Published in final edited form as:

Bioorg Med Chem Lett. 2011 September 1; 21(17): 5078–5083. doi:10.1016/j.bmcl.2011.03.100.

Clusters of ligands on dendrimer surfaces

Kristian H. Schlick^a, Joel R. Morgan^a, Julianna J. Weiel^a, Melissa S. Kelsey^a, and Mary J. Cloninger^{a,*}

^aMontana State University, 103 Chemistry & Biochemistry Building, Bozeman, MT 59717, USA

Abstract

The development of methodology that is designed to allow a significant increase in the patterning and in the functionalization of the dendrimer is the ultimate goal of the research described here. Glycoside clusters based on TRIS were formed using click chemistry and were attached to PAMAM dendrimers. A series of dendrimers bearing tris-mannoside and an ethoxyethanol group was synthesized, and the binding interactions of these dendrimers with Concanavalin A were evaluated using inhibition ELISAs. The results of the inhibition ELISAs suggest that tris-mannoside clusters can replace individual sugars on the dendrimer without loss of function. Since tris-mannoside clustering allows for a redistribution of the dendrimers' surface functionalities, from this chemistry one can envision patterned dendrimers that incorporate multiple groups to increase the function and utility of the dendrimer.

Keywords

Glycodendrimers; Dendrimers; Cluster Mannosides; Tris-mannosides; Multivalency

Dendrimers are being studied for a wide variety of applications.¹ Among other uses, dendrimers are being developed to serve as light harvesting devices and fluorescent sensors,^{2–4} and as catalysts.^{5,6} Biomedical applications of dendrimers include their use as gene delivery vectors,⁷ as contrast agents for magnetic resonance imaging,⁸ as antigen carriers and adjuvants in vaccine development,⁹ and as targeting and drug delivery agents^{10–12} for diseases such as cancer.¹³

In many cases, dendrimers bearing more than one functional group at their termini are highly desirable. For example, partial functionalization of PAMAMs with polyethylene glycol (PEG) groups reduced the cytotoxicity of the dendrimers,¹⁴ and the co-functionalization of a dendrimer with an imaging agent and a targeting ligand or a therapeutic molecule has been reported.^{15–18} As the need for dendrimers bearing multiple functional groups has emerged, several noteworthy synthetic strategies to afford heterobifunctional dendrimers have been reported.^{19–21}

The simplest way to heterobifunctionalize a dendrimer is to either sequentially or simultaneously add two compounds to form a random distribution.^{22–25} This method has the

© 2011 Elsevier Ltd. All rights reserved

*Corresponding author. Tel.: 406-994-3051; fax: 406-994-5407; mcloninger@chemistry.montana.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Supplementary data Supplementary data associated with this article can be found, in the online version.

advantage that the ratio of endgroups can be systematically varied over a wide range of endgroup ratios. However, the random distribution of endgroups that is sufficient for many applications may not be preferred when patterned dendrimer surface architectures are desired. Catalytic or recognition groups may need to be proximal in order for them to be fully effective.^{26,27}

Here, we report the synthesis of dendrimers bearing trimers of mannosides (Figure 1). We chose to use mannoside clusters because of their biological relevance and also because of the prevalence of existing examples of tri-mannoside clusters that have been reported. Mannose-specific bacterial adhesion has been studied by Lindhorst and co-workers using mannoside clusters because of the important interaction of the FimH protein on the type 1 fimbriae of *E. coli* with host cell mannosides.^{28–31} Roy and co-workers used small mannose functionalized dendrimers to study this interaction,^{32–34} and heptyl mannosides have also been reported to reduce bacterial binding to human bladder cells *in vitro*.³⁵ Additional examples of tri-mannoside clusters have also been reported,^{34,36–41} and an interesting subset of these reports use TRIS (tris(hydroxymethyl)aminomethane) as the foundation of the tri-mannoside.^{34,36–38} Several of the mannoside-clusters have been synthesized using Cu(I) mediated Huisgen triazole formation, or “click” chemistry.^{34,42–46}

Although the synthesis of tris-mannoside functionalized dendrimers that is reported here retains the ease of functionalization that is the hallmark of random heterobifunctionalization pathways and of click chemistry, this methodology enables a significant increase in the clustering of the endgroups on the dendrimer surface. Using the endgroup cluster, more endgroups are made available so that prodrugs, solubilizing and immunomodulating groups, and imaging agents can ultimately be added to the dendrimer. This is shown schematically in Figure 2, where replacing the original glycodendrimer (Figure 2a) with a patterned glycodendrimer leaves many vacant amines on the PAMAM surface that could be further elaborated (Figures 2b and c). In this report, an inhibition enzyme-linked immunosorbent assay (ELISA) was performed to compare the binding of the patterned dendrimers with that of unpatterned dendrimers toward Concanavalin A, a mannose binding lectin. Dendrimers bearing clusters of carbohydrates have comparable activity with the lectin to that of dendrimers that have sugars scattered across the dendrimer.

Synthesis of the sugar cluster begins with TRIS. Boc protection of the amine followed by propargylation of the three hydroxyl groups afforded **1**.³⁴ Removal of the Boc group and conversion of the amine to an isothiocyanate afforded **2**, the trispropargyl ether that was used as the alkyne component in the Cu(I)-mediated 1,3-dipolar cycloaddition with three equivalents of azido-functionalized peracetylated mannose³⁰, forming **3** (Scheme 1). The click reaction is quantitative; washing with EDTA is performed to remove copper after completion of the reaction.

PAMAM dendrimers were partially functionalized with 2-(2-isothiocyanatoethoxy)ethanol,⁴⁷ followed by functionalization with sugar cluster **3** (Scheme 2). Within each generation, a series of different cluster loadings were prepared (Table 1). The 2-(2-isothiocyanatoethoxy)ethanol group was added to the dendrimer for two reasons. First, the ethoxyethanol group helps with solubility. Tris-mannoside functionalized G(3) and G(6) dendrimers were insoluble in aqueous buffer solutions unless ethoxyethanol was added. Second, the ethoxyethanols serve as blocking groups for the primary amino termini of the PAMAMs, reducing the overall charge of the dendrimers at the requisite pH for the dendrimer/lectin studies.

After the first step of partial functionalization with 2-(2-isothiocyanatoethoxy)ethanol, MALDI-TOF analysis was performed. Then, a small excess of the tris-mannoside cluster **3**

was added to saturate the remaining amines. It should be noted that, with smaller, less hindered isothiocyanates, the order of addition of the two components can be reversed without consequence; however, the isothiocyanate functionality on **3** is neopentyl, and **3** is relatively large, so a 1.2 to 1.5 mole excess was required for the addition of **3** to ensure that the desired dendrimer loading was achieved. Deacetylation using Zemplén conditions in 1:1 H₂O:MeOH was performed to afford dendrimers **4–7**.

The degree of functionalization of the dendrimers with carbohydrates was determined by MALDI-TOF MS. The average numbers of endgroups that were incorporated are shown in Table 1. The loadings were determined by the change in M_w upon addition of the ethoxyethanol and of the tris-mannoside groups and by the change in M_w upon deacetylation. We compared the peak width at half height for the starting dendrimer and the functionalized dendrimers to determine the homogeneity of the compounds.²⁴ Polydispersity and sample heterogeneity of the compounds do not increase to any appreciable amount upon dendrimer functionalization.

The peracetylated dendrimers were purified by dialysis in DMSO with a regenerated cellulose membrane (MWCO 10 kDa). After extensive dialysis, some of the lower molecular weight glycodendrimers were still mixed with excess tri-smannose clusters. Fortunately, this mixture could be easily recognized due to a small change in the chemical shift of the acetyl protons in the NMR spectrum between peracetylated mannose on the dendrimer and the free peracetylated mannose cluster (not shown). In cases where dialysis was insufficient, polymer bound ethylene diamine was stirred with the crude dendrimers to remove excess **3**, which was then simply filtered from the product solution. The deacetylated dendrimers were purified by dialysis (MWCO 1000 Da) against Millipore water and lyophilized.

For comparison, unclustered mannose functionalized dendrimers **8–11** bearing only a single mannoside residue per dendrimer endgroup were synthesized as previously described (Figure 3).⁴⁷ Comparison of binding results of glycodendrimers **8–11** with those of tris-mannoside dendrimers **4–7** allows for evaluation of the effects of clustering sugar residues in close proximity versus a random functionalization of the dendrimer surface. Additional control compound **12** was also synthesized (structure is shown in Figure 3; experimental protocol is provided in the supporting information).

The inhibition efficacy of the tris-mannose-functionalized dendrimers was analyzed using ELISA inhibition experiments with Concanavalin A (Con A). Con A is a homotetrameric lectin that is known to selectively bind mannoside and glucoside residues and is a benchmark protein for the study of protein-carbohydrate interactions.^{48,49} RNase B, a high mannose glycoprotein, was used to coat the wells of a 96 well plate. After blocking the remainder of the surface with bovine serum albumin (BSA), pre-incubated mixtures of glycodendrimer and Con A were added to the plate and allowed to reach equilibrium. After washing to remove unbound Con A, an alkaline phosphatase/anti-Con A conjugate was added. 4-nitrophenyl phosphine was added as a substrate for the enzyme, and the amount of Con A that was bound to the surface was determined by measuring the absorbance at 410 nm.

ELISA inhibition experiments were performed in triplicate on glycodendrimers **4–11** (Figure 4 and Table 1). Data was fitted to a four-parameter dose-response curve to determine IC₅₀ values. Standard deviations for the log EC₅₀ values (from which the IC₅₀ values are derived) are given in Table S1 of the supplementary data. Monovalent methyl α -D mannoside was added as a control to each ELISA plate to ensure results from separate plates were comparable. Both tris-mannose functionalized and mannose functionalized dendrimers

were significantly more effective as inhibitors of the Con A/RNase B surface interaction than methyl α -D-mannoside was. Dendrimers **6f** and **7e**, which contain no mannose, did not inhibit Con A binding to the surface, suggesting that the Con A/surface interaction occurs with specificity and that the dendrimer architecture does not significantly increase non-specific binding. Compound **5g** also showed no inhibitory effect for the concentration ranges tested. For this dendrimer, the few mannose clusters are likely to be quite distant from one another and may even be buried in the interior of the large dendrimer, making them inaccessible to the Con A binding sites.

In two cases, compounds **6d** and **6e**, inhibition did not occur as expected. After an initial inhibition activity at lower concentrations, the signal increased rapidly, and even displayed activity above baseline. This suggests that with an increased amount of the glycodendrimer, Con A recruitment to the ELISA plate surface is promoted rather than inhibited. The glycodendrimers are apparently able to bind to Con A and then deliver the Con A to the surface, where it is retained through subsequent washing steps. Presumably, dendrimers **6d** and **6e** are of the size and shape needed to form a stable complex with Con A and are also able to anchor the Con A/dendrimer aggregate onto the surface of the ELISA plate, which links a larger amount of Con A to the surface of the ELISA plate than is bound when these glycodendrimers are not present.

When compound **12** was compared to methyl α -D-mannoside in a hemagglutination inhibition assay (HIA) with rabbit red blood cells and Con A, **12** had an inhibition potency that was only 2.3 times greater than that of methyl α -D-mannoside, while **9** had an inhibition potency that was 8900 times greater than methyl α -D-mannoside (see Figure S1 in the supporting information). The fact that the inhibition activity with Con A is low for both **12** and methyl α -D-mannoside relative to the glycodendrimer strongly suggests that the tris-mannoside unit is not a significantly more effective monovalent ligand for Con A than methyl α -D-mannoside is (details provided in the supporting information).

When IC₅₀ values are examined on a per mannoside basis, all of the generation three dendrimers (**4a–4d** and **8**) are comparable inhibitors. On a per dendrimer basis, **4a** and **4b** have IC₅₀ values comparable to the value for **8**. These three dendrimers also have approximately the same number of mannoses. Dendrimer **8** is only 2.6-fold more effective than dendrimer **4c** even though **4c** has 9 sugars (3.2-fold less) while **8** has 29 sugars on average. This suggests that, even for small generation dendrimers, a patterned arrangement of ligands on the dendrimer may allow systems to be as effective in binding events as dendrimers that have the same number of ligands displayed in an unpatterned presentation using far more of the dendrimer endgroups. Dendrimer **4d** with only one tris-mannoside on average is a surprisingly effective inhibitor, and we cannot rule out the possibility that this activity may be caused by a small subset of over-functionalized glycodendrimers.

In the generation four series of dendrimers, all of the compounds again have comparable IC₅₀ values when evaluated on a per mannoside basis, although dendrimers **5b** and **5c**, which are in the middle of the series in terms of amount of mannoses, have slightly lower IC₅₀ values than those of **9**. Dendrimers **5a**, **5d**, **5e**, **5f** are all slightly less effective inhibitors (on a per mannose basis) than **9**. On a per dendrimer basis, **5a**, **5b**, **5c**, and **9** are all comparable. Dendrimer **5d** is only 3-fold weaker than **9**, **5e** is only 6.1-fold weaker than **9**, and **5f** is 12.8-fold weaker than **9** (on a per dendrimer basis). The dramatically reduced number of mannoses that are required for inhibition (27, 15, and 9 for **5d**, **5e** and **5f** respectively compared to 55 sugars for **9**) suggest that patterned architectures can be useful for processes involving higher generation dendrimers. For example, **5d** shows good inhibition but only 9 of the PAMAM amino groups are functionalized with tris-mannoside, leaving 55 more amino termini available for functionalization with another group.

In the generation five series of dendrimers, all dendrimers **6a–6c** and **10** have comparable IC₅₀ values on a per mannoside basis. Dendrimer **6a** is 2-fold weaker (on a per mannose basis) but has twice as many sugars as the other dendrimers and is almost certainly over-functionalized (meaning that some tris-mannosides may be sterically inaccessible to Con A binding sites once some Con A/dendrimer interactions have occurred). On a per dendrimer basis, **6a–6c** and **10** all have comparable IC₅₀ values. Thus, adding the extra 29 tris-mannosides (87 mannosides) to convert **6b** to **6a** is not obviously advantageous.

In the generation six series, all dendrimers **7a–7d** and **11** have comparable IC₅₀ values on a per mannose basis, and all but **7d** have comparable IC₅₀ values on a per dendrimer basis. Even **7d** with nine carbohydrate clusters (27 mannosides) has only a 4-fold reduction in effectiveness as an inhibitor of the Con A/surface interaction relative to **11**. Reducing the number of clusters from 66 in **7a** (198 sugars) to 9 in **7d** (27 sugars) causes only a 3.5-fold reduction in the IC₅₀ value. Thus, the results from the sixth generation dendrimer series also suggest that dendrimers that are decorated with sugars in a clustered display, leaving many endgroups available for functionalization with other groups, are as effective as dendrimers where all the endgroups have been distributed across the dendrimer surface. Very little, if any, activity is lost when the carbohydrates are clustered.

Overall, IC₅₀ values were similar for all generations of dendrimers that were studied. This suggests that smaller dendrimers were as effective as larger dendrimers in inhibiting the binding of Con A to the surface. Larger dendrimers may only be needed when additional functionality is desired, such as described by Figure 2c. In all generations, the dendrimers with the clustered display of ligands were as effective as the dendrimers with monomeric ligands distributed across the endgroups.

In conclusion, tris-mannoside clusters have been synthesized using click chemistry and have been appended to G(3) through G(6)-PAMAM dendrimers. Varying amounts of tris-mannosides were used in conjunction with a solubilizing ethoxyethanol unit to form a series of glycodendrimers. ELISA inhibition experiments suggest that tris-clustered mannose residues can be used to replace individual sugars on the dendrimer without loss of function, freeing up a significant number of the endgroups on the dendrimers. Since tris-mannoside clustering allows for a redistribution of the dendrimers' surface functionalities, from this chemistry one can obtain patterned dendrimers that incorporate solubilizing groups (as described here), or groups to alter immunogenicity, imaging agents, prodrugs, and targeting ligands all on the same dendrimer (as depicted schematically in Figure 2c). The present study suggests an effective way to cluster the targeting carbohydrate groups so that additional groups can be added without loss of binding specificity or selectivity for the targeted lectin-carbohydrate interaction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This paper is dedicated to Carolyn R. Bertozzi on the occasion of her receipt of the Tetrahedron Young Investigator Award. This research was supported by NIH R01 GM62444. J. J. W. was supported by an undergraduate scholarship from the Beckman Foundation, and both J. J. W. and M. S. K. were supported by undergraduate scholarships from NIH P20 RR016455.

References

1. Astruc D, Boisselier E, Omelas C. Chem. Rev. 2010; 110:1857. [PubMed: 20356105]

2. Andronov A, Frechet JMJ. Chem. Commun. 2000:1701.
3. Nantalaksakul A, Reddy DR, Bardeen CJ, Thayumanavan S. Photosynth. Res. 2006; 87:133. [PubMed: 16408144]
4. Caminade A-M, Delavaux-Nicot B, Laurent R, Majoral J-P. Curr. Org. Chem. 2010; 14:500.
5. de Jesus E, Flores JC. Indust. Eng. Chem. Res. 2008; 47:7968.
6. Berger A, Gebbink RJMK, van Koten G. Top. Organomet. Chem. 2006; 20(Dendrimer Catalysis):1.
7. Dutta T, Jain NK, McMillan NAJ, Parekh HS. Nanomed. Nanotech. Biol. Med. 2010; 6:25.
8. Villaraza AJL, Bumb A, Brechbiel MW. Chem. Rev. 2010; 110:2921. [PubMed: 20067234]
9. Heegaard PMH, Boas U, Sorensen NS. Bioconj. Chem. 2010; 21:405.
10. Gupta U, Agashe HB, Asthana A, Jain NK. Nanomed. Nanotech. Biol. Med. 2006; 2:66.
11. Kannan, RM.; Perumal, OP.; Kannan, S. Dendrimers and Hyperbranched Polymers for Drug Delivery. In: Labhasetwar, V.; Leslie-Pelecky, DL., editors. Biomedical applications of nanotechnology. Wiley; Hoboken: 2007. p. 105
12. Svenson, S.; Tomalia, DA. Dendrimers as nanoparticulate drug carriers. In: Torchilin, VP., editor. Nanoparticulates as drug carriers. Imperial College Press; London: 2006. p. 277
13. Tekade R, Kumar PV, Jain NK. Chem. Rev. 2009; 109:49. [PubMed: 19099452]
14. Jevprasesphant R, Penny J, Jalal R, Attwood D, McKeown NB, D'Emanuele A. Int. J. Pharm. 2003; 252:263. [PubMed: 12550802]
15. Hourana R, Jain M, Maysinger D, Kakkar A. Chem. Eur. J. 2010 DOI: 10.1002/chem.201000241.
16. Swanson SD, Kukowska-Latallo JF, Patri AK, Chen C, Ge S, Cao Z, Kotlyar A, East AT, Baker JR. Int. J. Nanomed. 2008; 3:201.
17. Patri AK, Kukowska-Latallo JF, Baker JR. Adv. Drug Deliv. Rev. 2005; 57:2203. [PubMed: 16290254]
18. Lee CC, Gillies ER, Fox ME, Guillaudeu SJ, Fréchet JMJ, Dy EE, Szoka FC. Proc. Natl. Acad. Sci. U.S.A. 2006; 103:16649. [PubMed: 17075050]
19. Goodwin AP, Lam SS, Frechet JMJ. J. Am. Chem. Soc. 2007; 129:6994. [PubMed: 17489595]
20. Antoni P, Hed Y, Nordberg A, Nystrom D, von Holst H, Hult A, Malkoch M. Angew. Chem. Int. Ed. 2009; 48:2126.
21. Navath RS, Menjoge AR, Wang B, Romero R, Kannan S, Kannan RM. Biomacromol. 2010; 11:1544.
22. Wolfenden ML, Cloninger MJ. Bioconj. Chem. 2006; 17:958.
23. Wolfenden ML, Cloninger MJ. J. Am. Chem. Soc. 2005; 127:12168. [PubMed: 16131163]
24. Walter ED, Sebbly KB, Usselman RJ, Singel DJ, Cloninger MJ. J. Phys. Chem. B. 2005; 109:21532. [PubMed: 16853796]
25. Woller EK, Walter ED, Morgan JR, Singel DJ, Cloninger MJ. J. Am. Chem. Soc. 2003; 125:8820. [PubMed: 12862477]
26. Breinbauer R, Jacobsen EN. Angew. Chem. Int. Ed. 2000; 39:3604.
27. Mammen M, Choi S-K, Whitesides GM. Angew. Chem. Int. Ed. Engl. 1998; 37:2754–2794.
28. Patel A, Lindhorst TK. Carbohydr. Res. 2006; 341:1657. [PubMed: 16472787]
29. Rockendorf N, Sperling O, Lindhorst TK. Aust. J. Chem. 2002; 55:87.
30. Lindhorst TK, Kotter S, Krallmann-Wenzel U, Ehlers S. J. Chem. Soc., Perkin Trans. 2001; 1:823.
31. Kotter S, Krallmann-Wenzel U, Ehlers S, Lindhorst TK. J. Chem. Soc., Perkin Trans. 1998; 1:2193.
32. Touaibia M, Wellens A, Shiao TC, Wang Q, Sirois S, Bouckaert J, Roy R. ChemMedChem. 2007; 2:1190. [PubMed: 17589887]
33. Touaibia M, Shiao TC, Papadopoulos A, Vaucher J, Wang Q, Benhamioud K, Roy R. Chem. Commun. 2007:380.
34. Chabre YM, Contino-Pepin C, Placide V, Shiao TC, Roy R. J. Org. Chem. 2008; 73:5602. [PubMed: 18572921]
35. Gouin SG, Wellens A, Bouckaert J, Kovensky J. ChemMedChem. 2009; 4:749. [PubMed: 19343765]

36. Ashton PR, Hounsell EF, Jayaraman N, Nilsen TM, Spencer N, Stoddart JF, Young M. J. Org. Chem. 1998; 63:3429.
37. Kikkeri R, Garcia-Rubio I, Seeberger PH. Chem. Commun. 2009:235.
38. Kohn M, Benito JM, Mellet CO, Lindhorst TK, Fernandez JMG. ChemBioChem. 2004; 5:771. [PubMed: 15174159]
39. Al-Mughaid H, Grindley TB. J. Org. Chem. 2006; 71:1390. [PubMed: 16468786]
40. Sato K, Hada N, Takeda T. Carbohydr. Res. 2006; 341:836. [PubMed: 16530741]
41. Munoz EM, Correa J, Fernandez-Megia E, Riguera R. J. Am. Chem. Soc. 2009; 131:17765. [PubMed: 19919053]
42. Ortega-Munoz M, Lopez-Jaramillo J, Hernandez-Mateo F, Santoyo-Gonzalez F. Adv. Synth. Catal. 2006; 348:2410.
43. Franc G, Kakkar AK. Chem. Soc. Rev. 2010; 39:1536. [PubMed: 20419208]
44. Iha RK, Wooley KL, Nystrom AM, Burke DJ, Kade MJ, Hawker CJ. Chem. Rev. 2009; 109:5620. [PubMed: 19905010]
45. Huisgen, R. 1,3-Dipolar Cycloaddition—Introduction, Survey, Mechanism. Wiley; Hoboken: 1984. p. 1
46. Kolb HC, Finn MG, Sharpless KB. Angew. Chem. Int. Ed. 2001; 40:2004.
47. Woller EK, Cloninger MJ. Org. Lett. 2002;4, 7.
48. Lis H, Sharon N. Chem. Rev. 1998; 98:637. [PubMed: 11848911]
49. Bittiger, H.; Schnebli, HP. Concanavalin A as a Tool. John Wiley & Sons; New York: 1976.

Figure 1.
Tris mannose-functionalized PAMAM dendrimer. Top: schematic.

Figure 2. Schematics of (a) a functionalized dendrimer, (b) a glycodendrimer with clustered ligands; 2/3 of the termini remain available for additional elaboration, (c) heterogeneously functionalized dendrimer; multifunctionalization was enabled by ligand clustering.

Figure 3.
Mannose functionalized dendrimers 8–11 and control compound 12. Top: schematic of 8–11.

Figure 4.

ELISA inhibition graphs for compounds 4–11. The results are the averages of 3 replicate experiments. (a) Tris-mannoside functionalized G(3)-PAMAM 4, (b) Tris-mannoside functionalized G(4)-PAMAM 5, (c) Tris-mannoside functionalized G(5)-PAMAM 6, (d) Tris-mannoside functionalized G(6)-PAMAM 7, (e) Mannose functionalized G(3) to G(6)-PAMAMs 8–11.

Scheme 1.
Synthesis of isothiocyanato tris-mannoside **3**.

Scheme 2.

Synthesis of tris-mannoside functionalized dendrimers. Values for m and n are given in the supporting information; values for a and b are given in Table 1.

Table 1

MALDI-TOF MS data for dendrimers **4–7** and ELISA IC₅₀ values for **4–11** and for methyl α -D-mannoside.

Cmpd (gen ^a)	no. of ethoxy ethanol ^b	no. of 3 ^c (no. of man)	Mw (g/mol) ^d	IC ₅₀ ^e (/den)	IC ₅₀ ^e (/man)
4a (3)	7	13 (39)	22300	27 nM	1100 nM
4b (3)	16	8 (24)	17300	30 nM	720 nM
4c (3)	25	3 (9)	13400	95 nM	860 nM
4d (3)	27	1 (3)	12400	350 nM	1100 nM
8 (3)	0	NA ^f (29)	15900 ^g	37 nM	1100 nM
5a (4)	0	39 (117)	59000	11 nM	1300 nM
5b (4)	14	26 (78)	48000	11 nM	860 nM
5c (4)	28	20 (60)	41000	13 nM	780 nM
5d (4)	41	9 (27)	32500	54 nM	1500 nM
5e (4)	46	5 (15)	26300	110 nM	1700 nM
5f (4)	49	3 (9)	25300	230 nM	2100 nM
5g (4)	46	2 (6)	22800	ND ^h	ND
9 (4)	0	NA (55)	30600 ^g	18 nM	990 nM
6a (5)	0	68 (204)	101000	12 nM	2400 nM
6b (5)	29	39 (117)	69000	13 nM	1500 nM
6c (5)	50	30 (90)	64300	12 nM	1100 nM
6d (5)	63	12 (36)	49000	IM ⁱ	IM
6e (5)	86	8 (24)	44000	IM	IM
6f (5)	106	0 (0)	41000	ND	ND
10 (5)	0	NA (95)	55000 ^g	13 nM	1600 nM
7a (6)	48	66 (198)	135000	11 nM	2200 nM
7b (6)	116	31 (93)	107000	14 nM	1300 nM
7c (6)	122	22 (66)	98000	15 nM	990 nM
7d (6)	129	9 (27)	85000	38 nM	1000 nM
7e (6)	163	0 (0)	75000	ND	ND

Cmpd (gen ^a)	no. of ethoxy ethanol ^b	no. of 3 ^c (no. of man)	Mw (g/mol) ^d	IC ₅₀ ^e (/den)	IC ₅₀ ^e (/man)
11 (6)	0	NA (178)	103800 ^g	9 nM	1600 nM
Me man ^j	0	NA (1)		NA	12 mM

^aCompound (dendrimer generation).

^b“a” in Scheme 2; see supporting information for m.

^c“b” in Scheme 2; see supporting information for n.

^dMw is the weight averaged molecular weight.

^eThe reported IC₅₀ values emerge from triplicate experiments. Standard deviations for the log EC₅₀ values (from which the IC₅₀ values are derived) are given in Table S1 of the supplementary data.

^fNA = not applicable.

^gValues taken from ref. 46.

^hND = no determinable IC₅₀ value.

ⁱIM = IC₅₀ value was immeasurable.

^jMe man = methyl α-D-mannoside.