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The synthesis and immune stimulating action of mannose-capped lysine-based dendrimers

Ben W. Greatrex [a], Samuel J. Brodie [a], Richard H. Furneaux [a], Sarah M. Hook [b], Warren T. McBurney [b], Gavin F. Painter [a], Thomas Rades [b], Phillip M. Rendle [a,*]

[a] Industrial Research Limited, PO Box 31-310, Lower Hutt 5040, New Zealand
[b] School of Pharmacy, University of Otago, PO Box 913, Dunedin 9054, New Zealand

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ABSTRACT

The syntheses of monodisperse lysine dendrimers, capped with two to sixty-four mono-, di- and tri-α-D-mannopyranosyl residues are described. These syntheses used reactive *N*-hydroxysuccinimide esters to ensure complete reaction of dendrimer amines with the mannosylating reagents. The purities of the dendrimers were established by RP-HPLC and MALDI-TOF mass spectrometry and were found to be excellent. The relative ability of these glycodendrimers to induce dendritic cell maturation was measured, however, no significant trends were observed.

1. Introduction

Glycodendrimers are hyper-branched, well defined compounds that are recognized as ideal structures to exploit of the cluster glycoside effect and so influence cellular communication events.[1,2] The ability to construct dendrimers from a range of starting materials allows the fine-tuning of the size and shape of the dendrimer and the spacing of residues displayed on the outer shell. The use of L-lysine as a monomer for dendrimer synthesis is attractive for pharmaceutical targets as amide bond cleavage within the dendrimers yields the naturally occurring amino acid, thereby reducing the risk of metabolic toxicity. The L-lysine-based dendrimers were first described by Denkewalter et al. and can be synthesized in a stepwise divergent manner, which conserves the chirality of the α-stereocentre of the amino acid.[3]

The specific targeting of mannose-capped dendrimers to mannose receptors, highly expressed in cells of the immune system, has the potential to provide drug/antigen delivery systems for vaccination or treatment of diseases localized in macrophages and other antigen-presenting cells.[4] Due to the potential of this chemical class, the conjugation of α-D-mannopyranose units to dendritic supports has been performed by a number of groups.[1,5,6] Usually, the sugar is functionalized through the anomeric position by use, for example, of glycosyl isothiocyanates or *N*-hydroxysuccinimide (NHS) esters, which can react with amino groups located on the exterior of the dendrimer. Alternatively, it is possible to adopt thiol-radical addition and cycloaddition[7] strategies for the synthesis of these multivalent molecules. The choice of method used to attach the capping units to the dendrimers is crucial to obtain homogeneous products. The coupling must be highly efficient as slight deficiencies lead to heterogeneity in the resulting products, which is magnified for higher generations and separation of these impurities is impractical.

In this paper we report the synthesis of monodisperse mannose and 1-thiomannose mono- and oligosaccharide-capped lysine dendrimers developed from a benzhydrylamine core. We investigated the ability of our novel mannodendrimers to activate a specific type of immune cell, dendritic cells (DCs), by measuring the expression of activation markers MHC class II and CD86 using flow cytometry.

2. Results and discussion

2.1. Synthesis of mannose-capped dendrimers

Six generations of lysine-based dendrimers **G0** to **G5** (Fig. 1) containing two to sixty-four 'valence' amines, respectively, protected as Boc carbamates, were synthesized as previously described.[8] Capping the dendrimers required both amine-reactive mannosyl derivatives and a non-mannose hydroxy compound. The latter could be used to cap the dendrimers and provide controls with comparative physical properties for the DC assays. Mannosyl capping reagents included 1-thiomannose analogues, which are known for their greater stability to glycosidase action and their abilities to similarly bind to mannose specific receptors.[9] Mannobiosyl and mannotriosyl derived capping reagents were also prepared. The active esters **2**, **7**, **11**, **14**, **19** and **23**

* Corresponding author. Tel.: +64 4 931 3167; fax: +64 4 931 3055.
E-mail address: p.rendle@irl.cri.nz (P.M. Rendle).

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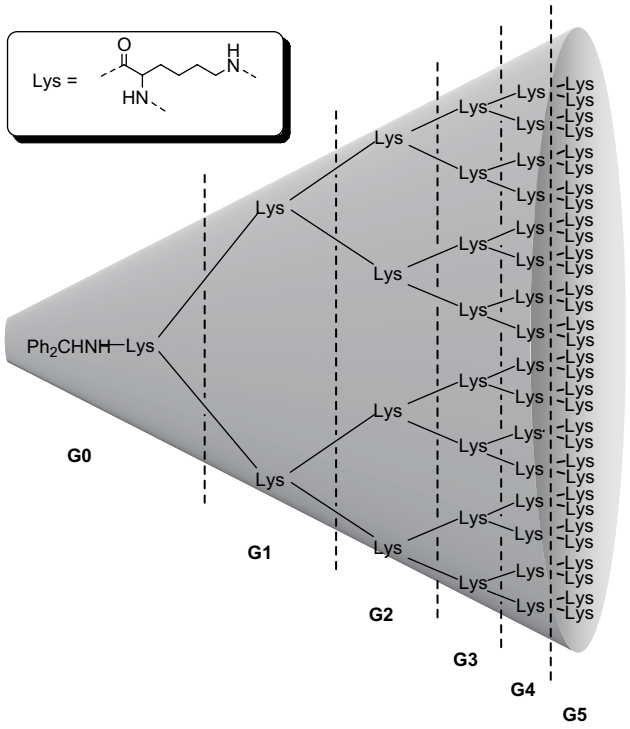
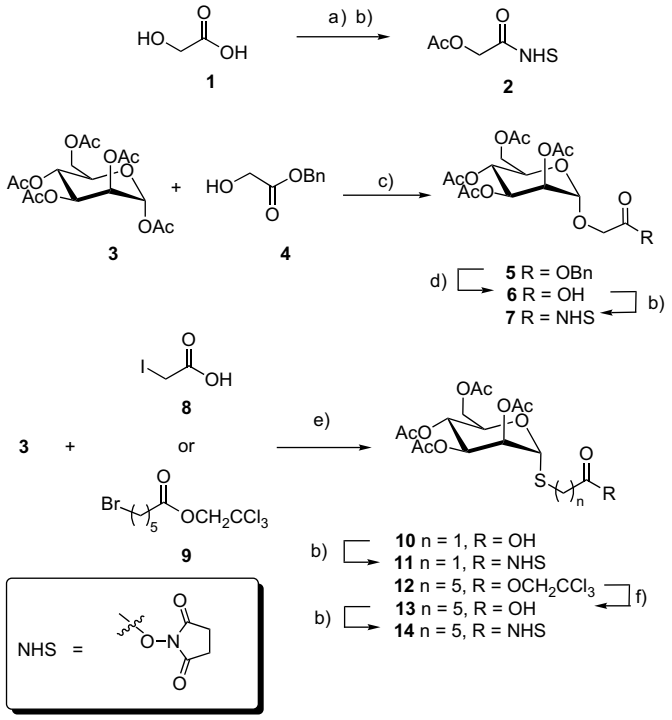


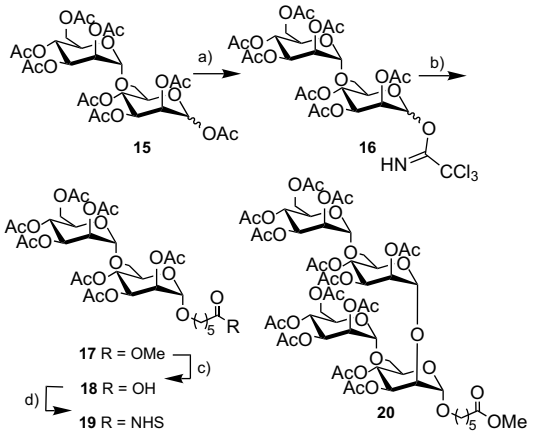
Figure 1. Structure of lysine dendrimers **G0**–**G5** with benzhydrylamine at the core.

were the key building blocks and their syntheses are outlined in Schemes 1–3.

Glycolic acid-derived capping reagent **2** was prepared as previously described (Scheme 1).[10] Reaction of peracetylated α-D-mannose (**3**) with benzyl glycolate (**4**) in the presence of boron trifluoride etherate gave the mannoside **5**, which on hydrogenolysis



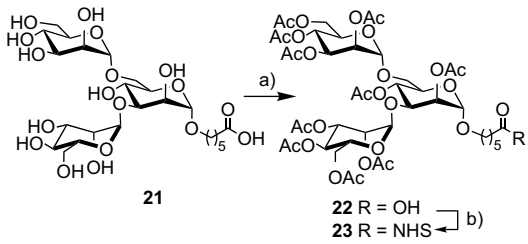
Scheme 1. (a) AcCl; (b) DCC, *N*-hydroxysuccinimide, EtOAc; (c) $BF_3{\cdot}OEt_2$, CH_2Cl_2; (d) H_2, 10% Pd/C solvent; (e) $BF_3{\cdot}OEt_2$, $(NH_2)_2CS$, CH_3CN, 3 h reflux then NEt_3 and **8** or **9**, 0 °C; (f) Zn, AcOH.



Scheme 2. (a) (i) $NH_2NH_2{\cdot}AcOH$, DMF; (ii) CCl_3CN, DBU; (b) (i) TMSOTf, CH_2Cl_2, $HO(CH_2)_5CO_2Me$, (ii) Pyridine, Ac_2O; (c) (i) NaOMe/MeOH; (ii) NaOH, H_2O; (iii) Pyridine, Ac_2O; (d) DCC, *N*-hydroxysuccinimide, EtOAc.

gave the free acid **6** from which the required active ester **7** was derived. For the synthesis of the 1-thiomannosides **11** and **14**, a one-pot procedure described by Ibatullin et al. was adapted.[11] Thus, peracetylated mannose **3** was allowed to react with thiourea in the presence of boron trifluoride etherate to give an intermediate isothiouronium salt, which was alkylated with the appropriate alkyl halide **8** or **9** in the presence of triethylamine, the latter halide being derived from 6-bromohexanoic acid. From acids **10** and **13**, the latter derived by zinc/acetic acid reduction of **12**, the required acylating reagents **11** and **14** were obtained by use of DCC and *N*-hydroxysuccinimide in ethyl acetate. The mannosides **7** and **11** with the glycolate-derived linker were too reactive for purification on silica gel and were instead partially purified by removing solids by filtration. Reactive ester **14**, with the longer alkyl linker, could be purified by flash silica column chromatography.

Roche et al. determined that the mannose disaccharide, α-D-Man-(1→6)-α-D-Man, was taken up most efficiently by the mannose receptor, which is one endocytic receptor expressed by DCs.[12] In light of this, the α-D-Man-(1→6)-α-D-Man capping reagent **19** was prepared (Scheme 2). Peracetylated α(1→6)-mannobiose **15** was converted to the acetylated glycosyl trichloroacetimidate **16**, which was treated with methyl 6-hydroxyhexanoate and TMSOTf to give the glycoside **17**. This perester was saponified to give the heptahydroxy free acid, which, when acetylated, gave heptaacetate **18** from which the required active ester **19**, was obtained after purification by flash chromatography. Although this multi-step process gave access to the target compound, a complication arose during the glycosylation of methyl 6-hydroxyhexanoate (1.5 equiv) with imidate **16** (1 mol equiv). In addition to the desired glycosidation reaction, transfer of the acetyl group from O-2 of the donor to the acceptor was observed. This 2-deacetylated hexa-*O*-acetylmannobiosyl glycoside also participated as a glycosylation acceptor and gave tetrasaccharide glycoside **20** as a by-product (34%). Such



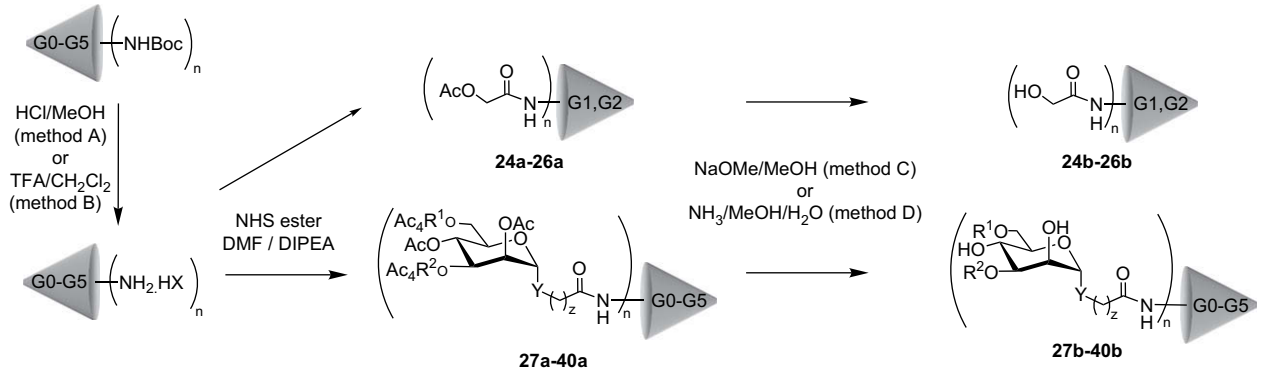
Scheme 3. (a) Pyridine, Ac_2O; (b) *N*-hydroxysuccinimide, DCC, EtOAc.

competitive reactions during glycosylations are not unknown.[13] In the present case this problem was ameliorated by repeating the reaction with excess of the acceptor and by acetylating the mixture when the reaction was complete. This both re-esterified the C-2 hydroxyl group of the donor and facilitated the chromatographic removal of the excess methyl 6-hydroxyhexanoate as its acetate. By this means the yield of glycoside **17** from imidate **16** was increased from 45% to 76%.

In previous studies involving the mannose binding proteins Con A and the Fimbrial lectin of *E. coli* α-D-Man-(1→6)[α-D-Man-(1→3)]α-D-Man was shown to be an avid ligand, which possessed a multivalent mode of binding.[6] We have previously described a synthesis of compound **21**[14] and wished to examine the properties of a mannodendrimer displaying this subunit in our DC assay. Thus, acetylation of the mannotrioside acid **21** using pyridine/acetic anhydride followed by aqueous workup afforded the deca-acetylated acid **22**, which, when allowed to react with *N*-hydroxysuccinimide in the presence of DCC, afforded ester **23** after isolation by flash chromatography (Scheme 3).

With a series of amine-reactive esters in hand, capping of the lysine dendrimers could proceed. The Boc protected dendrimers were deprotected using either HCl/MeOH or TFA/DCM, the latter yielding cleaner products for higher generation material. The resulting poly-amine salts could be stored for short periods. The coupling of the active esters **2**, **7**, **11**, **14**, **19** and **23** to these poly-amine salts **G0**–**G5** proceeded in good yield when facilitated by Hunig's base (Table 1) to give capped dendrimers **24a**–**40a**. To increase the likelihood of complete capping, DMF was used as solvent to ensure homogeneity and the reactions were left overnight. Precipitation of the products from the DMF using water or diethyl ether, filtration and purification by flash chromatography afforded material of good purity as indicated by RP-HPLC. Under-capped material having shorter retention times was clearly visible, and its disappearance was an excellent indication of reaction progress. The extent of mannosylation of each product **27a**–**40a** was readily determined from the ratios of the integrals of the ^{1}H NMR signals of the mannose anomeric resonances to those of the benzhydryl aromatic signals. Both of these signals were cleanly resolved from other resonances. Structure was also confirmed by electro-spray mass spectroscopy (ESMS). For larger generation protected glycodendrimers, ionization by ESMS was weak, however, MALDI-TOF MS of these products gave more intense molecular ions and therefore became the method of choice. It was difficult to achieve complete capping using 'short' linker active esters (**7** and **11** where Z=1) in even small dendrimers (n=4 and 8). Increasing the length of the linker (to Z=5) solved this problem and increased the yield of

Table 1
Capping of lysine dendrimers with mannose derived active esters



n	De-Boc method	NHS ester	Per-OAc	De-Ac method	Yield[a] (%)	Product	Y	Z	R^1	R^2	Purity[b]	MS m/z		Species[c]
												Calcd	Observed	
2	**A**	**2**	**24a**	**C**	35	**24b**	—	—	—	—	—[d]	450.20	450.20	$[M+Na]^+$
	A	**7**	**27a**	**C**	37	**27b**	O	1	H	H	—[d]	752.32	752.32	$[M+H]^+$
	A	**11**	**28a**	**C**	74	**28b**	S	1	H	H	>99	806.25	806.25	$[M+Na]^+$
4	**A**	**2**	**25a**	**C**	49	**25b**	—	—	—	—	99	800.41	800.41	$[M+H]^+$
	A	**7**	**29a**	**C**	27	**29b**	O	1	H	H	—[d]	724.81	724.89	$[M+2H]^{++}$
	A	**11**	**30a**	**C**	41	**30b**	S	1	H	H	>99	1534.5	1534.3	$[M+Na]^+$
	B	**14**	**31a**	**C**	60	**31b**	S	5	H	H	93	1758.7	1758.7	$[M+Na]^+$
	B	**19**	**32a**	**C**	62	**32b**	O	5	Man	H	99	2343.0	2343.2	$[M+Na]^+$
	A	**23**	**33a**	**C**	53	**33b**	O	5	Man	Man	98	2991.2	2991.3	$[M+Na]^+$
8	**A**	**2**	**26a**	**D**	29	**26b**	—	—	—	—	—[d]	772.91	772.91	$[M+2H]^{++}$
	A	**11**	**34a**	**C**	20	**34b**	S	1	H	H	>99	2991.0	2991.1	$[M+Na]^+$
	B	**14**	**35a**	**C**	63	**35b**	S	5	H	H	99	3439.5	3439.3	$[M+Na]^+$
	B	**19**	**36a**	**C**	72	**36b**	O	5	Man	H	90	4608.1	4606.1	$[M+Na]^+$
	B	**19**	**36a**	**D**	72	**36b**	O	5	Man	H	97			
16	**B**	**14**	**37a**	**C**	63	**37b**	S	5	H	H	90	6806.4	6805.3	$[M+Na]^+$
	B	**19**	**38a**	**D**	45	**38b**	O	5	Man	H	>97	9186	9143	$[M+Na]^+$
32	**B**	**14**	**39a**	**C**	61	**39b**	S	5	H	H	—[e]	13,525	13,558	$[M+Na]^+$
64	**B**	**14**	**40a**	**C**	97	**40b**	S	5	H	H	—[e]	26,991	26,811	$[M+Na]^+$

[a] Isolated combined yield for the capping and deprotection reactions.
[b] Purity determined by analytical RP-HPLC at 220 nm and results do not take into account differences in extinction coefficients.
[c] Molecular species observed in the mass spectrum.
[d] Purity not determined by HPLC although samples pure by NMR.
[e] Purity could not be unambiguously established by HPLC due to peak broadening.

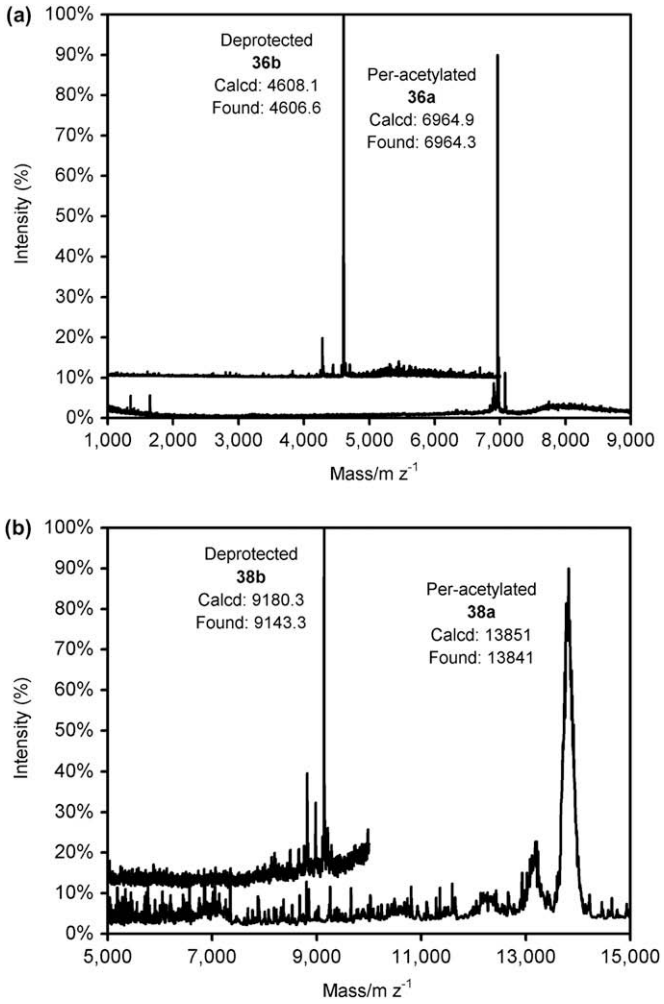


Figure 2. MALDI-TOF MS of a [**G2**] glycodendrimer, acetylated (**36a**) and deprotected (**36b**), and a [**G3**] glycodendrimer, acetylated (**38a**) and deprotected (**38b**).

fully capped dendrimers. This observation is consistent with the approximation that area on the surface of the dendrimer 'ball' per capping group falls with increasing generation. This effect can be negated by increasing the radius of the ball by increasing linker length.

Deacetylation of the dendrimers **24a**–**40a** using standard procedures gave glycolated dendrimers **24b**–**26b** and glycodendrimers **27b**–**40b**. The use of aqueous ammonia in methanol gave improved yield and purity for larger generation glycodendrimers (for example, octavalent **36b**). As expected, water solubility of the deprotected glycodendrimers increased with generation and number of sugars per capping group. MALDI-TOF measurements could be used informatively, as with protected compounds, to analyze product homogeneity and to confirm structure identity. MALDI-TOF measurements were more useful than NMR for visualizing impurities, especially on higher generation materials. A broadened peak was observed for the **G5** glycodendrimer **40b**, with a *m*/*z* range of 25,570–27,990 and an intensity maximum at *m*/*z* 26,810. While this is not unambiguous proof of the structure, the calculated *m*/*z* for glycodendrimer **40b** of 26,991 ($M+Na^+$) falls within the range observed.

As representative examples, the MALDI-TOF mass spectra of the octavalent and hexadecavalent α-D-Man-(1→6)-α-D-Man capped dendrimers **36b** and **38b** and the intermediate acetylated products **36a** and **38a**, respectively, are presented in Figure 2. When fully acetylated, the molecules have sodiated molecular ions at *m*/*z* 6964 and 13,851 (base peak). No isotopic distribution patterns were seen at high molecular weights on the instrument used. Also observed for **36a** was a peak corresponding to loss of a single acetate (6906, 10% base peak height) and a peak at 7078 (10% base peak height, M+114). Altering the laser intensity had no effect on the ratio of these three peaks. Once deprotected, a sodiated molecular ion was observed at *m*/*z* 4606.6 for **36b**, which had an isotope distribution pattern matching that expected for the molecular formula. Glycosidic bond cleavage giving molecular ions with loss of both one and two mannose units were observed in the both the mass spectra for **36b** and **38b**. Since glycosidic bond cleavage was not observed in the MALDI-TOF mass spectra of **36a** and glycosidic bonds are stable to the deprotection conditions employed, this cleavage is thought to be due to the acidic matrix (α-cyano-cinnamic acid/dihydroxybenzoic acid) used in the MALDI technique. No impurity directly resulting from the M+114 species noted in **36a** was observed in **36b**. The peak for **38a** was broad and two lower *m*/*z* broad peaks were observed that could correspond to singly and doubly under-capped products, however, no impurities directly resulting from these under-capped species were observed in **38b**.

2.2. Effects of mannose-capped dendrimers on DC activation

Mannodendrimers[15] and other poly-mannose neoglycoconjugates[16,17] can be used in the targeting of molecules to DCs via the mannose receptor and/or DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin). Mannose-capped dendrimers bind to DC-SIGN with micromolar affinity, an important receptor involved in DC–T-cell interactions and HIV virion transport.[18] Having at hand a range of mannose-capped dendrimers with different linkers, 'valencies' and number of sugars per capping group, we investigated their relative ability to activate DCs derived from the bone marrow of mice (BMDC). This was established by measuring the expression of activation markers MHC Class II and CD86 following a two-day incubation period. The expression of activation markers was determined by flow cytometry and the results expressed as the increase in mean fluorescence intensity (MFI) over background (i.e., unstimulated DCs), Figure 3. The mannose-capped dendrimers failed to significantly enhance DC maturation in vitro relative to LPS making it difficult to elucidate trends of activity based on structure. There was also little difference between dendrimers displaying mannose and those 'control' molecules capped with glycolic acid.

3. Conclusions

A series of discrete mannose-capped lysine dendrimers have been described. Variables include: two linker lengths, 1-thiomannosides, mono- versus oligo-mannosides and 'valencies' ranging from two to sixty-four. The mannodendrimers were compared with glycolated dendrimers and all dendrimers investigated lacked significant activity compared to LPS in a DC maturation assay. Mannose coated dendrimers do not appear to induce DC activation required for an immune response in vitro in mice. Other uses for mannose coated dendrimers are being investigated.

4. Experimental

4.1. General methods

Analytical TLC was carried out on pre-coated 0.25 mm thick Merck 60 F_{254} silica gel plates and visualization was by thermal development after dipping in ammonium molybdate and cerium(IV) sulfate in dilute H_2SO_4. Flash column chromatography was conducted using silica gel 60 (40–60 μm). Analytical RP-HPLC was conducted on a Waters Symmetry300™ C18 5 μm, 3.9×150 mm

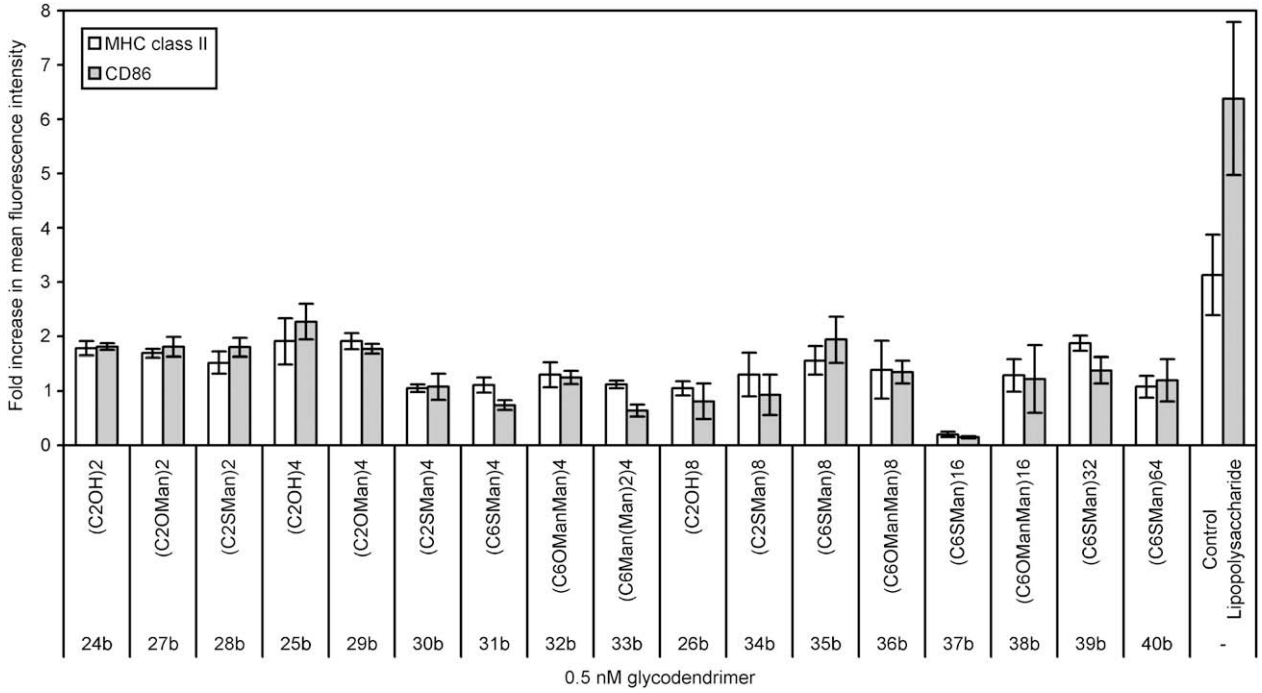


Figure 3. Activation of murine bone marrow derived dendritic cells (BMDCs) in response to glycodendrimer constructs. Results are expressed as fold increase in the mean fluorescence index (MFI) of the sample over the background (unstimulated DCs) following incubation with 0.5 nM of glycodendrimer for 48 h. The results represent the mean±the standard deviation of triplicate experiments.

column eluting with 0.1% trifluoroacetic acid in water/methanol gradients. Melting points were measured on a Reichert hot-stage microscope and are uncorrected. Optical rotations were measured using a Perkin Elmer 241 polarimeter and $[\alpha]_D$ values are reported in 10^{-1} deg cm^2 g^{-1}. Microanalyses were carried out at the Campbell Microanalytical Laboratory, University of Otago, New Zealand. ^{1}H and ^{13}C NMR spectra were recorded at 300 MHz and 75 MHz, respectively, and run in $CDCl_3$ with TMS as an internal standard unless otherwise stated; *J* values are given in hertz. Mass spectra were recorded on a Applied Biosystems Voyager-DE PRO MALDI-TOF (MALDI) and externally calibrated or on a Water Micromass Q-Tof Premier (ESI) mass spectrometer as specified. Dendrimers were named according to the recommendations set out by Friedhoven and Vögtle.[19]

4.2. (Benzyloxycarbonyl)methyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (5)

To a stirred solution of penta-*O*-acetyl-α-D-mannopyranose (**3**) (1.87 g, 4.78 mmol) and benzyl glycolate (**4**) (3.12 g, 18.8 mmol) in CH_2Cl_2 (30 cm^3) at 0 °C under Ar, $BF_3{\cdot}Et_2O$ (1.1 cm^3, 8.94 mmol) was added. After 1 h at 0 °C, the ice bath was removed and the reaction mixture stirred for 72 h, diluted with CH_2Cl_2 and the organic layer washed with satd $NaHCO_3$ (aq), brine, dried ($MgSO_4$) and concentrated in vacuo. Purification of the resulting residue by flash chromatography (80:20 to 50:50 EtOAc/hexane) afforded a colourless oil (1.78 g) contaminated with benzyl (2-acetoxy)-acetate. The crude mixture was taken up in pyridine (3 cm^3) and acetic anhydride (1.5 cm^3) was added, the mixture was stirred overnight and concentrated in vacuo. Purification of the residue by flash chromatography afforded the pure title compound as a colourless oil (1.32 g, 55%). $[\alpha]_D^{18}$ +53.4 (*c* 0.25, $CHCl_3$); Found: C, 55.8; H, 5.9. $C_{23}H_{28}O_{12}$ requires C, 55.6; H, 5.7%; δ_H 7.40–7.31 (5H, m, $C_6H_5CH_2$), 5.41–5.24 (3H, m, H-2, H-3, H-4), 5.19 (2H, br s, $C_6H_5CH_2$), 4.95 (1H, d, *J* 1.2, H-1), 4.30 (1H, d, *J* 16.2, OCH*H*CO_2), 4.24 (1H, dd, *J* 11.9 and 5.1, H-6a), 4.20 (1H, d, *J* 16.2, OC*H*HCO_2), 4.14 (1H, ddd, *J* 9.4, 5.1 and 1.8, H-5), 4.05 (1H, dd, *J* 11.9 and 2.0, H-6b), 2.14 (3H, s, COCH_3), 2.07 (3H, s, COCH_3), 2.03 (3H, s, COCH_3), 1.99 (3H, s, COCH_3); δ_C 170.4, 169.6, 169.6, 168.8, 135.0, 128.6, 128.5, 128.3, 97.9 ($J_{C\text{-}1,H\text{-}1}$ 173.8), 69.1, 68.8, 66.8, 65.8, 64.6, 62.2, 20.7, 20.5, 20.5; *m*/*z* (ESI) 514.1892 ($[M+NH_4]^+$. $C_{23}H_{32}O_{12}N$ requires 514.1919).

4.3. (*N*-Succinimidyloxycarbonyl)methyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (7)

To a stirred solution of glycoside **5** (1.32 g, 2.66 mmol) in EtOAc (85 cm^3) was added 10% Pd on carbon (10%, 525 mg). The flask was flushed with hydrogen several times and a hydrogen balloon was fitted. After 2 h, the reaction mixture was diluted with EtOAc and filtered through Celite. The solids were washed with EtOAc and the filtrate and washings were concentrated in vacuo to give carboxymethyl tetra-*O*-acetyl-α-D-mannopyranoside (**6**) (594 mg, 55%). R_f 0.21 (0.1% AcOH in ethyl acetate); δ_H 7.99 (1H, br s, CO_2H), 5.41–5.33 (2H, m, H-2, H-3), 5.28 (1H, dd, *J* 9.6 and 9.6, H-4), 4.93 (1H, br s, H-1), 4.30–4.08 (H-5, m, 5H, H-6a, H-6b, OCH_2CO), 2.15 (3H, s, COCH_3), 2.09 (3H, s, COCH_3), 2.05 (3H, s, COCH_3), 1.99 (3H, s, COCH_3); δ_C 174.0, 170.9, 170.2, 169.9, 169.7, 97.7, 69.4, 68.8, 68.8, 66.2, 65.1, 62.5, 20.8, 20.6, 20.6.

To a stirred solution of acid **6** (682 mg, 1.68 mmol) dissolved in EtOAc (35 cm^3), *N*-hydroxysuccinimide (200 mg, 1.74 mmol) was added, followed by DCC (360 mg, 1.74 mmol). The reaction mixture was stirred for 2 h and was filtered. The solids were washed with EtOAc and the filtrate and washings were concentrated in vacuo. The residue was taken up in cold CH_2Cl_2, the solution was filtered and the filtrate concentrated in vacuo to give the title compound as a colourless gum (861 mg, 98%) containing dicyclohexylurea (approx. 9% w/w). R_f 0.62 (0.1% AcOH in EtOAc); $[\alpha]_D^{22}$ +48.8 (*c* 0.25, $CHCl_3$); δ_H 5.38–5.26 (3H, m, H-2, H-3, H-4), 4.97 (d, *J* 1.3, 1H, H-1), 4.63 (1H, d, *J* 16.9, OC*H*HCO), 4.53 (1H, d, *J* 16.9, OCH*H*CO), 4.28 (1H, dd, *J* 12.2 and 4.7, H-6a), 4.16–4.06 (2H, m, H-5, H-6b), 2.88–2.80

(4H, m, CO(CH_2)$_2$CO), 2.14 (3H, s, COCH_3), 2.09 (3H, s, COCH_3), 2.03 (3H, s, COCH_3), 1.98 (3H, s, COCH_3); δ_C 170.5, 169.6, 169.5, 168.4, 164.7, 98.2 ($J_{C\text{-}1,H\text{-}1}$ 172.9), 69.4, 68.9, 68.6, 65.6, 62.6, 62.0, 25.4, 20.6, 20.5, 20.5; *m*/*z* (ESI) 521.25 ([M+NH_4]$^+$. $C_{20}H_{29}N_2O_{14}$ requires 521.16).

4.4. Carboxymethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside (10)

Penta-*O*-acetyl-α-D-mannopyranose (**3**) (11.6 g, 30 mmol, azeotropically dried with toluene 2×50 cm^3) and thiourea (2.49 g, 33 mmol,) were suspended in MeCN (50 cm^3, anhydrous grade). $BF_3\cdot Et_2O$ (13.2 cm^3, 14.8 g, 104 mmol) was added and the mixture heated under reflux for 2.5 h. The reaction solution was cooled to 0 °C and Et_3N (18.5 cm^3, 13.5 g, 134 mmol, 4.5 mol equiv) was added dropwise over 15 min, followed by iodoacetic acid (6.08 g, 33 mmol, 1.1 mol equiv). The reaction mixture was stirred at rt overnight, and concentrated to a viscous, oily slurry by rotary evaporation. The residue was partitioned between H_2O (350 cm^3) and CH_2Cl_2 (200 cm^3), the organic layer was separated and the aqueous layer extracted with further CH_2Cl_2 (4×100 cm^3). The combined organic extracts were washed with H_2O (200 cm^3), brine (200 cm^3), dried ($MgSO_4$), filtered and concentrated in vacuo. This crude product was purified by flash chromatography (98:0.25:2 to 98:0.85:2 CH_2Cl_2/MeOH/AcOH) to give fractions that were taken to dryness and then evaporated with toluene (×2), to give acid **10** as a pale yellow oil (6.0 g, 48%). R_f 0.57 (94:5:1, CH_2Cl_2/MeOH/AcOH); $[\alpha]_D^{24}$ +109.5 (*c* 1.0 in $CHCl_3$); (Found: C, 45.2; H, 5.4; S, 7.3. $C_{16}H_{22}O_{11}S$ requires C, 45.5; H, 5.25; S, 7.6%); δ_H 5.45 (1H, d, *J* 1.1, H-1), 5.39 (1H, dd, *J* 3.3 and 1.4, H-2), 5.34 (1H, dd, *J* 10.0 and 9.5, H-4), 5.25 (1H, dd, *J* 10.0 and 3.4, H-3), 4.37 (1H, ddd, *J* 9.5, 4.9 and 2.2, H-5), 4.30 (1H, dd, *J* 12.2 and 4.9, H-6a), 4.08 (1H, dd, *J* 12.1 and 2.2, H-6b), 3.52 (1H, d, *J* 15.6, SCH*H*CO$_2$), 3.32 (1H, d, *J* 15.6, SC*H*HCO$_2$), 2.17 (3H, s, COCH_3), 2.10 (3H, s, COCH_3), 2.06 (3H, s, COCH_3), 1.99 (3H, s, COCH_3); δ_C 174.3, 171.3, 170.3, 170.3, 170.1, 82.6 ($J_{C\text{-}1,H\text{-}1}$ 171.0), 70.6, 69.8, 69.8, 66.5, 62.7, 32.2, 21.2, 21.1, 21.0, 20.9; *m*/z (ESI$^-$) 421.078 ([M−H]$^+$. $C_{16}H_{21}O_{11}S$: requires 421.080).

4.5. (*N*-Succinimidyloxycarbonyl)methyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside (11)

Acid **10** (5.75 g, 14 mmol, azeotropically dried with toluene) was dissolved in EtOAc (40 cm^3, dried over K_2CO_3) and the solution was cooled to 0 °C. *N*-Hydroxysuccinimide (1.57 g, 14 mmol) and then DCC (2.81 g, 14 mmol) were added and the mixture was stirred at rt for 1 h. The reaction mixture was filtered, the filtrate taken to dryness under reduced pressure, dissolved in CH_2Cl_2 (15 cm^3, dried over K_2CO_3) and filtered again. The filtrate was concentrated to give the title compound **11** as a pale yellow foam (6.95 g, 98%); ^{1}H NMR indicated contamination with 5–10% w/w of dicyclohexylurea. R_f 0.60 (95:5, CH_2Cl_2/MeOH); δ_H 5.52 (1H, d, *J* 1.4, H-1), 5.41–5.33 (2H, m, H-2, H-4), 5.23 (1H, dd, *J* 10.0 and 3.5, H-3), 4.41–4.32 (2H, m, H-5, H-6a), 4.03 (1H, m, H-6b), 3.74 (1H, d, *J* 15.8, SCH*H*CO), 3.53 (1H, d, *J* 15.8, SC*H*HCO), 2.85 (4H, s, (CH_2)$_2$), 2.17 (3H, s, COCH_3), 2.11 (3H, s, COCH_3), 2.06 (3H, s, COCH_3), 2.00 (3H, s, COCH_3); δ_C 171.0, 170.2, 170.1, 170.0, 169.1, 165.5, 82.8 (C-1, $J_{C\text{-}1,H\text{-}1}$ 170.5), 70.3, 70.0, 69.7, 66.3, 62.4, 29.5, 25.9, 21.2, 21.1, 21.1, 21.0; *m*/*z* (ESI) 537.1377 ([M+NH_4]$^+$. $C_{20}H_{29}N_2O_{13}S$ requires 537.1385).

4.6. 2,2,2-Trichloroethyl 6-bromohexanoate (9)

A solution of 6-bromohexanoic acid (5.92 g, 30.3 mmol), 2,2,2-trichloroethanol (6.8 g, 45 mmol) and *p*-TsOH (250 mg) in benzene (100 cm^3) was heated under reflux using a Dean–Stark trap for 30 h to remove H_2O. Upon cooling, the mixture was diluted with ether (100 cm^3) and washed with satd $NaHCO_3$ (50 cm^3, aq) then H_2O (20 cm^3) and brine (20 cm^3). The organic layer was dried ($MgSO_4$) and the volatiles were removed. Distillation of the product gave the title compound as a colourless oil (8.82 g, 89%) with data consistent with the literature.[20] Bp 124 °C, 1.0 mmHg; δ_H 4.74 (2H, s, OCH_2CCl$_3$), 3.41 (2H, t, *J* 6.6, CH_2Br), 2.49 (2H, t, *J* 7.4, CH_2CO), 1.88 (2H, tt, *J* 6.9 and 6.7), 1.74 (2H, tt, *J* 6.3 and 6.3), 1.58–1.48 (2H, m); δ_C 172.1, 95.3, 74.3, 34.0, 33.6, 32.6, 27.9, 24.2.

4.7. 5-(2,2,2-Trichloroethoxycarbonyl)pentyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside (12)

To a stirred solution of penta-*O*-acetyl-α-D-mannopyranose (**3**) (9.1 g, 23 mmol) in dry CH_3CN (50 cm^3), thiourea (1.92 g, 25 mmol) and $BF_3\cdot Et_2O$ (11.5 g, 81 mmol) were added. The mixture was heated under reflux for 3 h then cooled to 0 °C. Et_3N (10.5 g, 103 mmol) was slowly added over 15 min followed by 2,2,2-trichloroethyl 6-bromohexanoate (**9**) (8.2 g, 25.2 mmol). The reaction mixture was stirred for 16 h at rt and H_2O (100 cm^3) was added. The aqueous layer was extracted with $CHCl_3$ (2×150 cm^3) and the combined organic layers were dried ($MgSO_4$), concentrated in vacuo and the residue was purified by flash chromatography to give the title compound (**12**) as a crystalline colourless solid (4.8 g, 35%). Mp 77.1–79.4 °C; R_f 0.56 (60:40, hexane/EtOAc); $[\alpha]_D^{22}$ +66.2 (*c* 1.0, MeOH); δ_H 5.34–5.24 (4H, m, H-1, H-2, H-3, H-4), 4.75 (2H, s, CH_2CCl$_3$), 4.37–4.29 (2H, m, H-5, H-6a), 4.10 (1H, dd, *J* 11.6 and 2.0, H-6b), 2.71–2.53 (2H, m, SCH_2), 2.48 (2H, t, *J* 7.1, CH_2CO), 2.16 (3H, s, CH_3CO), 2.10 (3H, s, CH_3CO), 2.05 (3H, s, CH_3CO), 1.99 (3H, s, CH_3CO), 1.76–1.62 (4H, m), 1.52–1.43 (2H, m); δ_C 172.0, 170.8, 170.3, 170.0, 170.0, 95.4, 82.8 ($J_{C\text{-}1,H\text{-}1}$ 166.95), 42.2, 71.5, 69.8, 69.3, 66.7, 62.8, 34.0, 31.3, 29.3, 28.4, 24.5, 21.2, 21.0, 20.9, 20.9; *m*/*z* (ESI) 626.0968 ([M+NH_4]$^+$. $C_{22}H_{35}Cl_3NO_{11}S$ requires 626.0990).

4.8. 5-Carboxypentyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside (13)

To a suspension of zinc dust (1.5 g) in acetic acid (10 cm^3), a solution of mannopyranoside **12** (592 mg, 1 mmol) in acetic acid (5 cm^3) was added. The reaction mixture was stirred for 6 h, filtered and the filtered solids were washed with EtOAc. The filtrate and washings were concentrated and the residue was purified by flash chromatography to give the title compound **13** as a crystalline white solid (471 mg, 98%). Mp 90.5–92.7 °C; R_f 0.05 ($CHCl_3$); $[\alpha]_D^{22}$ +79.1 (*c* 1.0, MeOH); δ_H 8.70 (1H, br s, CO$_2$*H*), 5.27–5.17 (4H, m, H-1, H-2, H-3, H-4), 4.40–4.35 (1H, br m, H-5), 4.32 (1H, dd, *J* 12.0 and 4.8, H-6a), 4.11 (1H, dd, *J* 12.0 and 1.8, H-6b), 2.73–2.54 (2H, m, SCH_2CH$_2$), 2.37 (2H, t, *J* 7.5, CH_2CO$_2$H), 2.17 (3H, s, COCH_3), 2.10 (3H, s, COCH_3), 2.06 (3H, s, COCH_3), 2.00 (3H, s, COCH_3), 1.73–1.60 (4H, m, SCH$_2$CH_2, CH_2CH$_2$CO$_2$), 1.51–1.39 (2H, m, CH$_2$CH_2CH$_2$); δ_C 178.3, 170.6, 170.0, 169.8, 169.7, 82.4, 77.4, 77.0, 76.5, 71.1, 69.4, 68.9, 66.3, 62.4, 33.6, 31.0, 28.9, 28.0, 24.0, 20.8, 20.6, 20.5; *m*/*z* (ESI) 496.1829 ([M+NH_4]$^+$. $C_{20}H_{34}NO_{11}S$ requires 496.1847).

4.9. 5-(*N*-Succinimidyloxycarbonyl)pentyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside (14)

To a stirred solution of the acid **13** (455 mg, 0.95 mmol) in EtOAc (3 cm^3), *N*-hydroxysuccinimide (114 mg, 0.99 mmol) followed by DCC (204 mg, 0.99 mmol) were added. The resulting solution was stirred for 3 h and filtered through cotton wool. Evaporation of the solvents and purification by flash chromatography afforded the reactive ester **14** as a colourless oil, which slowly crystallized upon standing (439 mg, 81%). Mp 83–84 °C (from CH_2Cl_2/hexane/ether); R_f 0.50 (30:70, hexane/EtOAc); $[\alpha]_D^{22}$ +73.7 (*c* 1.2, $CHCl_3$); Found: C, 50.25; H, 5.8; N, 2.4. $C_{24}H_{33}O_{13}NS$ requires C, 50.1; H, 5.8; N, 2.4%; δ_H 5.33–5.25 (4H, m, H-1, H-2, H-3, H-4), 4.39–4.33 (1H, m, H-5), 4.31 (1H, dd, *J* 12.0 and 5.4, H-6a), 4.09 (1H, dd, *J* 12.0 and 3.9, H-6b), 2.83

(4H, s, CO(CH_2)$_2$CO), 2.72–2.54 (2H, m, SCH_2), 2.61 (2H, t, *J* 6.9, CH_2CO_2N), 2.16 (3H, s, COCH_3), 2.09 (3H, s, COCH_3), 2.09 (3H, s, COCH_3), 1.98 (3H, s, COCH_3), 1.79 (2H, tt, *J* 7.2 and 7.5), 1.68 (2H, tt, *J* 7.2 and 7.2), 1.57–1.46 (2H, m, $CH_2CH_2CH_2$); δ_C 170.9, 170.3, 170.1, 170.1, 169.4, 82.8, 71.5, 69.8, 69.3, 66.7, 62.8, 31.2, 31.1, 29.1, 28.1, 25.9, 24.4, 21.2, 21.0, 21.0, 20.9; *m*/*z* (ESI) 593.1981 ($[M+NH_4]^+$. $C_{24}H_{37}O_{13}N_2S$ requires 593.2010).

4.10. 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (16)

To a stirred solution peracetylated α(1→6)-mannobiose **15**[21] (6.0 g, 8.8 mmol) in DMF (15 cm^3), hydrazine acetate (978 mg, 10.6 mmol) was added. The reaction mixture was warmed at 50 °C for 30 min and an additional portion of hydrazine acetate (100 mg) was added. After a further 30 min, the reaction mixture was cooled to rt and H_2O (100 cm^3) was added. The aqueous mixture was extracted with EtOAc (150 cm^3) and the organic extracts were washed with $NaHCO_3$ (2×50 cm^3, aq) and then NaCl solution (10 cm^3, aq). The combined aqueous washings were extracted with EtOAc (20 cm^3) and the combined organic extracts were dried ($MgSO_4$) and concentrated to give tetra-*O*-acetyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-α,β-D-mannopyranose as a colourless foam (5.49 g, 98% corrected for DMF and EtOAc content). δ_H (major anomer) 5.44 (1H, dd, *J* 10.1 and 3.3), 5.37 (1H, dd, *J* 9.7 and 3.4), 5.32–5.19 (5H, m), 4.86 (1H, d, *J* 1.3), 4.28–4.04 (4H, m), 3.96 (1H, br s), 3.78 (1H, dd, *J* 11.7 and 6.3, H-6a), 3.61 (1H, dd, *J* 11.7 and 2.6, H-6b), 2.16 (3H, s, COCH_3), 2.16 (3H, s, COCH_3), 2.11 (3H, s, COCH_3), 2.07 (3H, s, COCH_3), 2.06 (3H, s, COCH_3), 2.00 (3H, s, COCH_3), 1.99 (3H, s, COCH_3). This 1-hydroxy compound (5.49 g, 8.66 mmol) was dissolved in CH_2Cl_2 (14 cm^3) and trichloroacetonitrile (1.52 g, 10.5 mmol) in DBU (133 mg, 0.88 mmol) was added. The reaction mixture was allowed to stir at rt for 30 min and, without evaporation, was applied to a column of silica gel and purified by flash chromatography to give, after removal of the solvent, the α-trichloroacetimidate **16** as a colourless foam (5.10 g, 76%). R_f 0.53 (40:60, hexane/EtOAc); $[\alpha]_D^{22}$ +57.7 (*c* 1.0, $CHCl_3$); δ_H 8.82 (1H, s, C(N*H*)CCl_3), 6.23 (1H, d, *J* 1.8, H-1), 5.47 (1H, dd, *J* 2.6 and 2.0, H-2), 5.44–5.34 (2H, m), 5.31–5.23 (3H, m), 4.82 (1H, d, *J* 1.3, H-1′), 4.28 (1H, dd, *J* 12.5 and 5.2, H-6a′), 4.17–4.06 (3H, m, H-5, H-5′, H-6b′), 3.78 (1H, dd, *J* 11.0 and 5.4, H-6a), 3.63 (1H, dd, *J* 11.0 and 2.4, H-6b), 2.20 (3H, s, COCH_3), 2.14 (3H, s, COCH_3), 2.10 (3H, s, COCH_3), 2.08 (3H, s, COCH_3), 2.04 (3H, s, COCH_3), 2.01 (3H, s, COCH_3), 1.97 (3H, s, COCH_3); δ_C 170.6, 179.9, 169.8, 169.6, 169.6, 159.7, 97.5 ($J_{C\text{-}1,H\text{-}1}$ 173.9), 94.3 ($J_{C\text{-}1,H\text{-}1}$ 180.2), 90.5, 71.9, 69.4, 68.9, 68.8, 68.5, 67.9, 66.3, 66.0, 65.8, 62.3, 20.8, 20.7, 20.7, 20.6; *m*/*z* (ESI) 802.0889 ($[M+Na]^+$. $C_{28}H_{36}NaO_{18}$ requires 802.0896).

4.11. 5-(Methoxycarbonyl)pentyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-α-D-mannopyranoside (17)

To a stirred solution of trichloroacetimidate **16** (2.12 g, 2.71 mmol) and methyl 6-hydroxyhexanoate (790 mg, 5.4 mmol) in CH_2Cl_2 (21 cm^3), activated 4 Å molecular sieves (2.5 g) were added. The reaction mixture was stirred at rt for 45 min and then cooled to −40 °C and TMSOTf (71 mg, 0.32 mmol) was added. After 15 min, the cold bath was removed and the mixture was allowed to warm to rt. Stirring was continued for 1 h, CH_2Cl_2 (25 cm^3) was added, the reaction was quenched with NEt_3 and the mixture filtered through Celite. The solids were washed with CH_2Cl_2 and the filtrate and washings were taken to dryness and the residue was taken up in pyridine (5 cm^3). Acetic anhydride (5 cm^3) was added and the solution was left for 16 h. The volatiles were removed under high vacuum and the residue was purified by flash chromatography to give the title glycoside **17** as a colourless gum (1.46 g, 71%). R_f 0.40 (30:70, hexane/EtOAc); $[\alpha]_D^{22}$ +58.7 (*c* 1.16, $CHCl_3$); δ_H 5.38–5.21 (6H, m, H-2, H-3, H-4, H-2′, H-3′, H-4′), 4.87 (1H, d, *J* 1.3, H-1 or H-1′), 4.78 (1H, d, *J* 1.4, H-1 or H-1′), 4.26 (1H, dd, *J* 12.1 and 5.3, H-6a′), 4.14 (1H, dd, *J* 12.1 and 2.1, H-6b′), 4.11–4.05 (1H, m, H-5′), 3.95 (1H, ddd, *J* 9.5, 5.7 and 2.4, H-5), 3.78 (1H, dd, *J* 10.9 and 5.8, H-6a), 3.73 (1H, ddd, *J* 9.5, 6.5 and 6.5, OC*H*HCH_2), 3.68 (3H, s, CO_2CH_3), 3.57 (1H, dd, *J* 10.9 and 2.2, H-6b), 3.46 (1H, ddd, *J* 9.5, 6.5 and 6.5, OCH*H*CH_2), 2.34 (2H, t, *J* 7.5), 2.17 (3H, s), 2.16 (3H, s, COCH_3), 2.12 (3H, s, COCH_3), 2.07 (3H, s, COCH_3), 2.05 (3H, s, COCH_3), 2.00 (3H, s, COCH_3), 1.99 (3H, s, COCH_3), 1.71–1.61 (4H, m, $CH_2CH_2CH_2$), 1.47–1.37 (2H, m, $CH_2CH_2CH_2$); δ_C 174.0, 170.5, 170.1, 169.8, 169.8, 169.7, 169.5, 97.4 ($J_{C\text{-}1,H\text{-}1}$ 171), 97.2 ($J_{C\text{-}1,H\text{-}1}$ 171), 69.6, 69.4, 69.2, 69.2, 68.9, 68.6, 68.0, 66.6, 66.6, 66.0, 62.4, 51.4, 33.8, 28.9, 25.6, 24.6, 20.7, 20.6; *m*/*z* (ESI) 787.2639 ($[M+Na]^+$. $C_{33}H_{48}NaO_{20}$ requires 787.2637).

4.12. 5-(Methoxycarbonyl)pentyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)]-3,4-di-*O*-acetyl-α-D-mannopyranoside (20)

The tetrasaccharide by-product was also isolated from the first attempt at the above reaction. R_f 0.17 (30:70, hexane/ethyl acetate); $[\alpha]_D^{22}$ +59.6 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.44–5.24 (m, 9H), 5.23 (dd, *J* 3.0 and 1.8, 1H), 5.16 (dd, *J* 9.6 and 9.6, 1H), 4.95 (d, *J* 1.7, 1H), 4.87 (d, *J* 1.2, 1H), 4.84 (d, *J* 1.5, 1H), 4.81 (d, *J* 1.5, 1H), 4.30 (dd, *J* 8.3 and 5.0, 1H), 4.27 (dd, *J* 8.6 and 5.2, 1H), 4.18–4.00 (m, 7H), 3.93 (ddd, *J* 9.7, 6.6 and 2.4, 1H), 3.85–3.69 (m, 3H), 3.67 (s, 3H), 3.60 (dd, *J*=11.6 and 2.2, 1H), 3.56 (dd, *J*=10.8 and 2.6, 1H), 3.51–3.42 (m, 1H), 2.37–2.29 (m, 2H), 2.17 (s, 3), 2.15 (s, 3H), 2.15 (s, 3H), 2.13 (s, 3H), 2.10 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.73–1.59 (m, 4H), 1.47–1.35 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.9, 170.6, 170.5, 170.2, 170.0, 169.9, 169.8, 169.7, 169.6, 169.4, 98.8, 98.3, 98.0, 97.3, 70.5, 70.0, 69.5, 69.2, 69.0, 68.9, 68.6, 67.9, 67.4, 67.3, 66.3, 66.1, 65.9, 62.4, 62.2, 51.4, 33.8, 29.0, 25.6, 24.6, 20.8, 20.6; *m*/*z* (MALDI-TOF) 1363.50 ($[M+Na]^+$. $C_{57}H_{80}Na_1O_{36}$ requires 1363.43).

4.13. 5-Carboxypentyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-α-D-mannopyranoside (18)

Mannopyranoside **17** (850 mg, 1.11 mmol) was dissolved in dry MeOH (10 cm^3) and 30% sodium methoxide in MeOH (seven drops) was added. The reaction mixture was stirred at rt for 30 min and then concentrated to 1 cm^3 under reduced pressure. The solution was diluted with H_2O (10 cm^3) and NaOH (10 cm^3, 2 M) was added. The mixture was stirred overnight and neutralized with Amberjet 1200H resin. After filtration, the filtrate was concentrated in vacuo to give a colourless foam, which was dissolved in pyridine (5 cm^3). Acetic anhydride (3.96 g, 38.8 mmol) was added and the solution was left overnight. The volatiles were removed under high vacuum and the residue taken up in $CHCl_3$ (50 cm^3) and washed with HCl (2×30 cm^3, 1.0 M). The acidic washings were extracted with $CHCl_3$ (50 cm^3), the extracts were dried ($MgSO_4$), filtered and concentrated in vacuo to give the title compound **18** as a colourless foam (810 mg, 97%). R_f 0.30 (hexane/EtOAc/AcOH, 40:60:1); $[\alpha]_D^{22}$ +58.9 (*c* 1.20, $CHCl_3$); δ_H 5.38–5.19 (6H, m), 4.86 (1H, d, *J* 1.4), 4.78 (1H, d, *J* 1.4), 4.26 (1H, dd, *J* 12.3 and 5.1), 4.15 (1H, dd, *J* 12.2 and 2.2), 4.09 (1H, ddd, *J* 9.0, 5.1 and 2.0), 3.96 (1H, ddd, *J* 9.0, 5.8 and 2.2), 3.82–3.69 (2H, m), 3.56 (1H, dd, *J* 10.8 and 2.4), 3.52–3.44 (1H, m), 2.39 (2H, t, *J* 7.1), 2.17 (3H, s), 2.17 (3H, s), 2.12 (3H, s), 2.07 (3H, s), 2.05 (3H, s), 2.01 (3H, s), 1.99 (3H, s), 1.78–1.62 (4H, m), 1.52–1.40 (2H, m); δ_C 178.0, 170.6, 170.1, 170.0, 169.9, 169.8, 169.7, 169.7, 97.4,

97.2, 69.6, 69.4, 69.2, 69.2, 68.9, 68.6, 67.9, 66.4, 66.4, 66.0, 62.4, 33.6, 28.8, 25.4, 24.3, 20.8, 20.8, 20.6, 20.6, 20.6, 20.6, 20.5; *m*/*z* (ESI) 749.2547 ($[M-H]^-$. $C_{32}H_{45}O_{20}$ requires 749.2510).

4.14. 5-(*N*-Succinimidyloxycarbonyl)pentyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-α-D-mannopyranoside (19)

To a stirred solution of acid **18** (810 mg, 1.07 mmol) in EtOAc (8 cm^3), *N*-hydroxysuccinimide (147 mg, 1.28 mmol) followed by DCC (263 mg, 1.28 mmol) were added. The reaction mixture was stirred for 16 h, filtered to remove precipitated urea and the filtrate was taken to dryness under reduced pressure. Purification of the residue by flash chromatography (hexane/EtOAc, 30:70) afforded title compound **19** as a colourless foam (622 mg, 69%). R_f 0.34 (30:70, hexane/ethyl acetate); δ_H 5.38–5.20 (6H, m), 4.86 (1H, d, *J* 1.4), 4.78 (1H, d, *J* 1.4), 4.26 (1H, dd, *J* 11.9 and 5.2), 4.16 (1H, dd, *J* 11.9 and 2.2), 4.09 (1H, ddd, *J* 9.6, 5.3 and 2.1), 3.96 (1H, ddd, *J* 9.4, 6.0 and 2.3), 3.82–3.70 (2H, m), 3.57 (1H, dd, *J* 10.7 and 2.3), 3.52–3.44 (1H, m), 2.84 (4H, br s), 2.65 (2H, t, *J* 7.2), 2.16 (3H, s), 2.16 (3H, s), 2.12 (3H, s), 2.07 (3H, s), 2.05 (3H, s), 2.01 (3H, s), 1.99 (3H, s), 1.81 (2H, pent, *J* 7.0), 1.74–1.63 (2H, m), 1.59–1.47 (2H, m); δ_C 170.5, 170.1, 169.8, 169.6, 169.1, 168.4, 97.3, 97.2, 69.6, 69.4, 69.2, 68.8, 68.5, 67.7, 66.6, 66.5, 66.0, 62.4, 30.7, 28.6, 25.5, 25.2, 24.2, 20.7, 20.6, 20.5; *m*/*z* (ESI) 870.2633 ($[M+Na]^+$. $C_{36}H_{45}NNaO_{22}$ requires 870.2644).

4.15. 5-(*N*-Succinimidyloxycarbonyl)pentyl 2,4-di-*O*-acetyl-3,6-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (23)

A solution of 5-carboxypentyl (α-D-mannopyranosyl)-(1→3)-[(α-D-mannopyranosyl)-(1→6)]-α-D-mannopyranoside (**21**)[14] (300 mg, 0.48 mmol) was dissolved in pyridine (5 cm^3) and acetic anhydride (5 cm^3) was added. The solution was stirred for 16 h and the solvents were removed under reduced pressure. The residue was dissolved in $CHCl_3$ (30 cm^3) and washed with HCl (2×15 cm^3, M) and then H_2O (15 cm^3). The organic layer was dried and the volatiles were removed under reduced pressure to give crude 5-carboxypentyl 2,4-di-*O*-acetyl-3,6-di-*O*-(tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**22**) (466 mg, 93%). To a stirred solution of this material (215 mg, 0.20 mmol) and *N*-hydroxysuccinimide (36 mg, 0.31 mmol) in EtOAc (3 cm^3), a solution of DCC (64 mg, 0.31 mmol) in EtOAc (0.5 cm^3) was added. The reaction mixture was stirred for 16 h and then filtered through cotton wool. The volatiles were removed under reduced pressure and the product was purified by flash chromatography to give the title compound **23** as a foam upon drying under high vacuum (218 mg, 93%). R_f 0.50 (EtOAc/hexane, 90:10); $[\alpha]_D^{22}$ +36.2 (*c* 0.51, $CHCl_3$); δ_H 5.34–5.12 (7H, m), 5.05–5.00 (2H, m), 4.82 (1H, d, *J* 1.6), 4.79 (1H, d, *J* 1.5), 4.33–4.02 (7H, m), 3.90–3.70 (3H, m), 3.54–3.42 (2H, m), 2.83 (4H, br s), 2.63 (2H, t, *J* 7.2), 2.20 (3H, s), 2.14 (3H, s), 2.13 (3H, s), 2.13 (3H, s), 2.10 (3H, s), 2.05 (3H, s), 2.20 (6H, s), 1.98 (3H, s), 1.97 (3H, s), 1.78 (2H, tt, *J* 7.5 and 7.2), 1.71–1.59 (2H, m), 1.57–1.45 (2H, m); δ_C 170.9, 170.8, 170.5, 170.3, 170.1, 170.0, 169.5, 168.8, 99.2, 97.5, 75.2, 71.4, 70.3, 69.8, 69.2, 68.9, 68.7, 68.0, 67.0, 66.4, 66.3, 62.7, 31.2, 29.1, 25.9, 25.6, 24.6, 21.2, 21.0, 21.0; *m*/*z* (ESI) 1153.3876 ($[M+NH_4]^+$. $C_{48}H_{69}O_{30}N_2$ requires 1153.3929).

4.16. 1-Benzhydryl-L-lysinamide(*N*,*N*′):glycolyl$_2$-cascadane (24b)

To dendrimer [**G0**]–$[HCl]_2$ (105 mg, 0.273 mmol) and diisopropylethylamine (340 μL, 1.92 mmol) in anhydrous DMF (5 cm^3), a solution of the reactive ester **2** (160 mg, 0.744 mmol) in anhydrous DMF (5 cm^3) was added. After 2 h at rt, the solution was concentrated in vacuo, taken up in water and extracted with ether (×3). The combined organic extracts were concentrated in vacuo, dried and purified by flash silica chromatography (CH_2Cl_2/MeOH, 24:1) to give acetylated intermediate **24a** (59 mg); 55 mg of this material was dissolved in MeOH (10 cm^3) and the pH was adjusted to 12 with NaOMe in MeOH (30%). After 2 h, silica was added, the mixture concentrated in vacuo and purified by flash silica chromatography (CH_2Cl_2/MeOH, 9:1) to give the title compound as a white amorphous solid (39 mg, 84%). $[\alpha]_D^{20}$ −7.3 (*c* 0.52, MeOH); δ_H ($CDCl_3$/CD_3OH, 4:6) 7.36–7.22 (10H, m, Ph), 6.19 (1H, s, CHPh), 4.54 (1H, dd, *J* 5.9 and 7.8, NHC*H*CO), 4.02 (2H, s, CH_2OH), 3.97 (2H, s, CH_2OH), 3.20 (2H, dt, *J* 1.8 and 6.9), 1.88–1.66 (2H, m), 1.58–1.49 (2H, m), 1.40–1.29 (2H, m); δ_C ($CDCl_3$/CD_3OH, 4:6) 175.3, 175.2, 173.3, 143.1, 142.9, 130.2, 130.1, 129.2, 129.1, 129.1, 129.0, 63.2 (CH_2OH), 58.7 ($CHPh_2$), 54.2 (NHCHCO), 40.1, 34.0, 30.5, 24.3; *m*/*z* (ESI) 450.2000 ($[M+Na]^+$. $C_{23}H_{29}N_3NaO_5$ requires 450.2005).

4.17. 1-Benzhydryl-L-lysinamide(*N*,*N*′):{L-lysinyl(*N*,*N*′)}$^{G1}_{2x}$: glycolyl$_4$-cascadane (25b)

To dendrimer [**G1**]–$[HCl]_4$ (127 mg, 0.178 mmol) and diisopropylethylamine (404 μL, 2.31 mmol) in anhydrous DMF (2 cm^3), a solution of the reactive ester **2** (169 mg, 0.783 mmol) in anhydrous DMF (1 cm^3) was added. After 16 h at rt, the solution was concentrated in vacuo, taken up in water and extracted with $CHCl_3$ (×3). The combined organic extracts were concentrated in vacuo, dried and purified by flash silica chromatography ($CHCl_3$/MeOH, 19:1) to give acetylated intermediate **25a** (90 mg). MeOH (1 cm^3) and CH_2Cl_2 (1 cm^3) were added to half of this material and the pH was adjusted to 12 with NaOMe in MeOH (30%). After 1.5 h, the solution was neutralized with solid CO_2, concentrated in vacuo and purified by flash silica chromatography (CH_3CN/H_2O/concd NH_4OH, 6:1:1) to give the title compound as a white amorphous solid (35 mg, 49%). >99% pure (HPLC, 220 nm); $[\alpha]_D^{21}$ −15.6 (*c* 0.7, MeOH); δ_H (D_2O) 7.31–7.19 (10H, m, Ph), 6.02 (1H, s, CHPh), 4.36 (1H, t, *J* 7.2, NHC*H*CO), 4.29 (1H, t, *J* 7.1, NHC*H*CO), 4.21 (1H, t, *J* 7.1, NHC*H*CO), 4.05 (2H, s, CH_2OH), 4.04 (2H, s, CH_2OH), 3.99 (2H, s, CH_2OH), 3.97 (2H, s, CH_2OH), 3.18–3.03 (6H, m), 1.79–1.59 (6H, br m), 1.50–1.17 (12H, br m); δ_C (D_2O) 175.0, 174.8, 174.0, 173.9, 173.1, 141.2, 141.0, 129.3, 129.2, 128.3, 128.1, 127.9, 127.5, 61.3 (CH_2OH), 57.8 ($CHPh_2$), 54.3 (NHCHCO), 53.9 (NHCHCO), 53.5 (NHCHCO), 39.4, 38.9, 31.4, 31.0, 28.4, 28.2, 22.8, 22.7; *m*/*z* (ESI) 800.4193 ($[M+H]^+$. $C_{39}H_{58}N_7O_{11}$ requires 800.4189).

4.18. 1-Benzhydryl-L-lysinamide(*N*,*N*′):{L-lysinyl(*N*,*N*′)}$^{G1,G2}_{2x,4x}$: glycolyl$_8$-cascadane (26b)

Trifluoroacetic acid (2 cm^3) was added to [**G2**]–$[Boc]_8$ (122 mg, 0.065 mmol) in CH_2Cl_2 (2 cm^3) at 0 °C and the resulting solution was kept for 16 h at rt then dried in vacuo. The residue was azeotropically dried by the addition and evaporation of CH_2Cl_2 several times. To the freshly prepared [**G2**]–$[TFA]_8$ in anhydrous DMF (2 cm^3) were added Et^iPr_2N (362 μL, 2.08 mmol) and reactive ester **2** (223 mg, 1.04 mmol). The reaction mixture became viscous overnight and further anhydrous DMF (2 cm^3) was added. After a further 24 h, the acetylated intermediate **26a** was precipitated by the addition of water, washed with $CHCl_3$ and collected by filtration. The precipitate was suspended in MeOH (2 cm^3) and water (1 cm^3), and NH_4OH (1 cm^3, concd) was added. After 30 min stirring the precipitate had dissolved and after 4 h the solution was concentrated in vacuo and the residue purified by flash silica gel chromatography ($CHCl_3$/MeOH/concd NH_4OH/H_2O, 60:35:5:3) to give the capped dendrimer **26b** as a white solid (29 mg, 29%). $[\alpha]_D^{18}$ −32.7 (*c* 0.5, H_2O); δ_H (D_2O) 7.37–7.24 (10H, m, Ph), 6.07 (1H, s, CHPh), 4.66–4.14 (7H, m, NHC*H*CO), 4.09 (8H, s, CH_2OH), 4.01 (8H, s, CH_2OH), 3.20–3.03 (14H, m), 1.77–1.63 (14H, br m), 1.51–1.39 (14H, br m), 1.39–1.15 (14H, br m); δ_C (D_2O) 175.0, 174.9, 174.1, 173.9, 173.1,

141.2, 141.0, 129.3, 129.2, 128.3, 128.2, 127.9, 127.6, 61.4, 57.8, 54.4, 54.2, 53.8, 53.7, 53.5, 39.4, 39.0, 31.4, 31.0, 28.4, 28.2, 22.8; *m*/*z* (ESI) 772.9117 ($[M+2H]^+$. $C_{71}H_{115}N_{15}O_{23}$ requires 772.9140).

4.19. 1-Benzhydryl-L-lysinamide(*N*,*N*′):1-oxo-2-(α-D-mannopyranosyloxy)ethyl$_2$-cascadane (27b)

To a stirred solution of [**G0**]–$[HCl]_2$ (46 mg, 0.12 mmol) in DMF (5 cm^3), reactive ester **7** (148 mg, 0.29 mmol) and then diisopropylamine (0.15 cm^3, 0.11 g, 0.85 mmol) were added. The reaction mixture was stirred at rt for 16 h and then diluted with H_2O. The aqueous mixture was extracted with ether (×2) and the combined organic extracts were washed with H_2O, dried and concentrated in vacuo. Purification of the residue by flash chromatography ($CHCl_3$/MeOH, 99:1 to 95:5) gave the acetylated intermediate **27a** as a colourless foam (59 mg, 45%). $[\alpha]_D^{22}$ +36.7 (*c* 0.98, $CHCl_3$); δ_H 7.43 (1H, d, *J* 7.8), 7.37–7.19 (10H, m), 7.10 (1H, d, *J* 8.1), 6.58 (1H, dd, *J* 5.8 and 5.8), 6.25 (1H, d, *J* 8.2), 5.38–5.23 (6H, m), 4.88 (1H, d, *J* 1.2), 4.85 (1H, br s), 4.54 (1H, ddd, *J* 7.9, 7.5 and 6.2), 4.32–4.23 (2H, m), 4.19–3.93 (8H, m), 3.41–3.28 (1H, m), 3.23–3.12 (1H, m), 2.15 (3H, s), 2.14 (3H, s), 2.08 (3H, s), 2.08 (3H, s), 2.04 (3H, s), 2.01 (3H, s), 1.99 (3H, s), 1.94 (3H, s), 1.96–1.87 (1H, m), 1.83–1.69 (1H, m), 1.63–1.51 (2H, m), 1.45–1.28 (2H, m); *m*/z (ESI) 1088.4047 ($[M+H]^+$. $C_{51}H_{66}O_{23}N_3$ requires 1088.4081). The foam **27a** (44 mg, 404 μmol) was taken up in MeOH (5.5 cm^3) and sodium methoxide in MeOH (two drops, 30%) was added. The reaction was stirred at rt for 72 h and the reaction mixture was adsorbed onto flash silica gel and purified by flash chromatography to give title compound **27b** as a colourless glass (25 mg, 82%). R_f 0.21 (CH_3CN/H_2O/concd NH_4OH, 6:1:1); $[\alpha]_D^{22}$ +40.6 (*c* 0.18, H_2O); δ_H (D_2O) 7.44–7.23 (10H, m), 6.07 (1H, s), 4.84 (1H, br s), 4.80 (1H, br s), 4.41 (1H, dd, *J* 7.0 and 7.0), 4.22–4.08 (3H, m), 4.03–3.95 (3H, m), 3.86–3.77 (4H, m), 3.74–3.51 (6H, m), 3.20–3.07 (2H, m), 1.87–1.66 (2H, m), 1.53–1.40 (2H, m), 1.33–1.18 (2H, m); δ_C (75 MHz, D_2O) 173.2, 171.7, 141.1, 140.9, 129.3, 129.2, 128.3, 128.1, 127.8, 127.6, 100.4, 73.6, 70.8, 70.1, 66.9, 66.0, 61.2, 57.8, 53.9, 39.1, 31.4, 28.3, 22.6; *m*/*z* (ESI) 752.3209 ($[M+H]^+$. $C_{35}H_{50}N_3O_{15}$ requires 752.3236).

4.20. 1-Benzhydryl-L-lysinamide(*N*,*N*′):1-oxo-2-(α-D-mannopyranosylthio)ethyl$_2$-cascadane (28b)

To a stirred solution of [**G0**]–$[Boc]_2$ (71 mg, 0.14 mmol) in MeOH (3 cm^3), HCl (1.5 cm^3, concd) was added. After 30 min at 40 °C, the resulting solution was concentrated in vacuo, and evaporated with H_2O (×2) and then with MeCN (×2). Anhydrous DMF (5 cm^3) was added to the resulting white solid followed by Et_3N (135 μL, 0.97 mmol) and the reactive ester **11** (158 mg, 0.30 mmol) in DMF (3 cm^3). After 2.5 h at rt, the reaction mixture was diluted with H_2O (40 cm^3), stirred for 30 min, filtered and the precipitate was purified by flash chromatography ($CHCl_3$/MeOH, 98:2) to give acetylated intermediate **28a** (116 mg, 75%). δ_H 7.44 (1H, d, *J* 8.1), 7.37–7.17 (10H, m), 7.13 (1H, d, *J* 8.1), 6.73 (1H, dd, *J* 6.0 and 6.0), 6.18 (1H, d, *J* 8.1), 5.40 (1H, br d, *J* 1.2), 5.37–5.27 (5H, m), 5.23 (1H, dd, *J* 1.0 and 3.0), 5.23 (1H, dd, *J* 1.0 and 3.0), 4.49 (1H, ddd, *J* 7.9, 7.9 and 5.8), 4.36–4.23 (4H, m), 4.16–4.02 (2H, m), 3.47–3.03 (6H, m), 2.14 (3H, s), 2.13 (3H, s), 2.07 (3H, s), 2.06 (3H, s), 2.05 (3H, s), 2.04 (3H, s), 1.99 (3H, s), 1.98 (3H, s), 1.90–1.78 (1H, m), 1.78–1.60 (1H, m), 1.61–1.41 (2H, m), 1.41–1.20 (2H, m); δ_C 171.1, 171.0, 170.9, 170.4, 170.3, 170.2, 170.0, 168.7, 168.0, 141.8, 141.5, 129.1, 129.0, 127.9, 127.8, 82.6, 82.4, 77.6, 71.1, 70.9, 70.0, 69.7, 66.5, 66.4, 62.7, 57.4, 53.5, 39.8, 34.2, 33.7, 32.4, 29.2, 23.0, 21.2, 21.1, 21.0, 20.9. Acetylated intermediate **28a** (94 mg, 0.084 mmol) was dissolved in MeOH (3 cm^3), and the pH of the solution was adjusted to 12 with NaOMe in MeOH. (30%) After 30 min, the solution was neutralized with solid CO_2, concentrated in vacuo and purified by flash silica chromatography (CH_3CN/H_2O/concd NH_4OH, 6:1:1) to give dendrimer **28b** as a white amorphous solid (65 mg, 99%). >99% pure (HPLC, 220 nm); $[\alpha]_D^{22}$ +188.8 (*c* 0.5, MeOH); δ_H (D_2O) 7.05–6.97 (10H, m), 5.96 (1H, s), 5.27 (1H, s), 5.25 (1H, s), 4.43 (1H, br t), 4.01–3.97 (2H, m), 3.88–3.66 (10H, m), 3.32–3.15 (4H, m), 3.00 (2H, br s), 1.66 (2H, br m), 1.34 (2H, br m), 1.21 (2H, br m); δ_C (D_2O) 172.8, 171.8, 141.5, 141.1, 129.0, 127.8, 127.7, 85.3 (Man C-1), 73.9, 71.8, 71.6, 67.3, 61.2, 57.5, 54.3, 39.9, 34.2, 33.8, 31.8, 28.4, 23.0; *m*/*z* (ESI) 806.2570 ($[M+Na]^+$. $C_{35}H_{49}N_3NaO_{13}S_2$ requires 806.2599).

4.21. 1-Benzhydryl-L-lysinamide(*N*,*N*′):{L-lysinyl(*N*,*N*′)}$^{G1}_{2x}$:1-oxo-2-(α-D-mannopyranosyloxy)ethyl$_4$-cascadane (29b)

To a stirred solution of [**G1**]–$[HCl]_4$ (228 mg, 0.31 mmol) in DMF (12 cm^3), reactive ester **7** (861 mg, 1.71 mmol) and $^iPr_2NH$ (0.79 cm^3, 4.46 mmol) were added. The solution, under Ar, was stirred at rt for 72 h and diluted with H_2O. The aqueous mixture was extracted with ether (×3) and the combined organic extracts were washed with H_2O (×2), dried ($MgSO_4$) and dried in vacuo. Purification of the residue by flash chromatography ($CHCl_3$/MeOH, 99:1 to 95:5) gave acetylated intermediate **29a** as a colourless foam (243 mg, 36%). $[\alpha]_D^{20}$ +37.1 (*c* 1.8, $CHCl_3$); δ_H 7.74 (1H, d, *J* 8.2), 7.36–7.16 (12H, m), 7.09 (1H, d, *J* 7.5), 6.91 (1H, dd, *J* 5.7 and 5.7), 6.78 (1H, dd, *J* 5.8 and 5.8), 6.69 (1H, dd, *J* 6.0 and 6.0), 6.17 (1H, d, *J* 8.1), 5.40–5.23 (16H, m), 4.91 (1H, d, *J* 1.0), 4.87 (1H, d, *J* 0.9), 4.85 (1H, br s), 4.81 (1H, d, *J* 1.0), 4.53–4.33 (3H, m), 4.32–4.21 (4H, m), 4.18–3.92 (15H, m), 3.82 (1H, d, *J* 15.2), 3.34–3.19 (5H, m), 3.09–2.89 (1H, m), 2.15 (3H, s), 2.15 (6H, br s), 2.14 (3H, s), 2.08 (6H, br s), 2.08 (3H, s), 2.07 (3H, s), 2.04 (3H, s), 2.04 (3H, s), 2.03 (3H, s), 2.02 (3H, s), 2.00 (3H, s), 1.99 (3H, s), 1.99 (3H, s), 1.97 (3H, s), 1.93–1.62 (6H, m), 1.62–1.42 (6H, m), 1.42–1.25 (6H, m); δ_C 171.6, 171.2, 170.6, 170.5, 170.0, 169.4, 169.8, 169.8, 169.5, 169.5, 169.4, 168.4, 168.2, 168.0, 167.9, 141.4, 141.3, 128.5, 127.3, 97.8, 97.7, 97.5, 69.0, 68.8, 66.7, 66.6, 65.7, 65.7, 62.1, 56.8, 53.3, 53.0, 52.9, 52.7, 38.6, 38.4, 38.3, 31.7, 31.3, 31.3, 29.0, 28.7, 28.4, 22.8, 22.6, 22.2, 20.7, 29.6, 20.5; *m*/*z* (ESI) 1060.9053 ($[M+2H]^{2+}$. $C_{95}H_{131}N_7O_{47}$ requires 1060.9032). The solid **29a** (224 mg, 106 μmol) was taken up in MeOH (10 cm^3) and NaOMe in MeOH (two drops, 30%) was added. The mixture was stirred at rt for 16 h and neutralized with Amberlite IRC-50, filtered and concentrated in vacuo. The product was taken up in a small volume of H_2O, filtered through cotton wool and concentrated in vacuo to give the title compound **29b** as a colourless gum (113 mg, 74%). R_f 0.18 (MeCN/H_2O/concd NH_4OH, 3:1:1); $[\alpha]_D^{22}$ +24.0 (*c* 0.27, H_2O); δ_H (D_2O) 7.41–7.20 (10H, m), 6.05 (1H, s), 4.86–4.82 (4H, m), 4.38–4.28 (2H, m), 4.25–3.95 (13H, m), 3.89–3.51 (20H, m), 3.20–3.00 (6H, m), 1.88–1.57 (6H, m), 1.52–1.14 (12H, m); δ_C (D_2O) 173.8, 173.7, 173.1, 171.8, 171.7, 141.1, 140.9, 129.3, 129.2, 128.3, 128.0, 127.5, 100.4, 73.6, 70.8, 70.1, 67.0, 66.2, 66.0, 61.2, 57.8, 54.3, 53.9, 53.6, 39.4, 39.1, 31.4, 30.9, 28.4, 28.3, 28.1, 22.8, 22.6; *m*/*z* (ESI) 724.894 ($[M+2H]^{2+}$. $C_{63}H_{99}N_7O_{31}$ requires 724.819).

4.22. 1-Benzhydryl-L-lysinamide(*N*,*N*′):{L-lysinyl(*N*,*N*′)}$^{G1}_{2x}$:1-oxo-2-(α-D-mannopyranosylthio)ethyl$_4$-cascadane (30b)

To a stirred solution of [**G1**]–$[HCl]_4$ (90 mg, 0.126 mmol) in anhydrous DMF (2 cm^3), Et_3N (229 μL, 0.97 mmol) was added. After 10 min, a suspension formed to which was added a solution of reactive ester **11** (320 mg, 0.55 mmol) in DMF (2 cm^3) in two portions with 2 h between the additions. After being stirred overnight at rt, the reaction mixture was diluted with H_2O (25 cm^3), which caused a white precipitate to form. The stirring was resumed for 30 min and the precipitate was collected by vacuum filtration and washed with H_2O to give the acetylated intermediate **30a** (208 mg, 76%); δ_H 7.73 (1H, d, *J* 7.6), 7.57 (1H, d, *J* 8.0), 7.49 (1H, d, *J* 6.0), 7.38–7.11 (11H, m), 7.00 (1H, br dd, *J* 5.2 and 5.2), 6.90–6.77 (2H, m), 6.12 (1H, d, *J* 8.0), 5.44–5.11 (16H, m), 4.58 (1H, ddd, *J* 7.3, 7.0 and 7.0), 4.41–4.17 (10H, m), 4.15–4.01 (4H, m), 3.60–3.00 (14H, m),

2.17–1.96 (16×(3H, s)), 1.79–1.00 (18H, m). MeOH (5 cm^3) was added to the crude product (171 mg, 0.078 mmol) and the pH of the suspension was adjusted to 12 with 30% NaOMe in MeOH. After 2 h, the reaction was diluted with EtOH, filtered and the precipitate washed with EtOH (×3). The combined filtrates were concentrated in vacuo and purified by flash chromatography (3:1:1, CH_3CN/H_2O/concd NH_4OH) to give the capped dendrimer **30b** as a white solid (64 mg, 54%). >99% pure (HPLC, 220 nm); $[\alpha]_D^{21}$ +143.6 (*c* 0.5, H_2O); δ_H (D_2O) 7.30–7.19 (10H, m), 6.01 (1H, s), 5.28 (2H, s), 5.24 (2H, s), 4.37 (1H, t, *J* 7.1), 4.23 (1H, t, *J* 7.0), 4.13 (1H, t, *J* 7.0), 4.01 (4H, br s), 3.85–3.66 (20H, m), 3.92–2.97 (14H, m), 1.83–1.54 (6H, br m), 1.52–1.11 (12H, br m); δ_C (D_2O) 172.6, 171.8, 170.9, 170.8, 139.9, 139.7, 128.0, 127.1, 126.7, 126.3, 84.0, 72.6, 70.4, 70.3, 66.0, 59.9, 56.5, 53.5, 53.2, 53.0, 38.6, 38.1, 32.9, 32.5, 30.0, 29.8, 27.0, 21.6; *m/z* (MALDI-TOF) 1534.36 ($[M+Na]^+$. $C_{63}H_{97}N_7NaO_{27}S_4$ requires 1534.52).

4.23. 1-Benzhydryl-L-lysinamide(*N,N′*):{L-lysinyl(*N,N′*)}$^{G1}_{2X}$:1-oxo-6-(α-D-mannopyranosylthio)hexyl$_4$-cascadane (31b)

To a stirred solution of [**G1**]–$[TFA]_4$ (24 mg, 0.06 mmol) in DMF (0.5 cm^3), reactive ester **14** (118 mg, 0.20 mmol) was added followed by $^iPr_2NH$ (44 mg, 0.34 mmol). The reaction mixture was stirred for 16 h and diluted with H_2O (20 cm^3). The precipitated product was collected by vacuum filtration and washed with H_2O, then purified by flash chromatography ($MeOH/CHCl_3$, 1:99 to 5:95) to give acetylated intermediate **31a** as a white powder solid (62 mg, 76%). δ_H 7.56–7.45 (3H, m), 7.36–7.17 (10H, m), 7.05 (1H, dd, *J* 7.7 and 4.2), 6.57 (1H, d, *J* 8.4), 6.35 (1H, br t, *J* 5.6), 6.27 (1H, br t, *J* .54), 6.19 (1H, d, *J* 8.1), 5.37–5.21 (20H, m), 4.53 (1H, ddd, *J* 7.5, 7.5 and 7.5), 4.39–4.23 (13H, m), 4.15–4.05 (5H, m), 3.61–3.47 (1H, m), 3.27–3.12 (3H, m), 3.09–2.91 (2H, m), 2.71–2.49 (8H, m), 2.16 (6H, br s), 2.15 (3H, s), 2.13 (3H, s), 2.09 (9H, br s), 2.08 (3H, s), 2.05 (9H, br s), 2.04 (3H, s), 1.99 (6H, br s), 1.98 (3H, s), 1.96 (3H, s), 1.85–1.25 (42H, m); δ_C 174.1, 173.4, 173.1, 171.1, 170.9, 170.3, 170.1, 170.0, 141.9, 141.8, 128.9, 128.8, 127.8, 82.8, 71.5, 69.8, 69.3, 66.7, 62.8, 57.3, 53.3, 52.8, 39.4, 39.0, 36.7, 36.5, 31.4, 30.6, 29.7, 29.4, 28.6, 28.5, 25.5, 23.4, 23.1, 21.8, 21.2, 21.1, 21.0, 20.9. Capped dendrimer **31a** was dissolved in MeOH (5 cm^3) and CH_2Cl_2 (2 cm^3) and three drops of NaOMe in MeOH (30%) were added. The mixture was stirred for 30 min and solid CO_2 and H_2O (1 cm^3) were added to neutralize the reaction mixture. The MeOH was removed under reduced pressure and the precipitated solid triturated with cold H_2O to remove inorganic salts and give dendrimer **31b** as a white solid (40 mg, 68%). R_f 0.26 (6:1:1, $^iPr/H_2O/NH_4OH$); HPLC 93% pure, 220 nm; $[\alpha]_D^{22}$ +51.6 (*c* 0.5, MeOH); δ_H (MeOH-d_4) 7.39–7.25 (10H, m), 6.15 (1H, s), 5.24 (4H, s), 4.42 (1H, dd, *J* 8.3 and 5.9), 4.30 (1H, dd, *J* 8.0 and 6.1), 4.24 (1H, dd, *J* 8.3 and 5.9), 3.97–3.68 (24H, m), 3.17–3.12 (6H, m), 2.73–2.56 (8H, m), 2.26 (4H, t, *J* 7.0), 2.21 (4H, m), 1.90–1.41 (42H, m); δ_C (75 MHz, MeOH-d_4) 176.7, 129.8, 128.9, 128.8, 86.6, 75.0, 73.9, 73.3, 68.9, 62.7, 58.5, 55.0, 40.3, 37.2, 36.9, 32.7, 32.0, 30.4, 30.1, 29.4, 26.7, 26.6, 25.4, 24.3; *m/z* (MALDI-TOF) 1758.776 ($[M+Na]^+$. $C_{79}H_{129}N_7NaO_{27}S_4$ requires 1758.772).

4.24. 1-Benzhydryl-L-lysinamide(*N,N′*):{L-lysinyl(*N,N′*)}$^{G1}_{2X}$:1-oxo-6-(α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyloxy)hexyl$_4$-cascadane (32b)

To a stirred solution of [**G1**]–$[TFA]_4$ (24 mg, 0.024 mmol) in DMF (0.5 cm^3), reactive ester **19** (120 mg, 0.141 mmol) and $^iPr_2NH$ (30 mg, 0.24 mmol) were added. The mixture was stirred for 72 h and the volatiles were removed under high vacuum. Purification of the residue by flash chromatography ($CHCl_3$/MeOH, 97:3) gave acetylated intermediate **32a** as a colourless gum (66 mg, 79%). R_f 0.40 ($CHCl_3$/MeOH, 95:5); 94% pure (HPLC, 220 nm); δ_H 7.56 (1H, d, *J* 8.6), 7.49–7.44 (2H, m), 7.30–7.20 (10H, m), 7.05 (1H, dd, *J* 6.3 and 4.6), 6.73 (1H, d, *J* 8.0), 6.37 (1H, dd, *J* 5.2 and 5.2), 6.29 (1H, dd, *J* 5.3 and 5.3), 6.20 (1H, d, *J* 8.0), 5.36–5.18 (24H, m), 4.86–4.84 (4H, br s), 4.77–4.74 (4H, br s), 4.52 (1H, ddd, *J* 7.2, 7.2 and 7.2), 4.40–4.20 (6H, m), 4.17–4.04 (8H, m), 3.97–3.88 (4H, m), 3.82–3.66 (8H, m), 3.60–3.52 (4H, m), 3.48–3.40 (4H, m), 3.25–3.0 (4H, m), 2.40–1.80 (100H, m), 1.70–1.20 (40H, m); *m/z* (MALDI-TOF) 3519.20 ($[M+Na]^+$. $C_{159}H_{225}N_7NaO_{79}$ requires 3519.37). To a stirred solution of intermediate **32a** (65 mg, 18.5 μmol) in MeOH (5 cm^3), NaOMe in MeOH (five drops, 30%) was added. The reaction mixture was stirred for 30 min and sufficient ^iPrOH was added to precipitate the product. The white precipitate was collected, washed with a small amount of ^iPrOH, dissolved in H_2O and lyophilized to give the title compound **32b** as a fluffy white solid (34 mg, 79%). 99% pure (HPLC, 220 nm); δ_H (D_2O) 7.37–7.26 (10H, m), 6.06 (1H, s), 4.84 (4H, br s), 4.78 (4H, br s), 4.34 (1H, dd, *J* 6.3 and 6.3), 4.19 (1H, dd, *J* 6.3 and 6.3), 4.12 (1H, dd, *J* 6.9 and 6.9), 4.02–3.40 (56H, m), 3.16–3.05 (4H, br s), 3.00 (2H, br t, *J* 6.2), 2.29–2.10 (8H, m), 1.80–1.20 (42H, m); *m/z* (MALDI-TOF) 2343.20 ($[M+Na]^+$. $C_{103}H_{169}N_7NaO_{51}$ requires 2343.07).

4.25. 1-Benzhydryl-L-lysinamide(*N,N′*):{L-lysinyl(*N,N′*)}$^{G1}_{2X}$:1-oxo-6-{3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranosyloxy}hexyl$_4$-cascadane (33b)

To a stirred solution of [**G1**]–$[HCl]_4$ (10 mg, 0.014 mmol) in DMF (1 cm^3), reactive ester **23** (100 mg, 0.088 mmol) and Et^iPr_2N (19 mg, 0.14 mmol) were added. The reaction mixture was stirred for 16 h, diluted with H_2O and the precipitate was collected. The aqueous filtrate was extracted with $CHCl_3$ (15 cm^3) and the organic layer was dried and concentrated in vacuo. The residue was combined with the precipitate and triturated with hexane, to give a white amorphous solid. Purification by flash chromatography ($CHCl_3$/MeOH, 97:3 to 96:4) gave acetylated intermediate **33a** (46 mg, 68%). R_f 0.32 ($CHCl_3$/MeOH, 95:5). This solid was dissolved in dry MeOH (5 cm^3) and NaOMe in MeOH (three drops, 30%) was added. After stirring for 1 h, ^iPrOH (3 cm^3) was added to precipitate the deacetylated product, which was collected and washed with a small volume of cold isopropanol. The solid was dissolved in H_2O and lyophilized to give **33b** as a white solid (24 mg, 56%). >99% pure (HPLC, 220 nm); $[\alpha]_D^{22}$ +54.5 (*c* 0.079, H_2O); δ_H (D_2O) 7.39–7.27 (10H, m), 6.07 (1H, s), 5.07 (4H, s), 4.85 (4H, s), 4.77 (4H, s), 4.34 (1H, dd, *J* 7.2 and 7.2), 4.19 (1H, dd, *J* 7.1 and 7.1), 4.11 (1H, dd, *J* 7.1 and 7.1), 4.04–3.60 (76H, m), 3.49 (4H, br s), 3.01 (4H, br s), 3.01 (2H, br t, *J* 7.0), 2.24–2.51 (8H, m), 2.80–1.20 (42H, m); δ_C (D_2O) 176.9, 129.3, 128.0, 127.5, 79.0, 73.7, 73.1, 71.5, 71.5, 71.0, 70.8, 70.4, 70.1, 68.2, 67.1, 66.1, 65.7, 61.3, 39.3, 36.2, 28.6, 25.4; *m/z* (MALDI-TOF) 2991.38 ($[M+Na]^+$. $C_{127}H_{209}N_7NaO_{71}$ requires 2991.28).

4.26. 1-Benzhydryl-L-lysinamide(*N,N′*):{L-lysinyl(*N,N′*)}$^{G1,G2}_{2X,4X}$:1-oxo-2-(α-D-mannopyranosylthio)ethyl$_8$-cascadane (34b)

To a stirred solution of [**G2**]–$[Boc]_8$ (11 mg, 0.0059 mmol) in MeOH (4 cm^3), HCl (2 cm^3, concd) was added. After 10 min at 40 °C, the resulting solution was concentrated in vacuo, evaporated with H_2O (×2) and then MeCN to give [**G2**]–$[HCl]_8$. To the resulting solid, anhydrous DMF (1 cm^3) and Et^iPr_2N (51 μL, 0.293 mmol) were added. After the mixture was stirred for 10 min the HCl salt dissolved and reactive ester **11** (24 mg, 0.047 mmol) was added. After being left to stand overnight at rt, the solution was diluted with H_2O (5 cm^3) and stirred for 30 min. The precipitate was collected by vacuum filtration, washed with H_2O and purified by flash silica chromatography ($MeOH/CH_2Cl_2$, 7:93) to give acetylated intermediate **34a** (5 mg, 20%). δ_H 7.81 (1H, br d, *J* 6.0), 7.69 (1H, br d, *J* 7.8), 7.63–7.46 (5H, m), 7.36–7.16 (11H, m), 7.16–7.07 (3H, m), 7.02–6.83 (4H, m), 6.15 (1H, d, *J* 8.1), 5.47–5.13 (32H, m), 4.66–4.56 (1H, m), 4.55–4.19 (22H, m), 4.18–3.96 (8H, m), 3.53–2.97 (30H, m), 2.14 (24H, br s), 2.08 (24H, br s), 2.04 (24H, br s), 1.97 (24H, br s),

1.89–1.19 (42H, m). Intermediate **34a** (16 mg, 0.0037) was dissolved in MeOH (2 cm^3) and CH_2Cl_2 (2 cm^3) and the pH of the suspension was adjusted to 12 by addition of NaOMe in MeOH (30%). After 2 h, the solution was neutralized with ion exchange resin (Amberlite IRC 50, H^+ form), concentrated in vacuo and lyophilized to give the capped dendrimer **34b** as a white solid (11 mg, quant.). >99% pure (HPLC, 220 nm); $[\alpha]_D^{20}$ +36.2 (*c* 0.2, H_2O); δ_H (D_2O) 7.41–7.11 (10H, m), 6.06 (1H, s), 5.28 (4H, br s), 5.24 (4H, br s), 4.35 (1H, br dd, *J* 7.0 and 7.0), 4.28–4.08 (6H, m), 4.00 (8H, br s), 3.92–3.60 (40H, m), 3.40–3.00 (30H, m), 1.80–1.60 (14H, m), 1.53–1.37 (14H, m), 1.37–1.17 (14H, m); *m*/*z* (MALDI-TOF) 2991.1 ($[M+Na]^+$. $C_{119}H_{193}N_{15}NaO_{55}S_8$ requires 2991.0).

4.27. 1-Benzhydryl-L-lysinamide(*N*,*N*′):{L-lysinyl(*N*,*N*′)}$^{G1,G2}_{2x,4x}$:1-oxo-6-(α-D-mannopyranosylthio)hexyl$_8$-cascadane (35b)

To a stirred solution of [**G2**]–$[TFA]_8$ (126 mg, 0.06 mmol) dissolved in DMF (3 cm^3), reactive ester **14** (416 mg, 0.76 mmol) followed by Et^iPr_2N (245 mg, 1.90 mmol) were added. The reaction mixture was stirred for 24 h and diluted with H_2O. The precipitated product was collected by vacuum filtration and purified by flash chromatography (MeOH/$CHCl_3$, 1:99 to 5:95) to give the acetylated intermediate **35a** as a white solid (233 mg), which was dissolved in dry MeOH (5 cm^3) and CH_2Cl_2 (3 cm^3). NaOMe in MeOH (three drops, 30%) was added to the solution and the mixture was stirred for 30 min. Solid CO_2 (10 g) and H_2O (10 cm^3) were added, and when all the CO_2 had vaporized the volatiles were removed under reduced pressure (high vacuum) to leave the product, which was purified by flash chromatography. The resulting capped dendrimer **35b** was obtained as a white solid (137 mg, 63%). R_f 0.20 ($^iPr/H_2O/NH_{3(aq)}$, 3:1:1); 99% pure (HPLC, 220 nm); δ_H (CD_3OD/D_2O, 5:1) 7.37–7.26 (10H, m), 6.16 (1H, s), 5.25 (8H, s), 4.45 (1H, dd, *J* 5.4 and 8.1), 4.32–4.25 (6H, m), 3.95–3.69 (48H, m), 3.17–3.09 (14H, m), 2.71–2.56 (16H, m), 2.29 (8H, br m), 2.20 (8H, t, *J* 7.5), 1.80–1.28 (90H, m); δ_C (CD_3OD/D_2O, 5:1, resolved peaks only) 177.6, 177.2, 176.9, 130.1, 129.2, 129.0, 86.8, 75.2, 74.1, 73.5, 69.1, 55.3, 40.5, 37.5, 37.1, 33.1, 32.8, 32.3, 30.6, 30.3, 29.6, 26.9, 24.6; *m*/*z* (MALDI-TOF) 3439.32 ($[M+Na]^+$. $C_{151}H_{257}N_{15}NaO_{55}S_8$ requires 3439.54).

4.28. 1-Benzhydryl-L-lysinamide(*N*,*N*′):{L-lysinyl(*N*,*N*′)}$^{G1,G2}_{2x,4x}$:1-oxo-6-(α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyloxy)hexyl$_8$-cascadane (36b)

To a stirred solution of [**G2**]–$[TFA]_8$ (23 mg, 0.011 mmol) dissolved in DMF (0.5 cm^3), reactive ester **19** (120 mg, 0.141 mmol) followed by Et^iPr_2N (30 mg, 0.24 mmol) were added. The mixture was stirred for 72 h and the volatiles were removed under high vacuum. Purification of the residue by flash chromatography ($CHCl_3$/MeOH, 97:3) gave acetylated intermediate **36a** as a colourless gum (52 mg, 64%). R_f 0.42 ($CHCl_3$/MeOH, 95:5); 98% pure (HPLC, 220 nm); δ_H 8.0–7.73 (3H, m), 7.63–7.43 (3H, m), 7.36–7.14 (12H, m), 7.11–6.92 (3H, m), 6.72–6.56 (2H, m), 6.37–6.24 (2H, m), 6.20 (1H, d, *J* 8.0), 5.36–5.18 (48H, m), 4.88–4.84 (8H, br s), 4.80–4.74 (4H, br s), 4.74–4.66 (1H, m), 4.60–4.40 (4H, m), 4.40–4.20 (10H, m), 4.17–4.04 (16H, m), 3.97–3.88 (8H, br s), 3.83–3.64 (16H, m), 3.62–3.52 (8H, m), 3.49–3.40 (10H, m), 3.30–3.28 (12H, m), 2.30–1.90 (196H, m), 1.70–1.20 (78H, m); *m*/*z* (MALDI-TOF) 6964.3 ($[M+Na]^+$. $C_{311}H_{449}N_{15}NaO_{159}$ requires 6964.9 (average molecular mass)). To a stirred solution of intermediate **36a** (44 mg, 7.2 μmol) in MeOH (5 cm^3), NaOMe in MeOH (five drops, 30%) was added. The mixture was stirred for 30 min and ^{i}PrOH was added to precipitate the white product, which was collected, washed with a small amount of ^{i}PrOH, dissolved in H_2O and lyophilized to give capped dendrimer **36b** as a white solid (28 mg, 85%). 90% pure (HPLC, 220 nm); δ_H (D_2O) 7.36–7.26 (10H, m), 6.07 (1H, s), 4.85 (8H, br s), 4.78 (8H, br s), 4.36 (1H, dd, *J* 6.3 and 6.3), 4.23–4.14 (6H, m), 4.05–3.38 (112H, m), 3.20–3.00 (14H, m), 2.33–2.10 (16H, m), 1.80–1.20 (90H, m); *m*/*z* (MALDI-TOF) 4606.6 ($[M+Na]^+$, $C_{199}H_{337}N_{15}NaO_{103}$ requires 4608.1 (average molecular mass)).

4.29. 1-Benzhydryl-L-lysinamide(*N*,*N*′):{L-lysinyl(*N*,*N*′)}$^{G1,G2,G3}_{2x,4x,8x}$:1-oxo-6-(α-D-mannopyranosylthio)hexyl$_{16}$-cascadane (37b)

To a stirred solution of [**G3**]–$[TFA]_{16}$ (200 mg, 0.0509 mmol) in DMF (5 cm^3), reactive ester **14** (702 mg, 1.22 mmol) followed by Et^iPr_2N (395 mg, 3.05 mmol) were added. The reaction mixture was stirred for 16 h and concentrated in vacuo. The residue was dissolved in $CHCl_3$/MeOH and filtered through a bed of silica (R_f 0.50, $CHCl_3$/MeOH, 90:10) to give acetylated intermediate **37a** as a white solid. This solid was dissolved in dry MeOH (6 cm^3) and CH_2Cl_2 (3 cm^3) and NaOMe in MeOH (0.10 cm^3, 30%) was added to the solution. The solution was stirred for 30 min after which time the product precipitated as a white solid, which was collected on a frit and washed with a little cold MeOH/CH_2Cl_2 (1:1). The solid was dissolved in 1:1 MeOH/H_2O, the MeOH removed under reduced pressure and the product lyophilized to give capped dendrimer **37b** (219 mg, 63%). $[\alpha]_D^{22}$ +92.3 (*c* 0.31, MeOH/H_2O, 1:1); 90% pure (HPLC, 220 nm); δ_H (D_2O) 7.42–7.23 (10H, m), 6.16 (1H, s), 5.22 (16H, s), 4.50–4.20 (15H, m), 4.06–3.63 (96H, m), 3.13 (30H, br s), 2.60 (32H, br s), 2.37–2.13 (32H, m), 1.80–1.20 (186H, m); *m*/*z* (MALDI-TOF) 6805.3 ($[M+Na]^+$. $C_{295}H_{513}N_{31}NaO_{111}S_{16}$ requires 6806.4 (average molecular mass)).

4.30. 1-Benzhydryl-L-lysinamide(*N*,*N*′):{L-lysinyl(*N*,*N*′)}$^{G1,G2,G3}_{2x,4x,8x}$:1-oxo-6-(α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyloxy)hexyl$_{16}$-cascadane (38b)

To a stirred solution of [**G3**]–$[TFA]_{16}$ (34 mg, 8.6 μmol) in DMSO (1.0 cm^3), reactive ester **19** (176 mg, 0.207 mmol) followed by Et^iPr_2N (44 mg, 0.345 mmol) were added. The mixture was stirred for 96 h and the product was precipitated by the addition of water. The solid was collected by vacuum filtration and purified by flash chromatography ($CHCl_3$/MeOH, 100:0 to 70:30) to give acetylated intermediate **38a** as a colourless gum (65 mg, 55%). 88% pure (HPLC 220 nm); δ_H 8.37 (1H, s), 8.00–7.48 (10H, m), 6.83–6.54 (4H, m), 6.51–6.31 (4H, m), 6.21 (1H, d, *J* 8.4), 5.37–5.26 (80H, m), 5.21 (16H, br s), 4.88 (16H, br s), 4.78 (16H, br s), 4.64–4.40 (8H, br s), 4.43–4.32 (7H, br s), 4.27 (16H, dd, *J* 12.3 and 5.3), 4.16–4.04 (32H, m), 3.93 (16H, br s), 3.83–3.66 (32H, m), 3.63–3.55 (16H, m), 3.50–3.38 (22H, m), 3.24–2.96 (24H, m), 2.32–2.12 (32H, m), 2.16 (48H, s), 2.16 (48H, s), 2.11 (48H, s), 2.07 (48H, s), 2.06 (48H, s), 2.00 (48H, s), 1.98 (48H, s), 1.72–1.32 (186H, m); *m*/*z* (MALDI-TOF) 13,841 ($[M+Na]^+$. $C_{615}H_{897}N_{31}NaO_{319}$ requires 13,852 (average molecular mass)). To this white solid (65 mg, 4.7 μmol), methanolic ammonia (3 cm^3, 7 M) was added. After 3 h of stirring an oil precipitated from the solution and was dissolve by the addition of H_2O (3 cm^3). The mixture was stirred for 16 h and concentrated in vacuo. The residue was taken up in water (0.5 cm^3) and precipitated by the addition of ^{i}PrOH. The suspension was centrifuged (4000 rpm, 10 min), the pellet washed with a little ^{i}PrOH, then taken up in water and lyophilized to give the capped dendrimer **38b** as a white powder (35 mg, 81%). >95% pure (HPLC, 220 nm); $[\alpha]_D^{22}$ +35.1 (*c* 0.7, H_2O); δ_H (D_2O) 7.41–7.22 (10H, m), 6.11 (1H, s), 4.87 (16H, br s), 4.80 (16H, br s), 4.46–4.37 (1H, br m), 4.30–4.10 (14H, m), 4.00–3.62 (208H, m), 3.55–3.43 (16H, m), 3.13 (30H, br s), 2.32–2.14 (32H, br m), 1.84–1.19 (186H, m); *m*/*z* (MALDI-TOF) 9143 ($[M+Na]^+$. $C_{393}H_{675}N_{31}NaO_{208}$ requires 9186 (average molecular mass)).

4.31. 1-Benzhydryl-L-lysinamide(*N*,*N*′): {L-lysinyl(*N*,*N*′)}$^{G1,G2,G3,G4}_{2x,4x,8x,16x}$:1-oxo-6-(α-D-mannopyranosylthio)hexyl$_{32}$-cascadane (39b)

To a stirred solution of [**G4**]–[TFA]$_{32}$ (250 mg, 0.032 mmol) in DMF (5 cm^3), reactive ester **14** (884 mg, 1.22 mmol) followed by Et^{i}Pr$_2$N (330 mg, 2.56 mmol) were added. The reaction mixture was stirred for 16 h, ether (50 cm^3) was added and an oily product precipitated. Further stirring caused the oil to form a fine white precipitate, which was collected by vacuum filtration (473 mg, 78%). The solid was dissolved in dry MeOH (19 cm^3) and NaOMe in MeOH (seven drops, 30%) was added to the solution. The reaction mixture was stirred for 90 min and CH_2Cl_2 was added to precipitate the product. The oily precipitate was washed with a little cold MeOH, then dissolved in H_2O and the product was lyophilized to give the capped dendrimer **39b** (263 mg, 61%); $[\alpha]_D^{22}$ +89.8 (*c* 0.31, H_2O); δ_H (D_2O) 7.35–7.20 (10H, m), 6.16 (1H, m), 5.26 (32H, s), 4.20–4.40 (31H, m), 4.0–3.60 (192H, m), 3.14 (62H, br s), 2.80–2.57 (64H, m), 2.40–2.15 (64H, m), 1.80–1.20 (378H, m); *m*/*z* (MALDI-TOF) 13,558 ([M+Na]$^+$. $C_{583}H_{1025}N_{63}NaO_{223}S_{32}$ requires 13,525 (average molecular mass)).

4.32. 1-Benzhydryl-L-lysinamide(*N*,*N*′): {L-lysinyl(*N*,*N*′)}$^{G1,G2,G3,G4,G5}_{2x,4x,8x,16x,32x}$:1-oxo-6-(α-D-mannopyranosylthio)hexyl$_{64}$-cascadane (40b)

To a stirred solution of [**G5**]–[TFA]$_{64}$ (110 mg, 7.07 μmol) in DMF (3 cm^3), reactive ester **14** (390 mg, 0.687 mmol) followed by Et^{i}Pr$_2$N (148 mg, 1.14 mmol) were added. The reaction mixture was stirred for 16 h, water (30 cm^3) was added and the resulting precipitate collected by decanting off the aqueous layer. The solid was dried under vacuum, ether added and the solid collected by vacuum filtration. The solid was dissolved in dry MeOH/CH_2Cl_2 (6 cm^3, 5:1) and NaOMe in MeOH (30%) added. The reaction mixture was stirred for 60 min and a fine white precipitate formed. ^{i}PrOH (12 cm^3) was added and the precipitate isolated by vacuum filtration and washed with further ^{i}PrOH. The solid was then dissolved in H_2O and the product was lyophilized to give crude product, which was purified by RP-HPLC on a Phenomenex Synergi 4 μ Max-RP 80A, 30×250 mm column eluting with 0.1% trifluoroacetic acid in water/methanol to give capped dendrimer **40b** (84 mg, 44%). $[\alpha]_D^{22}$ +88.3 (*c* 0.6, H_2O); δ_H (D_2O) 7.36–7.20 (10H, m), 6.13 (1H, m), 5.27 (64H, s), 4.38–4.17 (63H, m), 4.03–3.64 (384H, m), 3.15 (126H, br s), 2.72–2.55 (128H, m), 2.35–2.13 (128H, m), 1.86–1.22 (762H, m); *m*/*z* (MALDI-TOF) 26,811 ([M+Na]$^+$. $C_{1159}H_{2049}N_{127}NaO_{447}S_{64}$ requires 26,991 (average molecular mass)).

4.33. Generation and activation of bone marrow dendritic cells (BMDCs)

Murine BMDCs were generated by culturing bone marrow derived stem cells from C57Bl/6J mice in complete Iscove's Modified Dulbecco's Medium (cIMDM; IMDM supplemented with 5% foetal bovine serum, 1% penicillin/streptomycin, 1% glutamax, and 0.01% 2-mercaptoethanol; from Invitrogen, CA, USA) with 20 ng/mL recombinant granulocyte/macrophage colony stimulating factor (clone kindly supplied by Dr G. Buchan, University of Otago) for 6 days at 37 °C, 5% CO_2. Cells were seeded at 5×10^5 cells/mL in cIMDM for pulsing with glycodendrimer formulations. Activation of BMDC was measured by flow cytometric analysis (FACScalibur, BD, USA). Cells were stained with the BMDC marker CD11c, and the expression of co-stimulatory activation markers CD86 and MHCII on CD11c^{+ve} cells was investigated 48 h after the addition of 0.5 nM of the various formulations or 10 μg/mL lipopolysaccharide (LPS) as a positive control. Data was analyzed using CellQuest Pro (BD, USA). The fold increase in the mean fluorescence intensity (MFI) of activation marker expression was determined by dividing the MFI of each sample by that of the negative control (media only, no formulation).

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