 (m, 12H), 1.29–1.50 (m, 16H), 1.41 (s, 36H; 4× *tert*-butyl), 1.57 (m, 2H), 1.70–1.95 (m, 24H), 2.29 (m, 6H), 2.83 (m, 8H), 3.15 (m, 4H), 6.40 (brs, 4H; 4× -CH$_2$-NHBoc), 7.63 (m, 4H; Ar-H), 7.87 (m, 2H; Ar-H), 7.97 (brs, 2H; 2× -CH$_2$-NHCOAr), 8.05 (m, 2H; Ar-H), 8.17 (m, 1H; Ar-H), 9.61 (s, 4H; 4× Ar-N-CO-), 9.69 (s, 2H; 2× Ar-NH-CO-), 12.43 ppm (brs, 1H; -COOH); MALDI-MS: m/z: 1678 [M+Na]$^+$; HR-MS: calcd for $C_{89}H_{130}N_{12}O_{18}$ [M+2Na]$^{2+}$ 850.4705; found 850.4708.

Preparation of 21: A mixture of **3** (2.5 g, 10.45 mmol), **20** (5.38 g, 20.90 mmol), HBTU (7.93 g, 20.90 mmol), and ethyldiisopropylamine (15.5 mL, 88.87 mmol) in DMF (50 mL) was stirred for 16 h at 20°C. The product was precipitated by slow addition of the crude mixture to well-stirred 1N HCl (300 mL). The solid was collected by filtration and washed with water, 1N NaOH, 1N HCl, and further water. It was then dissolved in a small amount of ethyl acetate and precipitated by addition to a mixture of cyclohexane (100 mL) and diethyl ether (300 mL). The precipitate was filtered off, washed, and dried. Compound **21** was isolated as a beige solid (3.64 g, 55%). ^{1}H NMR (400 MHz, [D$_6$]DMSO, 80°C): δ = 0.94 (m, 4H), 1.37–1.47 (m, 6H), 1.41 (s, 18H; 2× *tert*-butyl), 1.78 (m, 4H), 1.87 (m, 4H), 2.28 (m, 2H), 2.83 (m, 4H), 3.85 (s, 3H; -COOCH$_3$), 6.41 (brs, 2H; 2× -CH$_2$-NH-Boc), 7.90 (m, 2H; Ar-H), 8.19 (m, 1H; Ar-H), 9.71 ppm (s, 2H; 2× Ar-NH-CO-); HR-MS: calcd. for $C_{34}H_{52}N_4O_8$ [M+Na]$^+$ 667.3677; found 667.3676.

Preparation of 22: A mixture of **21** (1.60 g, 2.48 mmol), 2N NaOH (10 mL), and dioxane (10 mL) was stirred at 20°C for 16 h. The mixture was added dropwise to well-stirred 1N HCl (200 mL), and the resulting precipitate was filtered off, washed with diethyl ether, and dried. Compound **22** was isolated as a beige solid (1.22 g, 78%). ^{1}H NMR (400 MHz, [D$_6$]DMSO, 80°C): δ = 0.94 (m, 4H), 1.39–1.47 (m, 6H), 1.41 (s, 18H; 2× *tert*-butyl), 1.78 (m, 4H), 1.87 (m, 4H), 2.29 (m, 2H), 2.83 (m, 4H), 6.41 (brs, 2H; 2× -CH$_2$-NH-Boc), 7.86 (m, 2H; Ar-H), 8.16 (m, 1H; Ar-H), 9.68 (s, 2H; 2× Ar-NH-CO-), 12.45 ppm (brs, 1H; -COOH); HR-MS: calcd for $C_{33}H_{50}N_4O_8$ [M+Na]$^+$ 653.3521; found 653.3522.

Preparation of 23: A mixture of **21** (0.85 g, 1.31 mmol), TFA (20 mL), water (1.6 mL), and triethylsilane (0.8 mL) was stirred at 20°C for 3 h. The mixture was added dropwise to diethyl ether (200 mL), and the precipitate formed was filtered off and dried. The TFA salt of compound **23** was isolated as a beige solid (0.85 g, 91%). ^{1}H NMR (400 MHz, [D$_6$]DMSO/D$_2$O, 80°C): δ = 1.02 (m, 4H), 1.44 (m, 4H), 1.58 (m, 2H), 1.86 (m, 8H), 2.31 (m, 2H), 2.70 (m, 4H), 3.83 (s, 3H; -COOCH$_3$), 7.85 (d, ^3J(H,H) = 2.0 Hz, 2H; Ar-H), 8.15 ppm (t, ^3J(H,H) = 2.0 Hz, 1H; Ar-H); HR-MS: calcd for $C_{24}H_{36}N_4O_4$ [M+Na]$^+$ 467.2629; found 467.2630.

Preparation of 24: Ethyldiisopropylamine (1.5 mL) was added to a mixture of **27** (1.16 g, 2.00 mmol), **28** (0.62 g, 1.00 mmol), and HBTU (0.76 g, 2.00 mmol) in dry DMF (5 mL). The solution was stirred for 16 h at 20°C. Ethyl acetate (100 mL) was added and the mixture was extracted with 1N HCl (2×100 mL), 1N NaOH (2×100 mL), and brine (100 mL). The solution was dried with $MgSO_4$, the solvent was removed, the residue was redissolved in ethanol (5 mL), and this solution was added dropwise to diethyl ether (200 mL). The precipitate formed was filtered off and washed with diethyl ether. The methyl ester of **24** (1.30 g, 86%) was isolated as a beige solid. ^{1}H NMR (400 MHz, CD$_3$OD, 20°C, selected signals): δ = 1.41 (s, 36H; 4× *tert*-butyl), 2.38 (m, 12H; 6× -CH$_2$-CH_2-CO-), 3.03 (t, ^3J(H,H) = 7.0 Hz, 8H; 4× BocHN-CH_2-CH$_2$-), 3.38 (t, ^3J(H,H) = 7.0 Hz, 4H; 2× Ar-(CO)HN-CH_2-CH$_2$-), 3.87 (s, 3H; COOCH$_3$), 7.68 (d, ^3J(H,H) = 2.0 Hz, 4H; Ar-H), 7.94 (d, ^3J(H,H) = 2.0 Hz, 2H; Ar-H), 7.98 (t, ^3J(H,H) = 7.0 Hz, 2H; Ar-H), 8.07 ppm (t, ^3J(H,H) = 7.0 Hz, 1H; Ar-H); ES-MS: m/z: 1535 [M+Na]$^+$; HR-MS: calcd. for $C_{78}H_{120}N_{12}O_{18}$ [M+2Na]$^{2+}$ 779.4314; found 779.4310. Water (5 mL) was added dropwise to a solution of the methyl ester of **24** (1.00 g, 0.66 mmol) in DMF (12.5 mL). LiOH (0.28 g, 6.60 mmol) was then added and the mixture was stirred at room temperature for 16 h. Ethyl acetate (50 mL) and ethanol (5 mL) were added and the resulting mixture was washed with 1N HCl (2×50 mL), 1N NaOH (2×50 mL), and brine (50 mL). The solvent was removed and the residue was dissolved in a 5:1 mixture of ethyl acetate and ethanol (10 mL). This solution was added dropwise to diethyl ether (150 mL). The precipitate formed was filtered off, washed with diethyl ether and hexanes, and dried. Compound **24** was isolated as a colorless powder (0.94 g, 95%). ^{1}H NMR (400 MHz, CD$_3$OD, 20°C, selected signals): δ = 1.41 (s, 36H; 4× *tert*-butyl), 2.38

(m, 12H; 6× -CH_2-CH_2-CO-), 3.03 (t, ^3J(H,H) = 7.0 Hz, 8H; 4× BocHN-CH_2-CH_2-), 3.38 (t, ^3J(H,H) = 7.0 Hz, 4H; 2× Ar-(CO)HN-CH_2-CH_2-), 7.68 (d, ^3J(H,H) = 2.0 Hz, 4H; Ar-H), 7.95 (d, ^3J(H,H) = 2.0 Hz, 2H; Ar-H), 8.00 (t, ^3J(H,H) = 7.0 Hz, 2H; Ar-H), 8.10 ppm (t, ^3J(H,H) = 7.0 Hz, 1H; Ar-H); ES-MS: *m/z*: 1498 $[M-H]^-$; HR-MS: calcd. for $C_{78}H_{120}N_{12}O_{18}$ $[M+2Na]^{2+}$ 772.4236; found 772.4235.

Preparation of 26: Ethyldiisopropylamine (8 mL) was added to a mixture of **3** (2.33 g, 9.75 mmol), **25** (4.50 g, 19.50 mmol), and HBTU (7.39 g, 19.50 mmol) in dry DMF (32 mL). The solution was stirred for 72 h at 20 °C. Ethyl acetate (150 mL) was then added and the solution was extracted with 1 N HCl (2×150 mL), 1 N NaOH (2×150 mL), and brine (2× 100 mL). The organic phase was treated with decolorizing charcoal (2.5 g), dried with magnesium sulfate, and the solvent was removed. The residue was subjected to flash chromatography on SiO_2 (ethyl acetate/hexanes 2:1→4:1). Compound **26** was isolated as a colorless foam (4.70 g, 81%). ^{1}H NMR (400 MHz, CD_3OD, 20 °C): δ = 1.31 (m, 4H; 2× BocHN-CH_2-CH_2-CH_2-CH_2-CH_2-CO-), 1.31 (s, 18H; 2× *t*-butyl), 1.40 (quint., ^3J(H,H) = 7.5 Hz, 4H; BocHN-CH_2-CH_2-CH_2-CH_2-CH_2-CO-), 1.61 (quint., ^3J(H,H) = 7.5 Hz, 4H; BocHN-CH_2-CH_2-CH_2-CH_2-CH_2-CO-), 2.29 (t, ^3J(H,H) = 7.5 Hz, 4H; BocHN-CH_2-CH_2-CH_2-CH_2-CH_2-C(O)-), 2.93 (t, ^3J(H,H) = 7.5 Hz, 4H; BocHN-CH_2-CH_2-CH_2-CH_2-CH_2-C(O)-), 3.80 (s, 3H; CO_2CH_3), 7.88 (m, 2H; Ar-H), 8.03 ppm (m, 1H; Ar-H); HR-MS: calcd for $C_{30}H_{48}N_4O_8$ $[M+Na]^+$ 615.3364; found 615.3365.

Preparation of 27: Compound **26** (2.50 g, 4.22 mmol) was dissolved in a mixture of dioxane (20 mL) and water (25 mL). LiOH (1.76 g, 42.0 mmol) was added and the mixture was stirred for 16 h at 20 °C. Ethyl acetate (50 mL) was then added and the mixture was extracted with 1 N HCl (2×100 mL), water (2×100 mL), and brine (100 mL). The organic phase was dried with $MgSO_4$ and the solvent was removed. Compound **27** was isolated as a colorless foam (2.43 g, quant.). ^{1}H NMR (400 MHz, CD_3OD, 20 °C): δ = 1.31 (m, 4H; 2× BocHN-CH_2-CH_2-CH_2-CH_2-CH_2-CO-), 1.31 (s, 18H; 2× *t*-butyl), 1.40 (quint., ^3J(H,H) = 7.5 Hz, 4H; BocHN-CH_2-CH_2-CH_2-CH_2-CH_2-CO-), 1.62 (quint., ^3J(H,H) = 7.5 Hz, 4H; BocHN-CH_2-CH_2-CH_2-CH_2-CH_2-CO-), 2.29 (t, ^3J(H,H) = 7.5 Hz, 4H; BocHN-CH_2-CH_2-CH_2-CH_2-CH_2-CO-), 2.94 (t, ^3J(H,H) = 7.5 Hz, 4H; BocHN-CH_2-CH_2-CH_2-CH_2-CH_2-CO-), 7.87 (m, 2H; Ar-H), 8.06 ppm (m, 1H; Ar-H); HR-MS: calcd for $C_{29}H_{46}N_4O_8$ $[M+Na]^+$ 601.3208; found 601.3208.

Preparation of 28: Compound **26** (1.00 g, 1.69 mmol) was dissolved in a mixture of TFA (25 mL), water (0.7 mL), and triethylsilane (0.7 mL). The solution was stirred at 20 °C for 2 h. The solvents were removed and the residue was co-evaporated with diethyl ether (3×50 mL). Compound **28** (1.05 g, quant.) was obtained as a colorless foam. ^{1}H NMR (400 MHz, CD_3OD, 20 °C): δ = 1.37 (m, 4H; 2× H_2N-CH_2-CH_2-CH_2-CH_2-CH_2-CO-), 1.63 (m, 8H; H_2N-CH_2-CH_2-CH_2-CH_2-CH_2-CO-), 2.33 (t, ^3J(H,H) = 7.5 Hz, 4H; H_2N-CH_2-CH_2-CH_2-CH_2-CH_2-C(O)-), 2.84 (t, ^3J(H,H) = 7.5 Hz, 4H; H_2N-CH_2-CH_2-CH_2-CH_2-CH_2-C(O)-), 3.80 (s, 3H; CO_2CH_3), 7.85 (m, 2H; Ar-H), 8.06 ppm (m, 1H; Ar-H); HR-MS: calcd for $C_{20}H_{32}N_4O_4$ $[M+Na]^+$ 415.2316; found 415.2316.

Preparation of 29: Ethyldiisopropylamine (2.3 mL) was added to a mixture of **33** (0.45 g, 0.65 mmol), **4** (0.81 g, 1.30 mmol), and HBTU (0.52 g, 1.37 mmol) in dry DMF (13 mL). The solution was stirred for 16 h at 20 °C. Water (5 mL, dropwise) and LiOH monohydrate (1.40 g, 33 mmol) were added and the mixture was stirred for an additional 16 h at 20 °C. The solution was then added dropwise to a mixture of acetone and water (500 mL, 1:3). The precipitate formed was filtered off and washed with 1 N HCl, water, acetone, and CH_2Cl_2. Compound **29** was isolated as a beige solid (1.01 g, 94%). ^{1}H NMR (400 MHz, [D$_6$]DMSO, 80 °C): δ = 1.41 (s, 36H; 4× *tert*-butyl), 2.98 (s, 6H; 2× -CH_2-NCH_3-CO-), 4.22 (d, ^3J(H,H) = 6.0 Hz, 8H; 4× Ar-CH_2-NHBoc), 4.76 (s, 4H; 2× Ar-CH_2-NCH_3-CO-), 7.09 (brs, 4H; 4× Ar-CH_2-N*H*Boc), 7.39 (d, ^3J(H,H) = 8.0 Hz, 8H; Ar-H), 7.47 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 7.63 (m, 4H; Ar-H), 7.94 (d, ^3J(H,H) = 8.0 Hz, 8H; Ar-H), 8.03 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 8.12 (m, 2H; Ar-H), 8.38 (m, 2H; Ar-H), 8.57 (m, 1H; Ar-H), 10.15 (s, 4H; 4× Ar-CO-N*H*-), 10.24 ppm (s, 2H; 4× Ar-CO-NH-); MALDI-MS: *m/z*: 1670 $[M+Na]^+$; HR-MS: calcd for $C_{91}H_{98}N_{12}O_{18}$ $[M+2Na]^{2+}$ 843.3453; found 846.3454.

Preparation of 30: Ethyldiisopropylamine (1.6 mL) was added to a mixture of **40** (0.32 g, 0.46 mmol), **39** (0.60 g, 0.93 mmol), and HBTU (0.37 g, 0.98 mmol) in dry DMF (12 mL). The solution was stirred for 16 h at 20 °C. Water (5 mL, dropwise) and LiOH monohydrate (0.98 g, 23 mmol) were added and the mixture was stirred for an additional 16 h at 20 °C. The solution was then added dropwise to a mixture of acetone and 0.1 N HCl (500 mL, 1:3). The precipitate formed was filtered off and washed with water and diethyl ether. The solvent was removed and the residue was purified by flash chromatography (SiO_2, CH_2Cl_2/MeOH, 9:1). Compound **30** was isolated as a beige solid (0.45 g, 57%). ^{1}H NMR (400 MHz, [D$_6$]DMSO, 80 °C): δ = 1.36 (s, 36H; 4× *tert*-butyl), 3.20 (s, 12H; 4× Ar-NCH_3-CO-Ar), 3.21 (s, 6H; 2× Ar-NCH_3-CO-Ar), 4.09 (d, ^3J(H,H) = 6.0 Hz, 8H; 4× Ar-CH_2-NHBoc), 4.35 (d, ^3J(H,H) = 6.0 Hz, 4H; 2× Ar-CH_2-NHCOAr), 6.96 (brm, 4H; 4× Ar-CH_2-N*H*Boc), 7.12–7.22 (m, 26H; Ar-H), 7.26 (m, 1H; Ar-H), 7.45 (m, 6H; Ar-H), 8.64 ppm (t, ^3J(H,H) = 6.0 Hz, 2H; 2× Ar-CH_2-N*H*COAr); MALDI-MS: *m/z*: 1726 $[M+Na]^+$; HR-MS: calcd for $C_{95}H_{106}N_{12}O_{18}$ $[M+2Na]^{2+}$ 874.3766; found 874.3764.

Preparation of 31: A mixture of **3** (1.0 g, 4.18 mmol), **32** (2.2 g, 8.36 mmol), HBTU (3.17 g, 8.36 mmol), and ethyldiisopropylamine (6.2 mL, 35.5 mmol) in DMF (25 mL) was stirred for 16 h at 20 °C. The product was precipitated by dropwise addition of the crude mixture to well-stirred 1 N HCl (200 mL). The solid was collected by filtration and washed with water. It was dissolved in ethyl acetate (75 mL) and washed successively with 1 N HCl (3×20 mL), 1 N NaOH (3×20 mL), and brine (3×20 mL), and dried with Na_2SO_4. The solvent was removed and the residue was purified by flash chromatography (SiO_2, cyclohexane/EtOAc, 1:1) to give **31** as a beige solid (850 mg, 31%). ^{1}H NMR (400 MHz, [D$_6$]DMSO, 80 °C): δ = 1.44 (s, 18H; 2× *tert*-butyl), 2.83 (s, 6H; 2× -CH_2-NCH_3-CO-), 3.90 (s, 3H; -$COOCH_3$), 4.47 (s, 4H; 2× -CH_2-NCH_3-CO-), 7.37 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 7.99 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 8.15 (d, ^3J(H,H) = 2.0 Hz, 2H; Ar-H), 8.61 (t, ^3J(H,H) = 2.0 Hz, 1H; Ar-H), 10.25 ppm (s, 2H; 2× Ar-CO-NH-); HR-MS: calcd for $C_{36}H_{44}N_4O_8$ $[M+Na]^+$ 683.3051; found 683.3051.

Preparation of 32: NaH (60%, 3.18 g, 79.59 mmol) was added portionwise to **2** (5 g, 19.90 mmol) in THF (100 mL) and the resulting suspension was stirred for 30 min at 20 °C. Methyl iodide (9.9 mL, 159.2 mmol) was added and stirring was continued for 40 h. The mixture was then quenched with iced water and the pH was adjusted to 4 using 2 N HCl. The mixture was extracted with ethyl acetate (3×100 mL), and the combined organic phases were washed with brine (3×100 mL) and dried with Na_2SO_4. Filtration through SiO_2 (EtOAc) gave **32** (4.6 g, 87%) as a beige solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO, 80 °C): δ = 1.42 (s, 9H; *tert*-butyl), 2.81 (s, 3H; -CH_2-NCH_3-CO-), 4.45 (s, 2H; -CH_2-NCH_3-CO-), 7.32 (d, ^3J(H,H) = 8.0 Hz, 2H; Ar-H), 7.92 (d, ^3J(H,H) = 8.0 Hz, 2H; Ar-H), 12.47 ppm (brs, 1H; -COOH); HR-MS: calcd for $C_8H_{10}N_2O_2$ $[M+Na]^+$ 189.0635; found 189.0634.

Preparation of 33: Compound **31** (0.53 g, 0.80 mmol) was dissolved in a mixture of TFA (15 mL), water (1.2 mL), and triethylsilane (0.6 mL). The solution was stirred at 20 °C for 2 h. The solvents were removed and the residue was co-evaporated with diethyl ether (3×50 mL). Compound **33** (1.05 g, 89%) was obtained as a colorless solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO/D$_2$O, 20 °C): δ = 2.60 (s, 6H; 2× Ar-CH_2-NHCH_3), 3.87 (s, 3H; -$COOCH_3$), 4.20 (s, 4H; 2× Ar-CH_2-$NHCH_3$), 7.61 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 8.03 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 8.14 (m, 2H; Ar-H), 8.66 ppm (m, 1H; Ar-H); HR-MS: calcd for $C_{26}H_{28}N_4O_4$ $[M+Na]^+$ 483.2003; found 483.2004.

Preparation of 34: At 0 °C, DMF (2 drops) and oxalyl chloride (2.56 mL, 29.85 mmol) were successively added to a suspension of **2** (5 g, 19.90 mmol) in dry CH_2Cl_2 (150 mL) and the mixture was stirred at 0 °C for 4 h. The solvent was then removed, the residue was redissolved in CH_2Cl_2 (75 mL), and this solution was added at 0 °C to a solution of **35** (2.39 g, 8.95 mmol) and ethyldiisopropylamine (8.7 mL, 49.7 mmol) in CH_2Cl_2 (75 mL). The ice bath was removed and the mixture was stirred for 4 h. Water (100 mL) was then added, and the organic phase was separated, washed with 1 N HCl (3×100 mL), 1 N NaOH (3×100 mL), and brine (3×100 mL), and dried with Na_2SO_4. The solvent was removed and the residue was purified by flash chromatography (SiO_2, EtOAc/cyclo-

hexane, 6:4). Compound **34** was isolated as a colorless solid (3.1 g, 52%). ^{1}H NMR (400 MHz, [D$_6$]DMSO, 80°C): δ = 1.36 (s, 18H; 2× *tert*-butyl), 3.23 (s, 6H; 2× Ar-NC*H*$_3$-CO-Ar), 3.77 (s, 3H; -COOC*H*$_3$), 4.09 (d, ^3J(H,H) = 6.5 Hz, 4H; 2× Ar-C*H*$_2$-NHBoc), 6.96 (br m, 2H; 2× Ar-CH$_2$-N*H*Boc), 7.14 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 7.19 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 7.34 (t, ^3J(H,H) = 2.0 Hz, 1H; Ar-H), 7.44 ppm (d, ^3J(H,H) = 2.0 Hz, 2H; Ar-H); HR-MS: calcd for $C_{36}H_{44}N_4O_8$ [*M*+Na]$^+$ 683.3051; found 683.3050.

Preparation of 35: A mixture of **38** (9.6 g, 25.23 mmol) and HCl (37%, 15 mL) in methanol (150 mL) was heated at 64°C for 16 h. The crude solution was concentrated and then diethyl ether (500 mL) was added. The precipitate formed was filtered off, washed with diethyl ether, and dried. Compound **35** was isolated as a colorless powder (6.2 g, 92%). ^{1}H NMR (400 MHz, [D$_6$]DMSO/D$_2$O, 20°C): δ = 2.79 (s, 6H; 2× Ar-NHC*H*$_3$), 3.82 (s, 3H; -COOC*H*$_3$), 6.80 (m, 1H; Ar-H), 7.14 ppm (m, 2H; Ar-H); HR-MS: calcd for $C_{10}H_{14}N_2O_2$ [*M*+H]$^+$ 195.1128; found 195.1128.

Preparation of 37: Di-*tert*-butyl dicarbonate (30.1 g, 138.0 mmol) was added portionwise to a solution of **36** (10 g, 65.72 mmol), *tert*-butyl alcohol (75 mL), and 1 N NaOH (75 mL) and the mixture was stirred at 20°C for 16 h. The solution was then washed with cyclohexane (3×100 mL). The aqueous phase was acidified by dropwise addition of 1 N HCl and extracted with ethyl acetate (3×100 mL). The combined organic phases were washed with brine (3×100 mL) and dried with Na_2SO_4. The product was purified by filtration through SiO_2 (EtOAc). Compound **37** was isolated as a beige solid (20.2 g, 87%). ^{1}H NMR (400 MHz, [D$_6$]DMSO, 20°C): δ = 1.47 (s, 18H; 2× *tert*-butyl), 7.68 (m, 2H; Ar-H), 7.86 (m, 1H; Ar-H), 9.49 (s, 2H; 2× Ar-NHBoc), 12.82 ppm (s, 1H; -COOH); HR-MS: calcd for $C_{17}H_{24}N_2O_6$ [*M*+Na]$^+$ 375.1527; found 375.1526.

Preparation of 38: NaH (60%, 8.3 g, 206.4 mmol) was added portionwise to a solution of **37** (9.7 g, 27.53 mmol) in THF (100 mL). The resulting suspension was stirred for 1 h at 20°C. Methyl iodide (25.8 mL, 412.9 mmol) was added and stirring was continued for 16 h. Iced water (100 mL) was then carefully added to quench the reaction. The solution was acidified with 2 N HCl and extracted with ethyl acetate (3×150 mL). The organic phase was washed with 1 N HCl (3×100 mL) and brine (3× 100 mL). The solution was dried with Na_2SO_4 and the product was purified by filtration through SiO_2 (EtOAc). Compound **38** was isolated as a beige solid (9.7 g, 93%). ^{1}H NMR (400 MHz, [D$_6$]DMSO, 20°C): δ = 1.41 (s, 18H; 2× *tert*-butyl), 3.21 (s, 6H; 2× Ar-NCH$_3$Boc), 7.47 (m, 1H; Ar-H), 7.63 (m, 2H; Ar-H), 13.11 ppm (s, 1H; -COOH); HR-MS: calcd for $C_{19}H_{28}N_2O_6$ [*M*+Na]$^+$ 425.1659; found 425.1660.

Preparation of 39: A mixture of **34** (1.2 g, 1.82 mmol), 2 N NaOH (10 mL), and dioxane (10 mL) was stirred at 20°C for 16 h. The mixture was then added dropwise to well-stirred 1 N HCl (200 mL), and the resulting precipitate was filtered off, washed with diethyl ether, and dried. Compound **39** was isolated as a beige solid (1.05 g, 89%). ^{1}H NMR (400 MHz, [D$_6$]DMSO, 80°C): δ = 1.36 (s, 18H; 2× *tert*-butyl), 3.23 (s, 6H; 2× Ar-NC*H*$_3$-CO-Ar), 4.09 (d, ^3J(H,H) = 6.0 Hz, 4H; 2× Ar-C*H*$_2$-NHBoc), 6.96 (br m, 2H; 2× Ar-CH$_2$-N*H*Boc), 7.14 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 7.19 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 7.31 (m, 1H; Ar-H), 7.42 (m, 2H; Ar-H), 12.67 ppm (s, 1H; -COOH); HR-MS: calcd for $C_{35}H_{42}N_4O_8$ [*M*+Na]$^+$ 669.2895; found 669.2896.

Preparation of 40: A mixture of **34** (0.60 g, 0.91 mmol), TFA (15 mL), water (1.2 mL), and triethylsilane (0.6 mL) was stirred at 20°C for 3 h. The mixture was then added dropwise to diethyl ether (200 mL), and the precipitate formed was filtered off and dried. The TFA salt of compound **40** was isolated as a beige solid (0.53 g, 85%). ^{1}H NMR (400 MHz, [D$_6$]DMSO/D$_2$O, 20°C): δ = 3.20 (s, 6H; 2× Ar-NC*H*$_3$-CO-Ar), 3.73 (s, 3H; -COOC*H*$_3$), 3.98 (s, 4H; 2× Ar-C*H*$_2$-NH$_2$), 7.28 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 7.33 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 7.46 (m, 1H; Ar-H), 7.48 ppm (m, 2H; Ar-H); HR-MS: calcd for $C_{26}H_{28}N_4O_4$ [*M*+Na]$^+$ 483.2003; found 483.2004.

Modifications of dendrimer endgroups: *General procedure for the removal of Boc groups:* The Boc-protected dendrimer **4**, **6**, **7**, **8**, **9**, **10**, **11**, **19**, **24**, **29** or **30** (100 mg) was dissolved in a mixture of TFA (5 mL), water (0.4 mL), and mercaptoethanol (0.2 mL). The solution was stirred at 20°C for 3 h, then added dropwise to diethyl ether (50 mL), and the precipitate formed was filtered off. The TFA salts of the products were isolated as beige solids.

4N: Yield 78%; ^{1}H NMR (600 MHz, D$_2$O, 20°C): δ = 4.17 (s, 4H; 2× Ar-C*H*$_2$-NH$_2$), 7.48 (d, ^3J(H,H) = 8.5 Hz, 4H; Ar-H), 7.76 (d, ^3J(H,H) = 2.0 Hz, 2H; Ar-H), 7.79 (d, ^3J(H,H) = 8.5 Hz, 4H; Ar-H), 8.12 ppm (br t, ^3J(H,H) = 2.0 Hz, 1H; Ar-H); ES-MS: *m*/*z*: 419 [*M*+H]$^+$, 837 [2*M*+H]$^+$.

6N: Yield 93%; ^{1}H NMR (600 MHz, [D$_6$]DMSO/D$_2$O, 80°C): δ = 4.12 (s, 8H; 4× Ar-C*H*$_2$-NH$_2$), 4.57 (s, 4H; 2× Ar-C*H*$_2$-NH-CO-Ar), 7.48 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 7.56 (d, ^3J(H,H) = 8.0 Hz, 8H; Ar-H), 7.91 (m, 8H; Ar-H), 7.99 (d, ^3J(H,H) = 8.0 Hz, 8H; Ar-H), 8.05 (d, ^3J(H,H) = 2.0 Hz, 2H; Ar-H), 8.36 (m, 2H; Ar-H), 8.43 ppm (m, 1H; Ar-H); MALDI-MS (matrix: dihydroxybenzoic acid): *m*/*z*: 1220 [*M*+H]$^+$.

7N: Yield: 83%; ^{1}H NMR (600 MHz, [D$_6$]DMSO/D$_2$O, 80°C): δ = 4.11 (s, 16H; 8× Ar-C*H*$_2$-NH$_2$), 4.56 (s, 12H; 6× Ar-C*H*$_2$-NH-CO-Ar), 7.46 (m, 12H; Ar-H), 7.54 (d, ^3J(H,H) = 8.0 Hz, 16H; Ar-H), 7.88 (m, 24H; Ar-H), 7.95 (d, ^3J(H,H) = 8.0 Hz, 16H; Ar-H), 8.03 (d, ^3J(H,H) = 2.0 Hz, 2H; Ar-H), 8.25 (t, ^3J(H,H) = 2.0 Hz, 2H; Ar-H), 8.28 (d, ^3J(H,H) = 2.0 Hz, 4H; Ar-H), 8.31 ppm (t, ^3J(H,H) = 2.0 Hz, 1H; Ar-H); MALDI-MS (matrix: dihydroxybenzoic acid): *m*/*z*: 2822 [*M*+H]$^+$.

8N: Yield 91%; ^{1}H NMR (600 MHz, [D$_6$]DMSO/D$_2$O, 80°C): δ = 4.10 (s, 32H; 16× Ar-C*H*$_2$-NH$_2$), 4.55 (s, 28H; 14× Ar-C*H*$_2$-NH-CO-Ar), 7.47 (m, 28H; Ar-H), 7.54 (m, 32H; Ar-H), 7.85–7.95 (m, 88H; Ar-H), 8.02 (m, 2H; Ar-H), 8.23 (m, 6H; Ar-H), 8.27 (m, 8H; Ar-H), 8.33 ppm (m, 1H; Ar-H); MALDI-MS (matrix: α-cyano-4-hydroxycinnamic acid): *m*/*z*: 6025 [*M*+H]$^+$.

9N: Yield 92%; ^{1}H NMR (600 MHz, [D$_6$]DMSO/D$_2$O, 80°C): δ = 4.10 (s, 64H; 32× Ar-C*H*$_2$-NH$_2$), 4.52 (s, 60H; 30× Ar-C*H*$_2$-NH-CO-Ar), 7.35–7.55 (m, 124H; Ar-H), 7.77–8.00 (m, 186H; Ar-H), 8.10–8.25 ppm (m, 31H; Ar-H); no MS could be obtained neither by ES nor by MALDI.

10N: Yield 97%; ^{1}H NMR (600 MHz, [D$_6$]DMSO/D$_2$O, 80°C): δ = 4.11 (s, 128H; 64× Ar-C*H*$_2$-NH$_2$), 4.56 (s, 124H; 62× Ar-C*H*$_2$-NH-CO-Ar), 7.43–7.60 (m, 252H; Ar-H), 7.77–8.00 (m, 378H; Ar-H), 8.25–8.50 ppm (m, 63H; Ar-H); no MS could be obtained neither by ES nor by MALDI.

11N: Yield 97%; ^{1}H NMR (400 MHz, [D$_6$]DMSO, 80°C): δ = 2.32 (m, 6H; 3× -C*H*$_2$-CH(CH$_2$-NH-)$_2$), 2.42 (m, 3H; 3× -CH$_2$-C*H*(CH$_2$-NH-)$_2$), 3.37 (m, 12H; 3× -CH$_2$-CH(C*H*$_2$-NH-)$_2$), 4.11 (s, 8H; 2× Ar-C*H*$_2$-NH$_2$), 4.35 (d, ^3J(H,H) = 5.5 Hz, 4H; 2× Ar-C*H*$_2$-NH-), 7.35 (m, 4H; Ar-H), 7.54 (m, 8H; Ar-H), 7.79 (m, 4H; Ar-H), 7.90 (m, 8H; Ar-H), 8.25–8.40 ppm (m, 14H; 3× -CH$_2$-CH(CH$_2$-N*H*-)$_2$, 4× Ar-CH$_2$-N*H*$_2$); MALDI-MS: *m*/*z*: 1159 [*M*+H]$^+$.

19N: Yield 96%; ^{1}H NMR (400 MHz, [D$_6$]DMSO/D$_2$O, 20°C): δ = 0.99 (m, 12H), 1.40 (m, 12H), 1.54 (m, 6H), 1.84 (m, 24H), 2.30 (m, 6H), 2.68 (m, 8H), 3.09 (m, 4H), 7.61 (d, ^3J(H,H) = 2.0 Hz, 4H; Ar-H), 8.88 (d, ^3J(H,H) = 2.0 Hz, 2H; Ar-H), 7.13 (t, ^3J(H,H) = 2.0 Hz, 2H; Ar-H), 8.19 ppm (t, ^3J(H,H) = 2.0 Hz, 1H; Ar-H); MALDI-MS: *m*/*z*: 1255 [*M*+H]$^+$.

24N: Yield 93%; ^{1}H NMR (400 MHz, CD$_3$OD, 20°C): δ = 2.42 (m, 12H; 6× -CH$_2$-C*H*$_2$-CO-), 2.96 (t, ^3J(H,H) = 7.0 Hz, 8H; 4× H$_2$N-C*H*$_2$-CH$_2$-), 3.40 (t, ^3J(H,H) = 7.0 Hz, 4H; 2× Ar-CONH-C*H*$_2$-CH$_2$-), 7.70 (m, 4H; Ar-H), 7.95 (m, 2H; Ar-H), 8.02 (m, 2H; Ar-H), 8.10 ppm (m, 1H; Ar-H); ES-MS: *m*/*z*: 1100 [*M*+H]$^+$.

29N: Yield 96%; ^{1}H NMR (400 MHz, [D$_6$]DMSO/D$_2$O, 80°C): δ = 2.97 (s, 6H; 2× Ar-CH$_2$-NC*H*$_3$-CO-Ar), 4.13 (s, 8H; 4× Ar-C*H*$_2$-NH$_2$), 4.75 (s, 4H; 2× Ar-C*H*$_2$-NCH$_3$-CO-Ar), 7.46 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 7.60 (m, 12H; Ar-H), 8.01 (m, 12H; Ar-H), 8.09 (d, ^3J(H,H) = 2.0 Hz, 2H; Ar-H), 8.40 (m, 2H; Ar-H), 8.55 ppm (m, 1H; Ar-H); MALDI-MS: *m*/*z*: 1247 [*M*+H]$^+$.

30N: Yield 91%; ^{1}H NMR (400 MHz, [D$_6$]DMSO/D$_2$O, 20°C): δ = 3.18 (s, 18H; 6× Ar-NC*H*$_3$-CO-), 3.97 (s, 8H; 4× Ar-C*H*$_2$-NH$_2$), 4.29 (s, 4H; 2× Ar-C*H*$_2$-NHCOAr), 7.11–7.45 ppm (m, 33H; Ar-H); MALDI-MS: *m*/*z*: 1303 [*M*+H]$^+$.

General procedure for chloroacetylation: The TFA salt of the dendrimer **4N**, **6N**, **7N**, **8N**, **9N**, **10N**, **11N**, **19N**, **24N**, **29N** or **30N** (100 mg) was dissolved in a mixture of DMF (1 mL) and 2,6-lutidine (0.6 mL). A solution of chloroacetic anhydride (3.0 equiv./amino group) in DMF (1 mL) was added dropwise. The mixture was stirred at 20 °C for 3 h. Dropwise addition to 1 N HCl (50 mL) gave a beige precipitate, which was filtered off and washed with water, acetone, CH_2Cl_2, and diethyl ether.

4Cl: Yield 73 %; ^{1}H NMR (400 MHz, [D$_6$]DMSO, 20 °C): δ = 4.14 (s, 4H; 2× -CH_2-Cl), 4.41 (d, ^3J(H,H) = 6.0 Hz, 4H; 2× Ar-CH_2-NH-CO-CH_2-Cl), 7.43 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 7.97 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 8.12 (m, 2H; Ar-H), 8.62 (m, 1H; Ar-H), 8.83 (t, ^3J(H,H) = 6.0 Hz, 2H; NH), 10.41 ppm (s, 2H; NH); ES-MS: *m*/*z*: 569 [*M*+H]$^+$.

6Cl: Yield 93 %; ^{1}H NMR (600 MHz, [D$_6$]DMSO/D$_2$O, 80 °C): δ = 4.08 (s, 8H; 4× -CH_2-Cl), 4.38 (s, 8H; 4× Ar-CH_2-NH-CO-CH_2-Cl), 4.56 (s, 4H; 2× Ar-CH_2-NH-CO-Ar), 7.40 (d, ^3J(H,H) = 8.0 Hz, 8H; Ar-H), 7.48 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 7.92 (m, 16H; Ar-H), 8.08 (m, 2H; Ar-H), 8.32 (m, 2H; Ar-H), 8.42 ppm (m, 1H; Ar-H).

7Cl: Yield 83 %; ^{1}H NMR (600 MHz, [D$_6$]DMSO/D$_2$O, 80 °C): δ = 4.05 (s, 16H; 8× -CH_2-Cl), 4.36 (s, 16H; 8× Ar-CH_2-NH-CO-CH_2-Cl), 4.54 (s, 12H; 6× Ar-CH_2-NH-CO-Ar), 7.38 (d, ^3J(H,H) = 8.0 Hz, 16H; Ar-H), 7.46 (m, 12H; Ar-H), 7.87 (m, 40H; Ar-H), 8.06 (m, 2H; Ar-H), 8.25 (m, 6H; Ar-H), 8.33 ppm (m, 1H; Ar-H).

8Cl: Yield 91 %; ^{1}H NMR (600 MHz, [D$_6$]DMSO/D$_2$O, 80 °C): δ = 4.06 (s, 32H; 16× -CH_2-Cl), 4.36 (s, 32H; 16× Ar-CH_2-NH-CO-CH_2-Cl), 4.54 (s, 28H; 14× Ar-CH_2-NH-CO-Ar), 7.38 (m, 32H; Ar-H), 7.46 (m, 28H; Ar-H), 7.89 (m, 88H; Ar-H), 8.06 (m, 2H; Ar-H), 8.28 (m, 14H; Ar-H), 8.38 ppm (m, 1H; Ar-H).

9Cl: Yield 92 %; ^{1}H NMR (600 MHz, [D$_6$]DMSO/D$_2$O, 80 °C): δ = 4.07 (s, 64H; 32× -CH_2-Cl), 4.37 (s, 64H; 32× Ar-CH_2-NH-CO-CH_2-Cl), 4.55 (s, 60H; 30× Ar-CH_2-NH-CO-Ar), 7.39 (m, 64H; Ar-H), 7.47 (m, 60H; Ar-H), 7.92 (m, 184H; Ar-H), 8.07 (m, 2H; Ar-H), 8.33 ppm (m, 30H; Ar-H); the expected signal at $\delta \approx$ 8.38 ppm (m, 1H; Ar-H) could not be detected in the noise.

10Cl: Yield 97 %; ^{1}H NMR (600 MHz, [D$_6$]DMSO/D$_2$O, 80 °C): δ = 4.04 (s, 128H; 64× -CH_2-Cl), 4.35 (s, 128H; 64× Ar-CH_2-NH-CO-CH_2-Cl), 4.55 (s, 124H; 62× Ar-CH_2-NH-CO-Ar), 7.36 (m, 128H; Ar-H), 7.44 (m, 124H; Ar-H), 7.88 (m, 376H; Ar-H), 8.05 (m, 2H; Ar-H), 8.26 ppm (m, 30H; Ar-H); the expected signal at $\delta \approx$ 8.38 ppm (m, 1H; Ar-H) could not be observed in the noise.

11Cl: Yield 87 %; ^{1}H NMR (400 MHz, [D$_6$]DMSO/D$_2$O, 80 °C): δ = 2.29 (m, 6H; 3× -CH_2-CH(CH_2-NH-)$_2$), 2.37 (m, 3H; 3× -CH_2-CH(CH_2-NH-)$_2$), 3.35 (m, 12H; 3× -CH_2-CH(CH_2-NH-)$_2$), 4.08 (s, 8H; 4× -CO-CH_2Cl), 4.32 (s, 4H; 2× Ar-CH_2-NH-), 4.36 (s, 8H; 4× Ar-CH_2-NH-), 7.34 (m, 12H; Ar-H), 7.78 ppm (m, 12H; Ar-H); MALDI-MS: *m*/*z*: 1465 [*M*+H]$^+$.

19Cl: Yield 91 %; ^{1}H NMR (400 MHz, [D$_6$]DMSO, 80 °C): δ = 1.00 (m, 12H), 1.39–1.47 (m, 16H), 1.59 (m, 2H), 1.79–1.90 (m, 24H), 2.30 (m, 6H), 3.01 (m, 8H), 3.15 (m, 4H), 4.03 (s, 8H; 4× -COCH_2Cl), 7.63 (m, 4H; Ar-H), 7.86 (m, 2H; Ar-H), 7.89 (brs, 4H; 4× -CH_2-NHCOCH$_2$Cl), 7.97 (brs, 2H; 2× -CH_2-NHCOAr), 8.05 (m, 2H; Ar-H), 8.17 (m, 1H; Ar-H), 9.61 (s, 4H; 4× Ar-NH-CO-), 9.68 (s, 2H; 2× Ar-NH-CO-), 12.43 ppm (brs, 1H; Ar-COOH); MALDI-MS: *m*/*z*: 1583 [*M*+Na]$^+$.

24Cl: Yield 88 %; ^{1}H NMR (400 MHz, CD$_3$OD, 80 °C, selected signals): δ = 2.39 (m, 12H; 6× -CH_2-CH_2-CO-), 3.22 (t, ^3J(H,H) = 7.0 Hz, 8H; 4× Cl-CH_2(CO)NH-CH_2-CH_2-), 3.38 (t, ^3J(H,H) = 7.0 Hz, 4H; 2× Ar-(CO)HN-CH_2-CH_2-), 4.00 (s, 8H; 4× Cl-CH_2(CO)NH-), 7.69 (m, 4H; Ar-H), 7.94 (m, 2H; Ar-H), 7.97 (m, 2H; Ar-H), 8.10 ppm (m, 1H; Ar-H); ES-MS: *m*/*z*: 1427 [*M*+Na]$^+$.

29Cl: Yield 77 %; ^{1}H NMR (400 MHz, [D$_6$]DMSO/D$_2$O, 80 °C): δ = 2.96 (s, 6H; 2× Ar-CH_2-NCH_3-CO-Ar), 4.09 (s, 8H; 4× -CH_2Cl), 4.39 (s, 8H; 4× Ar-CH_2-NH-CO-CH_2Cl), 4.74 (s, 4H; 2× Ar-CH_2-NCH$_3$-CO-Ar), 7.41 (m, 12H; Ar-H), 7.60 (m, 4H; Ar-H), 7.92 (d, ^3J(H,H) = 8.0 Hz, 8H; Ar-H), 7.99 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 8.10 (m, 2H; Ar-H), 8.31 (m, 2H; Ar-H), 8.50 ppm (m, 1H; Ar-H); MALDI-MS: *m*/*z*: 1575 [*M*+Na]$^+$.

30Cl: Yield 97 %; ^{1}H NMR (400 MHz, [D$_6$]DMSO/D$_2$O, 80 °C): δ = 3.19 (s, 12H; 4× Ar-NCH_3-CO-Ar), 3.20 (s, 6H; 2× Ar-NCH_3-CO-Ar), 4.04 (s, 8H; 4× -CH_2-Cl), 4.26 (s, 8H; 4× Ar-CH_2-NH-CO-CH_2Cl), 4.32 (s, 4H; 2× Ar-CH_2-NH-CO-Ar), 7.11–7.19 (m, 26H; Ar-H), 7.29 (m, 1H; Ar-H), 7.42 ppm (m, 6H; Ar-H); MALDI-MS: *m*/*z*: 1631 [*M*+Na]$^+$.

General procedure for the functionalization with oligosaccharides: The chloroacetylated dendrimer **4Cl**, **6Cl**, **7Cl**, **8Cl**, **9Cl**, **10Cl**, **11Cl**, **19Cl**, **24Cl**, **29Cl** or **30Cl** (25 mg) and the trisaccharide αGal-SH[7a] or disaccharide Lac-SH[7a] (1.5 equiv/-CH_2Cl) were dissolved in degassed DMF (2.0 mL). At room temperature, DBU (5 equiv/-CH_2Cl) was added and the solution was stirred under nitrogen for 1 h. It was then added dropwise to a mixture of ethanol and diethyl ether (40 mL, 1:2). The precipitate that formed was collected, washed with diethyl ether (3×40 mL), dried, dissolved in water (to which 10 drops of 1 N HCl had been added), and subjected to ultrafiltration. Ultrafiltrations were performed using Amicon 8010 stirred cells (vol.: 10 mL, diam.: 25 mm) and Amicon YM3 disc membranes (molecular weight cut-off: 3000). Ultrafiltrations were repeated eight times (from 10 mL down to 2 mL) with the volume being made up with distilled water on each occasion. Lyophilization afforded the products as colorless solids. No ultrafiltration was performed for **4Gal**. Instead, the product was precipitated by dropwise addition of the reaction mixture to a mixture of ethanol and diethyl ether (1:1). The precipitate formed was collected and purified by chromatography on an RP-18 column; gradient $H_2O \rightarrow H_2O$/MeOH, 1:1.

4Gal: Yield 31 mg (50 %); ^{1}H NMR (400 MHz, [D$_6$]DMSO/D$_2$O, 80 °C, selected signals): δ = 1.83 (s, 6H; 2× -NHCOCH_3), 3.19 (s, 4H; 4× -CO-CH_2-S-), 4.31 (2× d, ^3J(H,H) = 8.0 Hz, 4H; 2× GlcNAc H-1, 2× βGal H-1), 4.36 (s, 4H; 2× Ar-CH_2-NH-CO-CH_2-S), 4.89 (d, ^3J(H,H) = 3.5 Hz, 2H; 2× αGal H-1), 7.40 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 7.89 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 8.05 (d, ^3J(H,H) = 2.0 Hz, 2H; 2× Ar-H), 8.33 ppm (t, ^3J(H,H) = 2.0 Hz, 1H; Ar-H); HR-MS: calcd for $C_{81}H_{118}N_8O_{40}S_2$ [*M*+2Na]$^{2+}$ 976.3336; found 976.3331.

6Gal: Yield 36 mg (70 %); ^{1}H NMR (600 MHz, [D$_6$]DMSO/D$_2$O, 80 °C, selected signals): δ = 1.81 (s, 12H; 4× -NHCOCH_3), 3.17 (s, 8H; 4× -CO-CH_2-S-), 4.28–4.34 (m, 16H; 4× Ar-CH_2-NH-CO-CH_2-S-, 4× GlcNAc H-1, 4× βGal H-1), 4.55 (s, 4H; 2× Ar-CH_2-NH-CO-Ar), 4.89 (d, ^3J(H,H) = 3.5 Hz, 4H; 4× αGal H-1), 7.37 (d, ^3J(H,H) = 8.0 Hz, 8H; Ar-H), 7.45 (d, ^3J(H,H) = 8.0 Hz, 4H; Ar-H), 8.19 ppm (m, 3H; Ar-H); HR-MS: calcd for $C_{185}H_{254}N_{20}O_{82}S_4$ [*M*−3H]$^{3-}$ 1397.4995; found 1397.4998.

7Gal: Yield 243 mg (80 %); ^{1}H NMR (600 MHz, [D$_6$]DMSO/D$_2$O, 80 °C, selected signals): δ = 1.81 (s, 24H; 8× -NHCOCH_3), 3.18 (s, 16H; 8× -CO-CH_2-S-), 4.29–4.35 (m, 32H; 8× Ar-CH_2-NH-CO-CH_2-S-, 8× GlcNAc H-1, 8× βGal H-1), 4.56 (s, 12H; 6× Ar-CH_2-NH-CO-Ar), 4.86 (d, ^3J(H,H) = 3.5 Hz, 8H; 8× αGal H-1), 7.39 (d, ^3J(H,H) = 8.0 Hz, 16H; Ar-H), 7.47 (m, 12H; Ar-H), 8.29 (m, 1H; Ar-H), 8.34 (m, 4H; Ar-H), 8.38 ppm (m, 2H; Ar-H); MALDI-MS: *m*/*z*: 8801 [*M*+Na]$^+$.

7Lac: Yield 8 mg (75 %); ^{1}H NMR (600 MHz, [D$_6$]DMSO/D$_2$O, 80 °C, selected signals): δ = 3.17 (s, 16H; 8× -CO-CH_2-S-), 4.11 (d, ^3J(H,H) = 8.0 Hz, 8H; 8× H-1 carbohydr.), 4.19 (m, 8H; 8× H-1 carbohydr.), 4.34 (m, 16H; 8× Ar-CH_2-NH-CO-CH_2-S-), 4.56 (s, 12H; 6× Ar-CH_2-NH-CO-Ar), 7.38 (d, ^3J(H,H) = 8.0 Hz, 16H; Ar-H), 7.47 (m, 12H; Ar-H), 8.26–8.29 ppm (m, 7H; Ar-H); MALDI-MS: *m*/*z*: 7176 [*M*+Na]$^+$.

8Gal: Yield 57 mg (70 %); ^{1}H NMR (600 MHz, [D$_6$]DMSO/D$_2$O, 80 °C, selected signals): δ = 1.80 (s, 48H; 16× -NHCOCH_3), 3.16 (s, 32H; 16× -CO-CH_2-S-), 4.28–4.34 (m, 64H; 16× Ar-CH_2-NH-CO-CH_2-S-, 16× GlcNAc H-1, 16× βGal H-1), 4.54 (s, 28H; 14× Ar-CH_2-NH-CO-Ar), 4.87 (d, ^3J(H,H) = 3.5 Hz, 16H; 16× αGal H-1), 7.37 (d, ^3J(H,H) = 8.0 Hz, 32H; Ar-H), 7.46 (m, 28H; Ar-H), 8.27 (m, 13H; Ar-H), 8.29 ppm (m, 2H; Ar-H); MALDI-MS: *m*/*z*: 17989 (broad signal) within experimental error for [*M*+K]$^+$.

9Gal: Yield 13 mg (90 %); ^{1}H NMR (600 MHz, [D$_6$]DMSO/D$_2$O, 80 °C, selected signals): δ = 1.81 (s, 96H; 32× -NHCOCH_3), 3.17 (s, 64H; 32× -CO-CH_2-S-), 4.28–4.35 (m, 128H; 32× Ar-CH_2-NH-CO-CH_2-S-, 32× GlcNAc H-1, 32× βGal H-1), 4.55 (s, 60H; 30× Ar-CH_2-NH-CO-Ar), 4.86 (d, ^3J(H,H) = 3.5 Hz, 32H; 32× αGal H-1), 7.38 (m, 64H; Ar-H), 7.47 (m, 60H; Ar-H), 8.30–8.35 ppm (m, 31H; Ar-H); no MS could be obtained by MALDI.

10Gal: Yield 13 mg (85%); ^{1}H NMR (600 MHz, [D$_6$]DMSO/D$_2$O, 80°C, selected signals): δ = 1.81 (s, 192H; 64× -NHCOCH_3), 3.17 (s, 128H; 64× -CO-CH_2-S-), 4.29–4.35 (m, 256H; 64× Ar-CH_2-NH-CO-CH$_2$-S-, 64× GlcNAc H-1, 64× βGal H-1), 4.56 (s, 124H; 62× Ar-CH_2-NH-CO-Ar), 4.86 (d, ^3J(H,H) = 3.5 Hz, 64H; 64× αGal H-1), 7.39 (d, ^3J(H,H) = 8.0 Hz, 128H; Ar-H), 7.47 (m, 124H; Ar-H), 8.30–8.35 ppm (m, 63H; Ar-H); no MS could be obtained by MALDI.

11Gal: Yield 38 mg (68%); ^{1}H NMR (400 MHz, [D$_6$]DMSO/D$_2$O, 80°C, selected signals): δ = 1.82 (s, 12H; 4× NHCOCH_3), 4.31–4.37 (m, 20H; 6× Ar-CH_2-NH-CO-, 4× GlcNAc H-1, 4× βGal H-1), 4.87 (d, ^3J(H,H) = 3.5 Hz, 4H; 4× αGal H-1), 7.33 (m, 12H; Ar-H), 7.78 ppm (d, ^3J(H,H) = 8.0 Hz, 12H; Ar-H); HR-MS: calcd for $C_{179}H_{266}N_{20}O_{82}S_4$ $[M-4H]^{4-}$ 1032.8963; found 1032.8968.

19Gal: Yield 41 mg (89%); ^{1}H NMR (400 MHz, [D$_6$]DMSO/D$_2$O, 80°C, selected signals): δ = 1.83 (s, 12H; 4× -NHCOCH_3), 3.13 (s, 8H; 4× -CH_2-S-), 4.32 (d, ^3J(H,H) = 7.0 Hz, 4H; 4× GlcNAc H-1), 4.36 (d, ^3J(H,H) = 8.0 Hz, 4H; 4× βGal H-1), 4.87 (d, ^3J(H,H) = 3.5 Hz, 4H; 4× αGal H-1), 7.60 (d, ^3J(H,H) = 2.0 Hz, 4H; Ar-H), 7.84 (m, 2H; Ar-H), 8.03 (m, 2H; Ar-H), 8.12 ppm (m, 1H; Ar-H); HR-MS: calcd for $C_{185}H_{290}N_{20}O_{82}S_4$ $[M-4H]^{4-}$ 1056.9432; found 1056.9426.

24Gal: Yield 42 mg (47%); ^{1}H NMR (400 MHz, D$_2$O, 20°C, selected signals): δ = 1.90 (s, 12H; 4× -NHCOCH_3), 3.05 (s, 8H; 4× -CO-CH_2-S-), 4.31 (d, ^3J(H,H) = 8.0 Hz, 4H; 4× GlcNAc H-1), 4.41 (d, ^3J(H,H) = 8.0 Hz, 4H; 4× βGal H-1), 5.03 (d, ^3J(H,H) = 3.5 Hz, 4H; 4× αGal H-1), 7.35–8.00 (m, 6H; Ar-H), 7.50 (m, 1H; Ar-H), 7.72 ppm (m, 2H; Ar-H); HR-MS: calcd for $C_{173}H_{278}N_{20}O_{82}S_4$ $[M-4H]^{4-}$ 1017.9198; found 1017.9206.

29Gal: Yield 25 mg (66%); ^{1}H NMR (400 MHz, [D$_6$]DMSO/D$_2$O, 80°C, selected signals): δ = 1.82 (s, 12H; 4× NHCOCH_3), 2.96 (s, 6H; 2× Ar-CH$_2$-NCH_3-CO-Ar), 3.18 (s, 8H; 4× -CO-CH_2-S-), 4.31–4.36 (m, 16H; 4× Ar-CH_2-NH-CO-CH$_2$-S-, 4× GlcNAc H-1, 4× βGal H-1), 4.73 (s, 4H; 2× Ar-CH_2-NCH$_3$-CO-Ar), 4.87 (d, ^3J(H,H) = 3.5 Hz, 4H; 4× αGal H-1), 7.59 (d, ^3J(H,H) = 2.0 Hz, 4H; Ar-H), 8.08 (m, 2H; Ar-H), 8.31 (m, 2H; Ar-H), 8.48 ppm (m, 1H; Ar-H); HR-MS: calcd for $C_{187}H_{258}N_{20}O_{82}S_4$ $[M-3H]^{3-}$ 1406.8433; found 1406.8426.

30Gal: Yield 43 mg (92%); ^{1}H NMR (400 MHz, [D$_6$]DMSO/D$_2$O, 80°C, selected signals): δ = 1.82 (s, 12H; 4× NHCOCH_3), 2.50 (s, 12H; 4× Ar-N(CH_3)-CO-), 2.54 (s, 6H; 2× Ar-N(CH_3)-CO-), 3.12 (s, 8H; -CO-CH$_2$-S-), 4.23 (s, 8H; 4× Ar-CH_2-NH-CO-CH$_2$-S-), 4.31–4.37 (m, 12H; 2× Ar-CH_2-NH-CO-Ar, 4× GlcNAc H-1, 4× βGal H-1), 4.57 (s, 4H; 2× Ar-CH_2-NH-CO-Ar), 4.87 (d, ^3J(H,H) = 3.5 Hz, 4H; 4× αGal H-1), 7.30 (m, 1H; Ar-H), 7.43 ppm (m, 6H; Ar-H); HR-MS: calcd for $C_{191}H_{266}N_{20}O_{82}S_4$ $[M+3Na]^{3+}$ 1449.5273; found 1449.5268.

Multi-angle light scattering (MALS): All determinations of the weight-average molar mass (MW_{Aggr}) and of dn/dc were performed at 20°C, unless otherwise stated. They were carried out by Wyatt Technology Deutschland GmbH and by Wellensiek Laborgeraete using a DAWN® EOS and a Wyatt Optilab 903 refractometer. Concentrations were 0.3 mg mL^{-1} for **4Gal**, **7Gal**, **7Lac**, **8Gal**, **9Gal**, **10Gal**, and **24Gal** and 0.06 mg mL^{-1} for **6Gal**, **11Gal**, **19Gal**, **29Gal**, and **30Gal**. Aqueous solutions were heated for 30 min at 80°C and then allowed to cool to 20°C. The samples were passed through Spartan 30 or Schleicher & Schuell 0.45 μm filters.

Atom force microscopy (AFM): Aqueous solutions (10 μL, 2 μg mL^{-1} for **6Gal**–**9Gal**, 0.2 μg mL^{-1} for **10Gal**) were spotted on freshly cleaved mica. The solvent was allowed to evaporate for at least 120 min. A Multi-Mode AFM with a Nanoscope III controller (Digital Instruments) was used to generate all of the images. The microscope was operated in tapping mode in air with Olympus etched silicon probes (cantilever length 120 μm, tip height 10 μm).

Transmission electron microscopy: An aqueous solution of **6Gal** (1 μL, 2 μg mL^{-1}) was diluted with 1 μL of a 2% sodium silicotungstate solution for negative contrasting. The mixture was spotted on a copper net with a Formvar (polyformaldehyde) surface and the solvent was allowed to evaporate. A transmission electron microscope (EM910/FA Leo) was used to generate the image.

Computational methods: All calculations were performed within Macromodel 8.5 (Schrödinger, L. L. C.) applying the AMBER* force field with a distance-dependent dielectric constant of 4.0 *R*. The starting structures were submitted to 10000 steps of Monte Carlo (MC) minimizations. Two sets of MC perturbations were applied: new starting structures were generated by randomly modifying torsion angles and by variations along random linear combinations of the 20 lowest vibrational modes (LMOD).[13] All rotatable bonds (those not adjacent to amide bonds) were included. To achieve a good balance between searching within one well of the potential energy surface and jumping from one well to another, a 50% mixture of both perturbations was applied. Of the minimized structures, only those within 200 kJ mol^{-1} of the current global minimum were accepted. Building and visualization of structures was performed in the framework of the molecular graphics program WitnotP (A. Widmer, unpublished results).

ELISAs: ***a) human serum***: On plate A, wells were coated with 0.5 μg of αGal-HSA conjugate in 100 μL of NaHCO$_3$ (100 mM, pH 9.6) for 2 h at 37°C. Blocking with 250 μL of PBS containing 0.5% Tween®20 for 2 h at ambient temperature was followed by removal of the liquid. On plate B, pooled human serum was diluted 1:20 in PBS containing 10% Seablock™ and 0.2% Tween®20 and incubated for 30 min with serial dilutions of the glycodendrimers. Thereafter, 100 μL from each well on plate B was transferred to plate A, incubated for 1 h at ambient temperature, and washed three times with PBS containing 0.15% Tween®20. Then, the mixtures were incubated with 100 μL of PBS containing anti-IgM secondary antibody peroxidase conjugate (diluted 1/2000), 10% Blocker-BSA™, and 0.2% Tween®20. After washing six times with 300 μL of PBS containing 0.1% Tween 20, color development was initiated by adding 100 μL of TMB substrate solution and stopped after 5 min by adding 50 μL of 1 M H_2SO_4. The A_{450} value was measured.

b) Cynomolgus monkey serum: Wells were coated with 0.5 μg of αGal-polymer[7] in 100 μL of PBS overnight at 4°C. Blocking with 300 μL of PBS containing 10% Seablock™ for 2 h at ambient temperature was followed by removal of the liquid. The wells were incubated for 30 min with 100 μL of serially diluted serum sample in PBS containing 10% Seablock™ and 0.2% Tween®20, and subsequently washed three times with PBS containing 0.15% Tween 20. Incubation with secondary antibody and color development were performed as described above.

Haemolysis assays: ***a) human serum***: Pig erythrocytes were obtained from heparinized pig blood, washed three times with CFD buffer (pH 7.4), and suspended in CFD at a concentration of 1×10^9 mL^{-1}. On low-bind U-shaped plates, seven serial dilutions of inhibitor were prepared in 50 μL of CFD. Human serum was serially diluted in 50 μL of CFD (ten dilutions assayed for each inhibitor concentration). Then, 50 μL of pig erythrocyte solution was added to each well and the plate was incubated for 60 min at 37°C with mild shaking. Plates were centrifuged for 10 min at 2000× g to precipitate the unlysed erythrocytes, the supernatant was transferred to a flat-bottomed plate, and released hemoglobin (A_{420}) was measured.

b) Cynomolgus monkey serum: As above, but with the addition of 50 μL of baby rabbit complement (heterologous complement source at a final dilution of 1:10) in CFD and 50 μL of pig erythrocyte solution to each well.

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