Synthesis and Biological Evaluation of α-D-Mannopyranoside-Containing Dendrimers[†]

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The extension of dendritic synthetic principles to the preparation of new kinds of dendrimer-based glycoconjugate systems has been investigated. A convergent growth approach to the synthesis of α-D-mannopyranoside-containing dendrimers of various generations has been used successfully to obtain high molecular weight and monodisperse dendrimers in which 3−36 identical copies of the mannopyranoside residues are located at their peripheries. A repetitive synthetic sequence for forming amide bonds is employed in the preparation of various dendritic wedges and their attachment to a 1,3,5-trisubstituted benzenoid core in the last step. The protecting groups on the saccharide residues are then finally removed to obtain free saccharide-containing dendrimers. The efficacy of these dendrimers in inhibiting the binding of concanavalin A lectin to a purified yeast mannan fraction (as measured by an enzyme-linked lectin assay) is shown to be about four times more pronounced on a molar basis for the dendrimers possessing nine, 18, and 36 mannose residues, when compared to a dendritic wedge possessing three mannose residues and methyl α-D-mannopyranoside as control inhibitors.

Introduction

A large number of biological events are related to the functional aspects of protein-bound saccharides.[1] The carbohydrate portions of glycoproteins and glycolipids on cell surfaces participate at a macromolecular level in many biological recognition processes,[2] such as those involving immune defense response, viral replication, parasite infection, cell−cell adhesion, and inflammation.[3] The specific biological functions of the oligosaccharide chains in glycoproteins are mediated by a range of carbohydrate-binding proteins—the so-called lectins. Generally, multiple copies of saccharide ligands are necessary in order to generate strong binding affinities to their constituent lectin receptor sites,[4] e.g., several endogeneous selectin ligands having multiple, densely spaced *O*-linked sugars with very high surface densities.[5] The phenomenon, referred to as the "glycoside cluster effect" has been well documented and verified for many neoglycoconjugates, i.e., synthetic analogues of naturally occurring oligosaccharide chains containing the active mono- or oligosaccharide components only.[6] The importance of intersaccharide distances and the branching mode in cluster glycosides has been demonstrated elegently by Lee et al.,[6c] and these requirements for synthetic cluster glycosides have been confirmed further by van Boom et al.[7] in the studies involving the attachment of cluster galactosides with the hepatic asialoglycoprotein receptor. In the quest to obtain multivalent cluster glycosides, strategies employed commonly include attachment of determinant sugar ligands to carriers derived from proteins[8] and polymers.[9] On the basis of the idea that the rapidly emerging field of dendrimer chemistry[10] could be utilized to create new and novel forms of glycomimetics, we[11] and others[12] have been developing synthetic routes to present multiple copies of sugar ligands on the surfaces of dendrimers and dendritic-like structures. Following the development of numerous

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elegant synthetic routes for the synthesis of different types of dendrimers,[10] using various kinds of building blocks, dendrimer chemistry now appears to offer the most highly controllable methodology for synthesizing large three-dimensional macromolecules with very precise chemical constitutions and increasingly well-defined molecular structures.

We report herein the synthesis of a series of α-D-mannopyranose-containing dendrimers in which these monosaccharides are located at the peripheries of the dendrimers with peptidic-like interiors. Central to the synthesis of these functionalized dendrimers is the choice of the appropriate synthetic methodology that will allow their facile preparation. Of the various methods that have now been identified, the so-called "convergent synthetic route" and the "divergent synthetic route" have been applied widely in the synthesis of many different dendrimers.[10] In view of the structural characteristics of carbohydrate-containing dendrimers, the convergent synthetic route has been applied by us in their synthesis in order to obtain the component dendritic wedges and the final dendrimers. These have extremely high purities and structural homogeneities associated with their macromolecules and also boast unique chemical constitutions. Recently, Roy et al.[13] have reported the synthesis and studies of mannosylated dendrimers, prepared by the modification of the peripheries of PAMAM dendrimers (generation 0−3) and also other dendritic structures.

Following the convergent synthesis of these new α-D-mannopyranoside-containing dendrimers, we have evaluated their biological potencies by an enzyme-linked lectin assay (ELLA), involving the inhibition of concanavalin A (Con A) binding to a purified yeast mannan fraction Sc500.[14] This fraction is of interest because of its strong binding of antibodies found in the serum of patients with Crohn's disease[14,15] and also because of its implication in the allergy to yeast mannan of bakers and brewers who are overexposed to yeast in their work.[14] Dendrimers of the type discussed herein may therefore find a use in the clinic when the optimum branching densities and lengths of branches have been determined. This investigation addresses the first question by reference to Con A binding that is known to be inhibited, if weakly, at the monosaccharide level.

Results and Discussion

The convergent synthetic methodology involves essentially the synthesis of dendritic wedges possessing carbohydrates—preferably lining a surface—as one of the structural components, followed by the attachment of these wedges to further branching components and then finally to core components in order to obtain the desired dendrimers. The structural components that constitute the branching regions of the dendritic wedges have been derived from (i) tris(hydroxymethyl)aminomethane (Tris) and (ii) 3,3′-iminodipropionic acid, while the core has been derived from 1,3,5-benzenetricarbonyl chloride. The desired synthetic sequences using these components rely on (i) glycosylation of the three hydroxyl groups in Tris so as to obtain a tris-branched saccharide-containing wedge, (ii) attachment of these wedges to the branched 3,3′-iminodipropionic acid derivatives by the formation of amide bonds, affording highly branched saccharide-containing dendritic wedges, and finally, (iii) attachment of these branched wedges to the core component derived from 1,3,5-benzenetricarbonyl chloride, once again by the formation of amide bonds. The syntheses of these dendrimers have been carried out by first constructing the respective dendritic wedges, followed by the coupling of these wedges to the central core component.

Synthesis of Tris−Mannopyranoside-Containing Dendritic Wedge (7). The tris-branched mannopyranoside derivative **7** was readily obtained (Scheme 1) by the reaction of 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide (**2**) with the Z-Tris derivative **1** in the presence of AgOTf and 2,4,6-collidine in $CH_2Cl_2/MeNO_2$. Under these conditions, the tris-glycosylation was stereoselective and afforded the Tris−α-D-mannopyranoside derivative **3** in 84% yield after column chromatography. The first step in the elaboration of the dendritic wedge **3** involved the modification of the protecting groups on the mannopyranoside residues. Removal of the *O*-benzoyl protecting groups in **3** using the Zemplén procedure gave **4**, which was protected with *O*-acetyl functions using Ac_2O/C_5H_5N to give **5** in 93% yield overall. This modification was required in order to reduce the steric bulkiness associated with the *O*-benzoyl protecting groups that, otherwise, would slow the reactions of the wedge components in the later stages of the dendrimer synthesis. The amine protecting group in **5** was then removed by hydrogenolysis (10% Pd on C) to give **7** in excellent yield. The completely deprotected "3-mer" wedge **6** was

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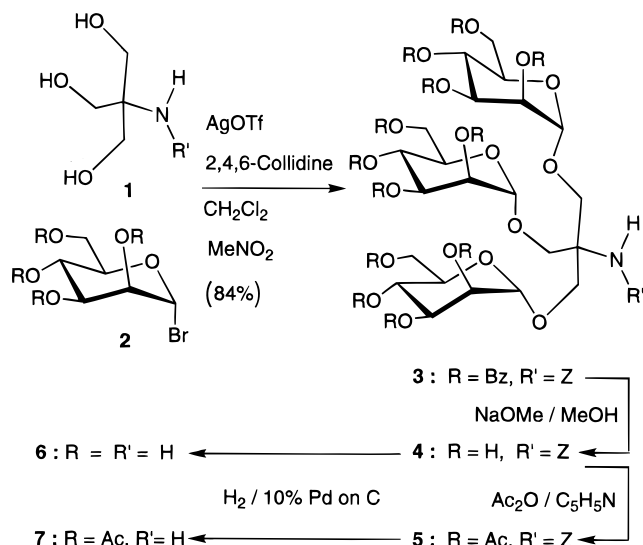
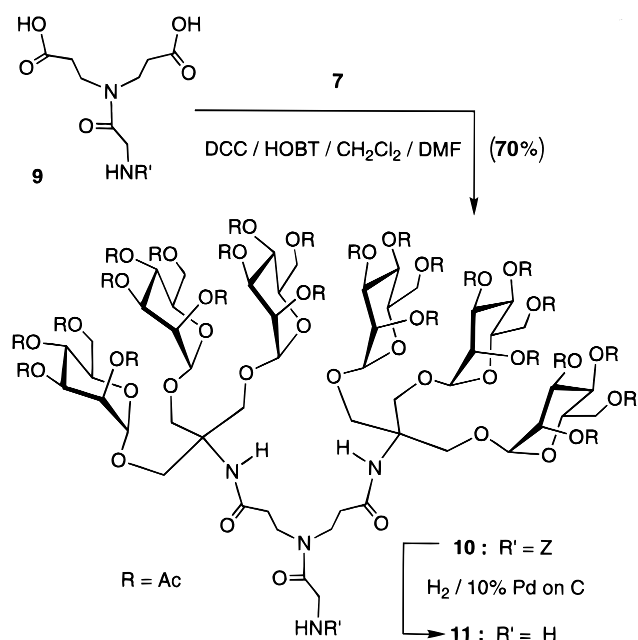
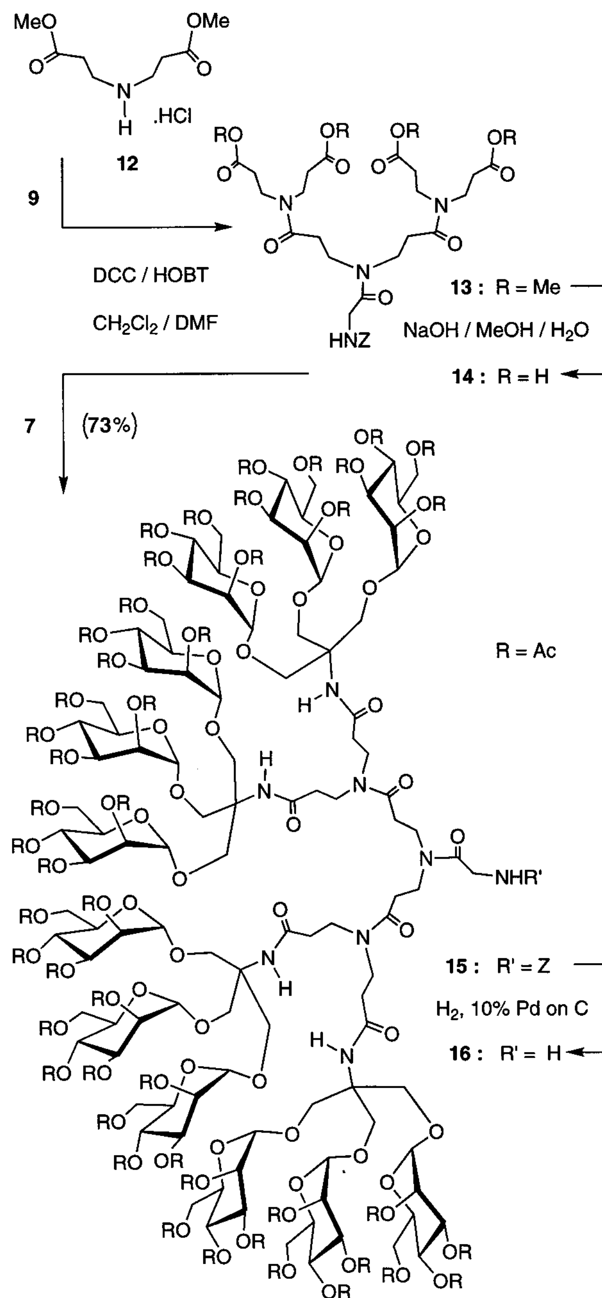
Scheme 1



Scheme 2



Scheme 3



obtained by the hydrogenolytic removal of the protecting group from **4**.

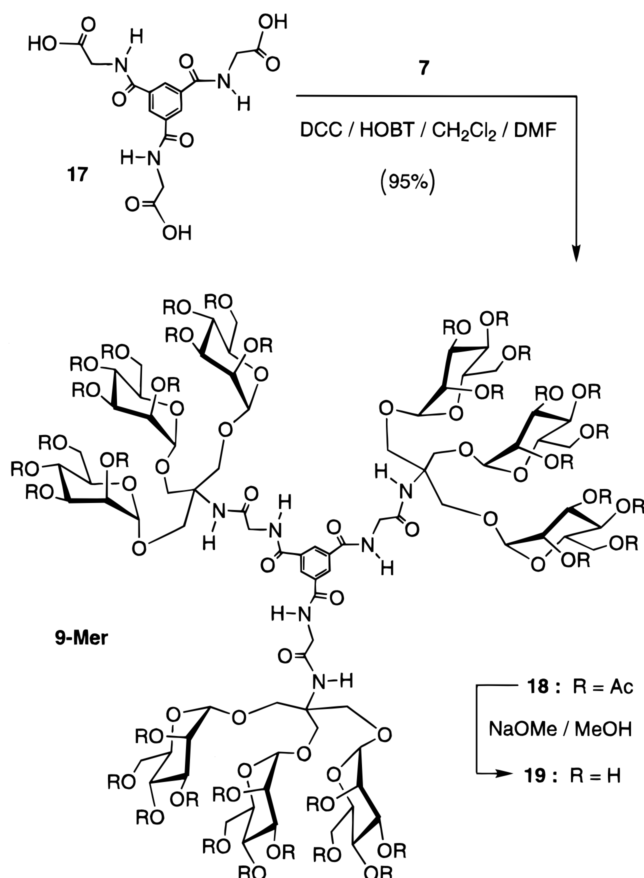
Synthesis of the Highly Branched Mannopyranoside-Containing Dendritic Wedges. These higher generation dendritic wedges were synthesized subsequently by the reaction of **7** with branched carboxylic acid derivatives acting as sources of further branching components. The amide bond-forming reactions were carried out in the presence of the DCC/HOBT coupling reagents. Thus, the reaction of **7** with **9** afforded (Scheme 2) the amino-protected “6-mer” wedge **10** in 70% yield.

The tetracarboxylic acid derivative **14** was obtained (Scheme 3) as follows: reaction of 2.0 mol equiv of **12** with 1.0 mol equiv of **9** afforded the tetramethyl ester **13**, which was then subjected to ester hydrolysis to give **14**. Amide bond formation between **7** and the tetracarboxylic acid **14** proceeded smoothly to give the amino-group protected “12-mer” wedge **15** in 73% yield. The amine protecting groups in **10** and **15** could both be easily removed by hydrogenolysis to produce the free amine wedges **11** and **16**, respectively, in good yields. All these dendritic wedges were purified by silica gel column chromatography and are stable with the exception of the free amine wedges, which lead to the formation of some intractable products when kept in solution for long periods of time.

Synthesis of the “9-Mer” 19. The tricarboxylic acid **17**, obtained by the reaction of 1,3,5-benzenetricarbonyl chloride with Gly-OMe, followed by hydrolysis of the ester functions, was used as the core for the attachment of the dendritic wedges. Amide bond formations were carried out in the presence of DCC/HOBT as the coupling reagents. The 9-mer **18** was obtained (Scheme 4) after reaction of **7** (3.3 mol equiv) with **17** (1.0 mol equiv). Removal of the *O*-acetyl protecting groups in **18** using NaOMe/MeOH afforded the final free α-D-mannopyranoside-containing 9-mer **19**.

Synthesis of the “18-Mer” 21. Following the convergent synthetic protocol, involving the minimum num-

Scheme 4



ber of reactions at the final stage of dendrimer synthesis, the preparation of the 18-mer **21** was carried out (Scheme 5) using **11** and **17** as the precursors. Reaction of the 6-mer **11** with **17**, in the presence of DCC/HOBT, afforded the 18-mer **20** in 88% yield after GPC. The facile nature of this coupling reaction was illustrated by the formation of the desired 18-mer as the exclusive product of the reaction. The free α-D-mannopyranoside-containing 18-mer **21** was obtained by deprotection (NaOMe/MeOH/H_2O).

Synthesis of the "36-Mer" 23. The synthesis of the 36-mer **23** was achieved (Scheme 6) by the so-called 12 × 3 reaction sequence, which involves the reaction of **16** (3.5 mol equiv) with **17** (1.0 mol equiv), once again in the presence of DCC/HOBT: it led to the isolation of the desired 36-mer dendrimer **22** in 39% yield, after GPC. The disubstituted product was also isolated in 30% yield. Deprotection of **22** afforded the free α-D-mannopyranoside-containing 36-mer **23**, which was subjected to GPC in order to remove salts and other trace impurities.

Structural Characterizations of Dendritic Wedges and Dendrimers. In their protected forms, all the dendritic wedges and dendrimers are soluble in a variety of organic solvents (CH_2Cl_2, EtOAc, MeOH, and THF), rendering their purifications by either column chromatographic (silica gel) or GPC (phenogel) techniques routine. In the case of the high molecular weight dendrimers, purification by GPC appears to be more suitable, since the molecular weight differences between the desired dendrimer products, other byproducts, and reagents allows their facile separation. The behavior of the elution profiles of the 36-mer **22**, 18-mer **20**, and the 9-mer **18** is shown in Figure 1. These retention times are relative to each other using a particular set of elution conditions and have not been calibrated to any commercially known standards. A similar trend of the elution profile was observed in the case of free saccharide-containing dendrimers also.

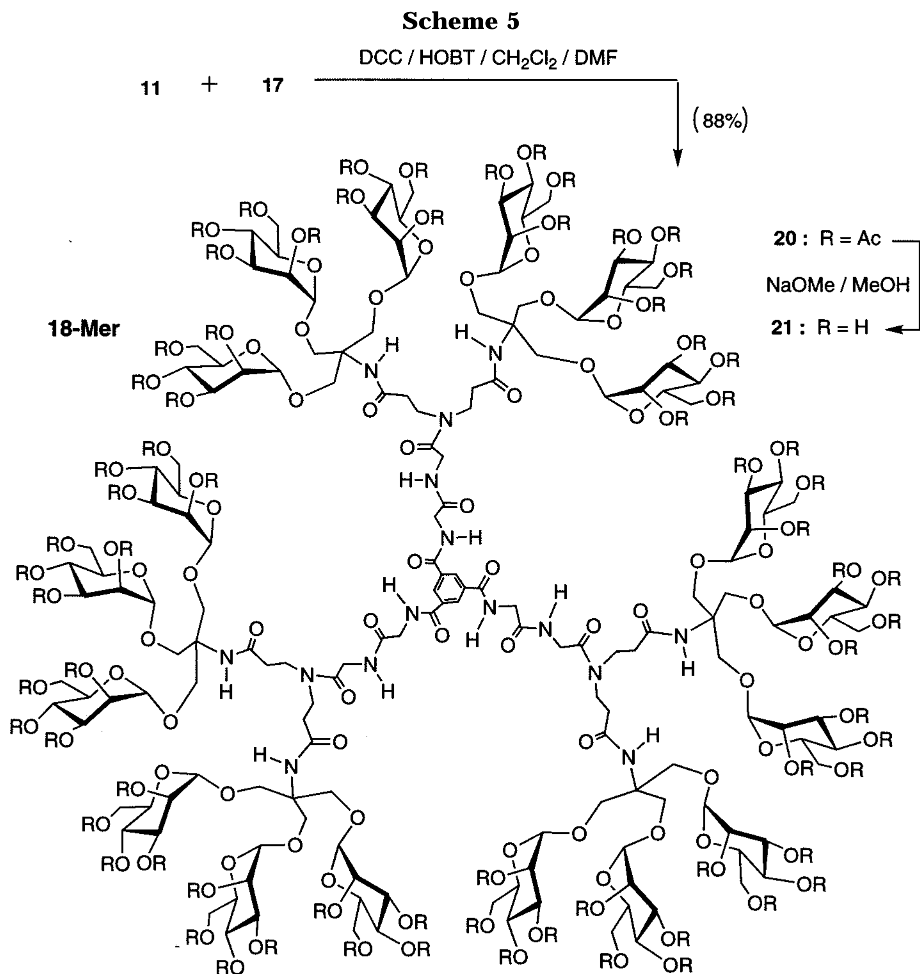
The structural characterizations of all the new compounds were achieved using common physical methods. The chirooptical properties, as measured by the specific and molar optical rotations of the dendritic wedges and dendrimers, do not change very significantly. They were found to be approximately proportional to the number of monosaccharide residues each compound possesses. The elemental analyses of all the compounds were in good agreement with the expected values.

Liquid secondary ion (LSI) and matrix-assisted laser-desorption time-of-flight (MALDI-TOF) mass spectrometries have been used to confirm the molecular weights of the dendritic wedges and dendrimers. While LSI-MS has been useful in the detection of the lower molecular weight compounds, the high molecular weight ones have been characterized by MALDI-TOF-MS. The MALDI-TOF mass spectrum of the 36-mer **22** is shown in Figure 2, wherein the protonated molecular ion peak, as well as its sodium adduct, can be observed.

^1H and ^{13}C NMR spectroscopies have also been indispensable in the characterization of all the dendritic wedges and the dendrimers. The protons associated with mannopyranosyl units, as well as with the nonsaccharide units, can be observed clearly in their ^1H NMR spectra. In keeping with our previous observations on glucopyranoside-containing dendrimers,[11a,b] the room-temperature ^1H NMR spectra of the 18-mer **20** and the 36-mer **22** were very broad, and elevated temperatures within the probe were necessary in order to obtain sharp and well-resolved resonances. ^1H and ^{13}C NMR spectra of the completely deprotected dendrimers **19**, **21**, and **23** were recorded in D_2O. A comparison of the ^1H and ^{13}C NMR chemical shift values of the dendritic wedge **6** and the dendrimers **19**, **21**, and **23** are given in Tables1 and 2, respectively. Broad-band decoupled ^{13}C NMR spectra of all dendritic wedges and the dendrimers shared the common features of the ^1H NMR spectra in that all of the resonances were sharp and "simple". In the case of the 18-mer **20** and the 36-mer **22**, the ^1H and ^{13}C NMR chemical shift assignments were confirmed further by heteronuclear multiple quantum coherence (HMQC) spectroscopy. Although all the resonances for the carbon atoms of lower molecular weight dendritic wedges and dendrimers could be observed clearly, those associated with the inner skeleton methylenes arising from glycine and propionamide residues in the higher molecular weight derivatives were difficult to detect because of their relatively small signal intensities. The observed chemical shift values and coupling constants values supported the assigned α-anomeric configuration to the D-mannopyranosyl residues present in all the dendritic wedges and dendrimers.

Biological Evaluation of the Dendrimers 6, 19, 21, and 23. Figure 3 shows the results of the ELLA with the various dendrimers as inhibitors of binding of Con A to the yeast mannan fraction Sc500. This biological evaluation of the dendrimers compares the inhibition activity to that of methyl α-D-mannopyranoside, which, in several assays, has given 50% inhibition (IC_{50}) with 0.5 mg mL^{-1} (2.5 mM). This inhibition activity of methyl α-D-mannopyranoside approximates to the activity shown

Scheme 5



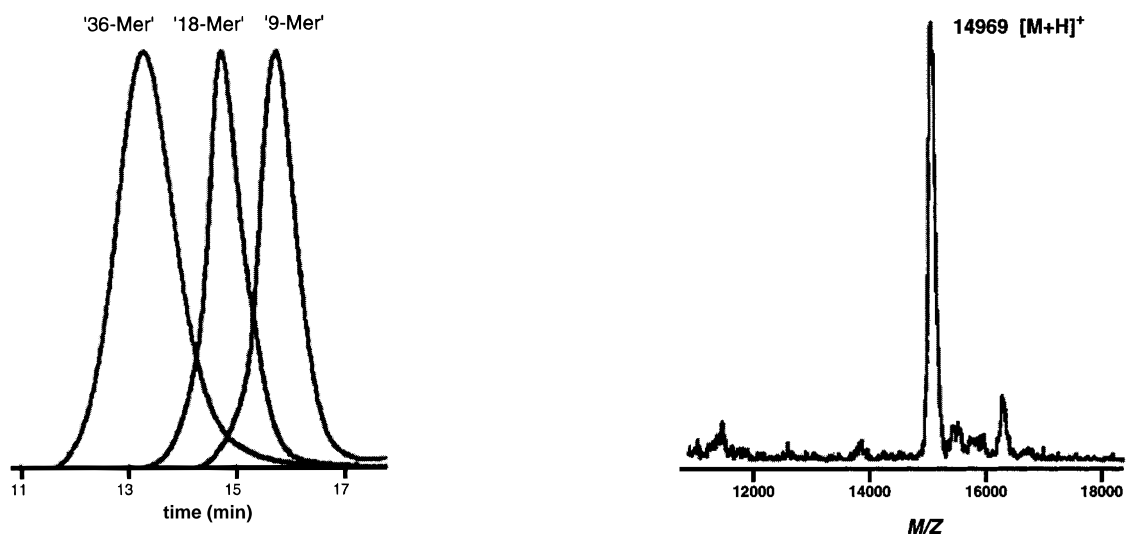


Figure 1. GPC traces of the *O*-acetylated dendrimers 9-mer **18**, the 18-mer **20**, and the 36-mer **22**.

Figure 2. MALDI-TOF mass spectrum of the 36-mer **22**.

in Figure 3 for the dendritic wedge **6** on a molar basis. The 9-mer **19** was the most active inhibitor at the mg mL^{-1} level with an IC_{50} of 0.65 mM. Thus, the inhibition potential of the 9-mer **19** is four times that of the 3-mer **6** and methyl α-D-mannopyranoside. This IC_{50} value of the 9-mer **19** compares very favorably with the increase in potency that other researchers have found for the inhibition of lectins: e.g., for sialic acid inhibition of hemagglutination of human erythrocytes by influenza virus, where the so-called "16-mer" possessing 16 sialic acid residues at the periphery of polylysine dendritic backbone, was twice as active as the "8-mer", which was found to be twice as active as the "4-mer", which, in turn, was twice as active as the dimer.[12a] Also, for Con A binding to unfractionated yeast mannan, inhibition using mannosylated poly(amidoamine) starburst dendrimers, which are most similar to the dendrimers reported in this paper, showed an increase in activity by a factor of 3.6 in going from a tri- to an octasaccharide derivative.[13c] In the present study, the 18-mer **21** and the 36-mer **23** both have similar activities when compared on a molar basis

Scheme 6

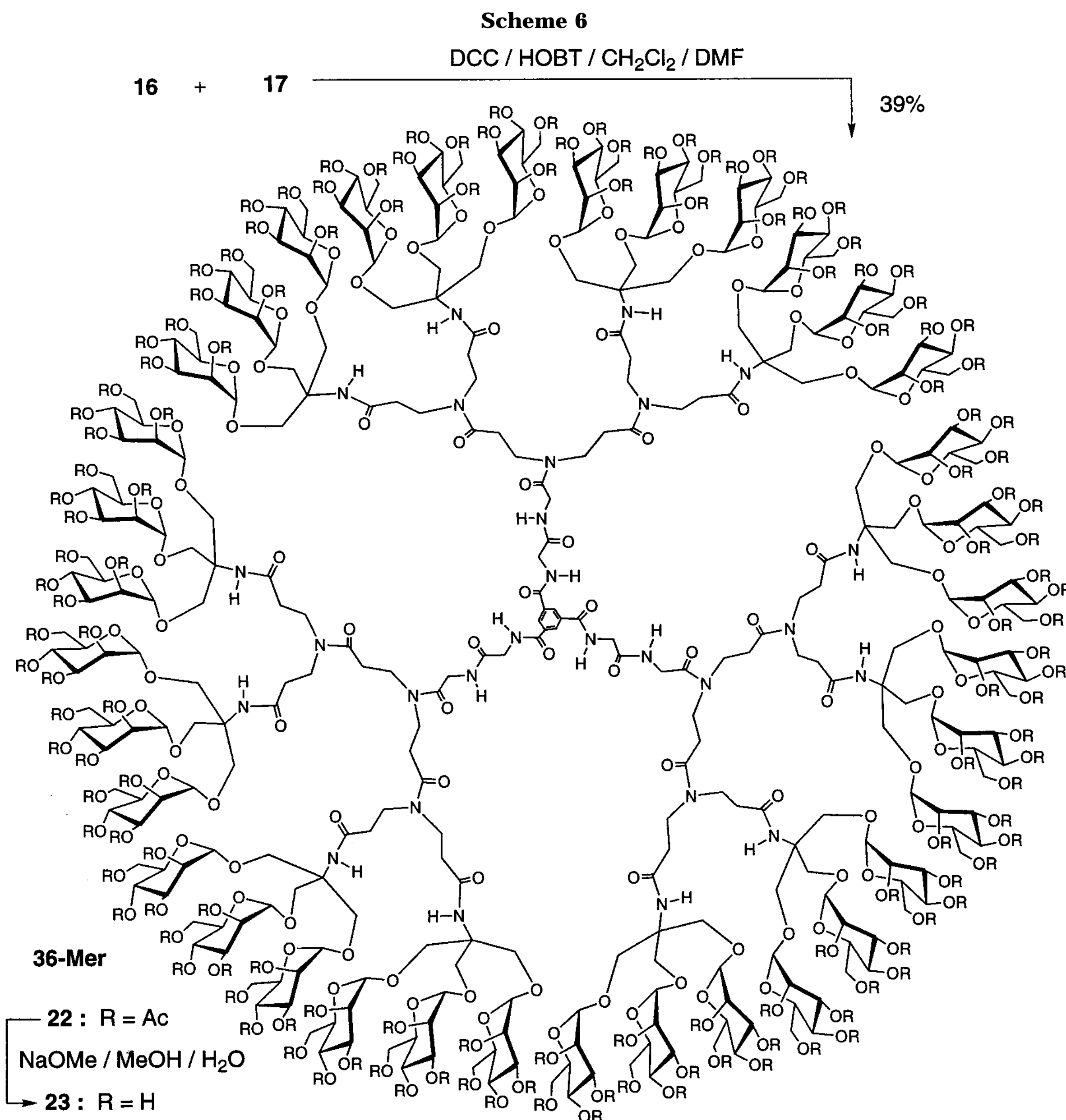


Table 1. **^{1}H NMR Spectroscopic Data (δ Values) of α-D-Mannopyranoside-Containing Dendrimers 6, 19, 21, and 23 at 300 MHz (6 and 19) and 500 MHz (21 and 23) in D_2O**

	6	19	21	23
H-1	4.74	4.76	4.72–4.76	4.76
H-2–H-6, CH_2C(quat), $GlyCH_2$, and/or CH_2CH_2	3.40–3.90	3.53–3.94, 4.03	3.42–3.92, 4.16	3.40–4.20
Ph		8.35	8.40	8.40
CH_2CH_2			2.40–2.64	2.32–2.90

with each other. Their activities are not much greater than the 9-mer **19**, suggesting that with the homogeneous, monodisperse, "complete" dendrimers reported here the clustering effect is most pronounced between the 9-mer **19** and the 18-mer **21**. Previous molecular modeling studies had shown that the peripheral monosaccharide residues cover the internal aglycon scaffold of dendrimers.[11a,b] Thus, the 9-mer **19** and the 18-mer **21** represent suitable model compounds for future studies on dendrimers having oligosaccharide sequences greater than one monosaccharide residue on each antenna for inhibition of antibodies that have a more specific binding site for functional groups, usually on tri- to heptasaccharides,[16] although some disaccharides may be active.[17]

In the present investigation, we evaluated the performance of the newly synthesized dendrimers as inhibitors of the binding of antibodies found in the serum of Crohn's patients, which recognize[15] the yeast mannan fraction Sc500. With a maximum dilution of 0.2 mg mL^{-1} of the dendrimers as inhibitors, we did not observe any inhibition. This observation was not surprising, given the fact

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Table 2. ^{13}C NMR Spectroscopic Data (δ Values) of α-D-Mannopyranoside-Containing Dendrimers 6, 19, 21, and 23 at 75.5 MHz (6 and 19) and 125.5 MHz (21 and 23) in D_2O

	6	19	21	23
C-1	102.9	103.0	102.9	102.9
C-2	72.5	72.6	72.5	72.5
C-3	73.2	73.3	73.2	73.2
C-4	69.5	69.5	69.3	68.9
C-5	75.5	75.7	75.6	75.6
C-6	63.5	63.6	63.4	63.4
*C*H$_2$C(quat)	71.5	68.6	68.1	68.2
C(quat)	56.5	56.5	62.1	62.1
GlyCH$_2$ and/or *C*H$_2$*C*H$_2$		46.2	36.7, 37.4, 43.5, 45.2,45.7, 46.2	33.2, 33.8, 36.8, 37.5, 37.7, 44.8,45.1, 45.4, 46.0, 46.9, 47.2
Ph		132.2, 137.0	132.3, 136.8	130.3, 131.4
CONH		171.5, 173.4	171.6, 172.5, 174.1, 175.3,176.1	175.1, 175.3, 175.8, 176.0, 176.1

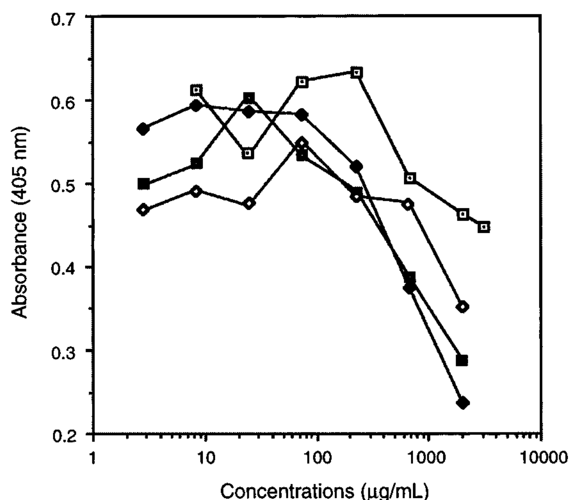

Figure 3. Inhibition of binding of biotin-labeled ConA to yeast mannan Sc500 by the free-saccharide 3-mer wedge (⊡) and the free-saccharide dendrimers 9-mer (**19**) (◆), 18-mer (**21**), (■) and 36-mer (**23**) (◇).

that more than a monosaccharide is recognized by these antibodies; all the antigenic activity is lost on acetolysis, very mild acid hydrolysis, or mild alkali elimination, which leaves significant amounts of mannopyranose residues linked to the remaining polymer, but we presume no intact Man 1 → 3 or 1 → 2 side chains. In addition to their involvement in Crohn's disease, yeast mannans are also a source of allergies for bakers and brewers who are overexposed to the yeast *Saccharomyces cerevisiae*. Although the exact nature of this epitope has not yet been established, it is most strongly expressed on the Sc500 yeast mannan fraction used in the present investigation and is therefore likely to be similar to the antigen present in Crohn's patients. The route of exposure would dictate which type of immunogenicity was invoked. In the case of the bakers' and brewers' allergies, the IgE hypersensitivity reaction can be tested by dendrimers having the appropriate di- or trisaccharide sequences in multivalent forms, and if shown to be active, these compounds could also be tried in clinical desensitization.

Conclusions

α-D-Mannopyranoside-containing dendrimers, carrying 3−36 copies of these monosaccharide units, have been synthesized using a convergent growth approach. By employing this methodology, not only have the desired dendrimers been obtained in good to excellent yields, but also a number of potentially useful multivalent dendritic wedge structures have been prepared. It should be possible, using this methodology, to synthesize very large dendritic structures by introducing additional building blocks, derived from saccharides or otherwise. The biological potencies of the 3-mer **6**, the 9-mer **19**, the 18-mer **21**, and the 36-mer **23** have been evaluated using an enzyme-linked lectin assay procedure and have been shown to exhibit most pronounced inhibitory effects between the 9-mer **19** and the 18-mer **21** in this particular assay, when compared with methyl α-D-mannopyranoside, the monomer analogue of the α-D-mannopyranoside-containing dendrimers.

Experimental Section

General Methods. Chemicals were purchased from Aldrich and used as received. Solvents were either used as purchased or dried according to procedures described in the literature.[18] TLC was carried out using aluminum sheets precoated with silica gel 60F. The plates were inspected by UV light or by treatment with 5% H_2SO_4 in EtOH, followed by heating the plates. Column chromatography was carried out using silica gel 60F (230−400 mesh). Gel permeation chromatography (GPC) of the fully protected dendrimers was carried out on a Phenogel (500 and 1000 Å) semipreparative columns (300 × 7.80 mm) attached to a high-performance liquid chromatography system fitted with a UV-detector. Detection was carried out at 254, nm and GPC-grade THF was used for the elutions. The fully deprotected dendrimers were purified on a Fractogel (25−40 µm) column (100 × 0.25 cm) fitted with a differential refractometer with deionized and degassed water as eluant. Microanalysis were performed by the University of North London Microanalytical Service. Liquid secondary ion mass spectra (LSI-MS) were recorded using *m*-nitrobenzyl alcohol matrix. Matrix-assisted laser desorption ionization-time-of-flight mass spectra (MALDI-TOF-MS) was performed using a *trans*-indoleacrylic acid matrix and an average of 50 laser shots per sample. Optical rotations were performed at 23 °C. ^{1}H NMR and ^{13}C NMR spectra were recorded on a 300 and 75.5 MHz spectrometer, a 400 and 100.6 MHz spectrometer, or a 500 and 125.5 MHz spectrometer with either the residual solvent or TMS as internal standards. For studies in D_2O, TSP was used as the external reference. The chemical shifts are expressed in ppm, and the coupling constants from the ^{1}H NMR spectra in hertz (Hz) and are within a ca. 0.2 Hz error range. The following abbreviations were used to explain the multiplicities: s, singlet; d, doublet; t, triplet; m, multiplet; dd, double doublet; app d, apparent doublet; app t, apparent triplet; band, several overlapping signals; br, broad. The ^{1}H and ^{13}C NMR chemical

(18) Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*, 3rd ed.; Pergamon Press: Oxford, 1988.

shift values of deprotected dendritic wedge **6** and deprotected dendrimers **19**, **21**, and **23** are given in Tables 1 and 2, respectively.

***N*-(Benzyloxycarbonyl)tris[[(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)oxy]methyl]methylamine (3)** A solution of 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide (**2**)[19] (6.0 g, 9.10 mmol) in CH_2Cl_2 (10 mL) was added dropwise over a period of 0.5 h to a stirring mixture of **1**[11a] (0.67 g, 2.63 mmol), AgOTf (2.34 g, 9.10 mmol), and 2,4,6-collidine (0.943 g, 7.78 mmol) in CH_2Cl_2 (20 mL) and $MeNO_2$ (20 mL) at −25 to −30 °C. Stirring was continued for 0.5 h at the same temperature, and then the reaction mixture was allowed to reach room temperature before being stirred overnight. C_5H_5N (2.0 mL) was then added to the reaction mixture, which was diluted with CH_2Cl_2 (50 mL) before being filtered over Celite. The filtrate was washed successively with aqueous $Na_2S_2O_3$ (1 M) solution (3 × 30 mL), aqueous $NaHCO_3$ (1 M) solution (3 × 30 mL), and H_2O (2 × 20 mL) and dried, before the solvents were evaporated off in vacuo. Purification by column chromatography (PhMe/EtOAc 95:5) afforded **3** (4.36 g, 84%) as a white foamy solid: TLC R_f (PhMe/EtOAc 9:1) 0.58 (UV, H_2SO_4); $[\alpha]_D$ −19.2° (*c* 1, $CHCl_3$); ^1H NMR (300 MHz, CD_3COCD_3) δ 4.38 (3H, d, *J* = 10.3 Hz), 4.67 (3H, d, *J* = 10.3 Hz), 4.73 (3H, dd, *J* = 2.5, 12.4 Hz), 4.88 (6H, band), 5.17 (1H, d, *J* = 12.1 Hz), 5.25 (1H, d, *J* = 12.1 Hz), 5.48 (3H, app s), 5.77 (1H, br), 5.97 (3H, app.t), 6.09 (3H, dd, *J* = 2.94, 10.1 Hz), 6.31 (3H, app t, *J* = 10.1 Hz), 7.03−8.30 (65H, m); ^{13}C NMR (75.5 MHz, CD_3COCD_3) δ 59.9, 63.4, 67.1, 67.3, 68.0, 70.4, 71.2, 71.6, 99.6, 126.1−138.4, 156.6, 165.8−166.4; MALDI-TOF-MS *m/z* 2012 $[M + Na]^+$. Anal. Calcd for $C_{114}H_{95}NO_{32}$: C, 68.78; H, 4.78; N, 0.70. Found: C, 68.84; H, 4.75; N, 0.74.

***N*-(Benzyloxycarbonyl)tris[(α-D-mannopyranosyloxy)-methyl]methylamine (4).** A solution of **3** (4.14 g, 2.08 mmol) in 0.1 M methanolic NaOMe (250 mL) was stirred at room temperature for 15 h before being neutralized with Amberlite IR-120 (H^+ form) ion-exchange resin and filtered. After the solvents had been removed in vacuo, the resulting mixture was dissolved in H_2O (15 mL) and extracted with Et_2O (2 × 20 mL), and the aqueous portion was evaporated before being dried thoroughly to obtain **4** (1.46 g, 95%): $[\alpha]_D$ +61.4° (*c* 1, H_2O); ^1H NMR (300 MHz, D_2O) δ 3.42−3.92 (39H, band), 5.05 (2H, s), 7.24−7.42 (5H, m); ^{13}C NMR (75.5 MHz, CD_3COCD_3) δ 61.1, 63.4, 68.5, 69.3, 72.5, 73.2, 75.6, 102.9, 130.3−139.1, 158.2; MALDI-TOF-MS *m/z* 764 $[M + Na]^+$. Anal. Calcd for $C_{30}H_{47}NO_{20}$: C, 48.58; H, 6.34; N, 1.89. Found: C, 48.61; H, 6.44; N, 1.87.

Tris[[(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-oxy]methyl]methylamine (7). Acetic anhydride (4.2 mL) was added to a solution of **4** (1.1) in C_5H_5N (7.24 mL), which was stirred at room temperature for 8 h before being evaporated in vacuo. The resulting residue was dissolved in EtOAc (70 mL), washed with saturated aqueous $NaHCO_3$ solution (3 × 25 mL) and H_2O (2 × 25 mL), and dried before the solvents were evaporated off to give **5** (1.72 g, 93%) as a foamy powder. A suspension of **5** (1.50 g) in EtOAc/MeOH (2:1) (50 mL) and 10% Pd/C (0.75 g) was subjected to hydrogenolysis by using a balloon filled with H_2 gas for 12 h. After filtration over Celite, the solvents were removed in vacuo to yield **7** (1.25 g, 94%) as a white foamy powder: TLC R_f (EtOAc) 0.19 (H_2SO_4); $[\alpha]_D$ +54.8° (*c* 1, $CHCl_3$); ^1H NMR (500 MHz, CD_3COCD_3) δ 1.93, 2.01, 2.03, 2.09 (36H, 4 × s), 3.52 (3H, d, *J* = 10.0 Hz), 3.80 (3H, d, 10.0 Hz), 4.07−4.13 (6H, band), 4.28 (3H, dd, *J* = 5.5, 12.0 Hz), 4.91 (3H, d, *J* = 1.8 Hz), 5.22−5.30 (9H, band); ^{13}C NMR (125.5 MHz, CD_3COCD_3) δ 20.6−20.7, 56.4, 63.1, 66.8, 69.8, 70.0, 70.1, 70.8, 99.1, 170.2−170.8; MALDI-TOF-MS *m/z* 1112 $[M + H]^+$. Anal. Calcd for $C_{46}H_{65}NO_{30}$: C, 49.73; H, 5.86; N, 1.26. Found: C, 49.75; H, 6.06; N, 1.27.

General Procedure for the Deacylations. A solution of the acylated dendritic wedges or dendrimers in MeOH or in MeOH/H_2O was treated with 1 M NaOMe and left stirring at room temperature before being neutralized with Amberlite IR-120 (H^+ form) ion-exchange resin and filtered. The solvents were then removed in vacuo, the resulting residue was dissolved in a minimum amount of H_2O and extracted with Et_2O, and the aqueous portion was evaporated in vacuo. The resulting residue was subjected to GPC purification to afford the completely deacylated dendritic wedges and dendrimers.

Tris[(α-D-mannopyranosyloxy)methyl]methylamine 3-Mer (6). A suspension of **4** (0.250 g) in MeOH/H_2O (8:2) (10 mL) containing 10% Pd/C (0.10 g) was subjected to hydrogenolysis using a balloon filled with H_2 gas for 16 h. After filtration over Celite, and the solvents were removed in vacuo to afford **6** (0.160 g, 76%): $[\alpha]_D$ +58.8° (*c* 0.9, H_2O); MALDI-TOF-MS *m/z* 630 $[M + Na]^+$.

General Procedure for the Amide Bond Formation. A solution of the amine component (1.0−1.2 mol equiv) in CH_2Cl_2 was added to a stirred solution of the carboxylic acid component, DCC (1.0−1.2 mol equiv), and HOBT (1.0−1.2 mol equiv) in CH_2Cl_2/DMF (2:1) at 0 °C under a N_2 blanket. The reaction mixture was stirred at room temperature and filtered, and the solvents were evaporated off. The resulting residue was dissolved in EtOAc and washed successively with 5% aqueous HCl solution (30 mL), saturated $NaHCO_3$ solution (30 mL), and H_2O (15 mL), before being dried. The solvents were evaporated off in vacuo, and the crude product was purified either by column chromatography or by gel permeation chromatography.

6-Mer Amine Wedge (11). A solution of **7** (0.60 g, 0.54 mmol) in CH_2Cl_2 (15 mL) was added dropwise to a stirred solution of **9**[11a] (0.091 g, 0.26 mmol), DCC (0.112 g, 0.54 mmol), and HOBT (0.073 g, 0.54 mmol) in CH_2Cl_2/DMF (2:1) (40 mL) at 0 °C under a N_2 blanket. The reaction mixture was allowed to stir at room temperature for 55 h before being worked up as described in the general procedure to obtain the *N*-(benzyloxycarbonyl) group-protected derivative **10** (0.470 g, 70%). A suspension of **10** (0.450 g) in EtOAc/MeOH (2:1) (15 mL) containing 10% Pd/C (0.250 g) was subjected to hydrogenolysis as described for **7** to afford **11** (0.40 g, 93%): $[\alpha]_D$ +49.4° (*c* 1, $CHCl_3$); ^1H NMR (400 MHz, CD_3COCD_3) δ 1.93, 2.01, 2.03, 2.09 (72H, 4 × s), 2.57 (4H, br), 3.59 (4H, br), 3.90 (6H, d, *J* = 9.9 Hz), 3.98−4.23 (20H, band), 4.29 (6H, dd, *J* = 3.7, 11.6 Hz), 4.89 (6H, d, *J* = 2.9 Hz), 5.19−5.31 (18H, band), 7.47 (2H, b); ^{13}C NMR (75.5 MHz, CD_3COCD_3) δ 20.3, 20.37, 20.41, 20.47, 25.5, 26.2, 34.3, 54.7, 59.8, 62.6, 66.3, 69.6, 69.7, 98.8, 169.9, 170.1, 170.2, 170.3, 170.6; MALDI-TOF-MS *m/z* 2428 $[M + Na]^+$. Anal. Calcd for $C_{100}H_{140}N_4O_{63}$: C, 49.92; H, 5.82; N, 2.33. Found: C, 49.88; H, 5.91; N, 2.30.

Tetracarboxylic Acid Wedge (14). Et_3N (1.67 mL, 12.0 mmol) was added to a stirred suspension of **12**·HCl (2.0 g, 8.89 mmol) in CH_2Cl_2 (15 mL) at 0 °C. After 0.75 h, Et_2O was added, the reaction mixture was filtered, and the solvents were removed to obtain 3,3′-iminobis(methyl propionate) **12** as an oil (1.42 g, 85%). A solution of **12** (1.14 g, 6.03 mmol) in CH_2Cl_2 was added dropwise to a stirred solution of **9** (0.91 g, 2.50 mmol), DCC (1.13 g, 5.50 mmol), and HOBT (0.74 g, 5.50 mmol) in CH_2Cl_2/DMF (2:1) (15 mL) at 0 °C. The reaction mixture was stirred at room temperature for 30 h before being worked up as described in the general procedure to obtain **13** (1.68 g, 93%). A solution of **13** (1.60 g, 2.30 mmol) in MeOH (35 mL) was added to a 2 M aqueous NaOH solution (9.7 mL) at 0 °C, and then it was stirred for 5 h. The precipitated material was dissolved in a minimum amount of H_2O. The aqueous solution was neutralized with Amberlite (H^+ form) ion-exchange resin, filtered, evaporated, and dried thoroughly to afford **14** (1.42 g, 95%) as an oil: ^1H NMR (300 MHz, CD_3SOCD_3) δ 2.45 (4H, br t), 2.58 (8H, br), 3.49 (8H, br), 3.59 (4H, br), 3.93 (2H, d), 5.05 (2H, s), 7.20 (1H, br t), 7.38 (5H, br s); ^{13}C NMR (75.5 MHz, CD_3SOCD_3) δ 30.6, 31.4, 32.1, 32.4, 33.0, 33.5, 41.5, 42.0, 42.4, 43.4, 51.5, 65.5, 127.8, 128.5, 137.2, 156.5, 168.6, 170.3, 171.7, 172.0, 172.8, 173.2; LSI-MS *m/z* 639 $[M + H]^+$.

12-Mer Amine Wedge (16). A solution of **7** (0.90 g, 0.81 mmol) in CH_2Cl_2 (20 mL) was added with stirring to a solution of **14** (0.123 g, 0.193 mmol), DCC (0.167 g, 0.81 mmol), and HOBT (0.110 g, 0.81 mmol) in CH_2Cl_2/DMF (2:1) (60 mL) under a N_2 blanket at 0 °C. The reaction mixture was stirred

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at room temperature for 72 h before being worked up as described in the general procedure to obtain **15** (0.70 g, 73%). A suspension of **15** (0.50 g) and 10% Pd/C (0.40 g) in EtOAc/MeOH (2:1) (25 mL) was subjected to hydrogenolysis for 30 h as described for **7** to afford **16** (0.402 g, 82%): $[\alpha]_D$ +52.8° (*c* 1.24, $CHCl_3$); ^1H NMR (400 MHz, CD_3COCD_3) δ 1.95, 2.02, 2.05, 2.10 (144H, 4 × s), 2.43, 2.58 (12H, 2 × br), 3.60 (12H, br), 3.86−3.97 (12H, band), 4.0−4.21 (38H, band), 4.29 (12H, dd, J = 5.20, 12.30 Hz), 4.95 (12H, br), 5.20−5.30 (36H, band), 7.42 (4H, br); ^{13}C NMR (75.5 MHz, CD_3COCD_3) δ 20.5, 20.6, 20.7, 25.6, 26.4, 34.4, 56.1, 60.0, 62.8, 62.9, 66.5, 66.6, 69.6, 69.7, 69.9, 70.0, 99.0, 170.1−170.8; LSI-MS *m/z* 4881 $[M + H]^+$. Anal. Calcd for $C_{204}H_{284}N_8O_{127}$: C, 50.21; H, 5.87; N, 2.30. Found: C, 50.15; H, 5.75; N, 2.21.

Peracetylated 9-Mer Dendrimer (18). A solution of **7** (0.30 g, 0.270 mmol) in CH_2Cl_2 (10 mL) was added dropwise to a stirred solution of **17**[11a] (0.031 g, 0.082 mmol), DCC (0.052 g, 0.253 mmol), and HOBT (0.034 g, 0.253 mmol) in CH_2Cl_2/DMF (2:1) (15 mL) at 0 °C under an N_2 blanket. The reaction mixture was left stirring at room temperature for 30 h before being worked up as described in the general procedure to obtain the acetylated dendrimer **18** (0.290 g, 96%): $[\alpha]_D$ +30.7° (*c* 0.7, $CHCl_3$); ^1H NMR (400 MHz, CD_3COCD_3) δ 1.95, 1.97, 2.02, 2.06 (108H, 4 × s), 3.92 (9H, d, J = 9.8 Hz), 4.01−4.14 (24H, band), 4.18 (9H, d, J = 9.8 Hz), 4.26 (9H, dd, J = 5.6, 12.0 Hz), 4.90 (9H, app s), 5.19−5.29 (27H, band), 7.48 (3H, s), 8.02 (3H, br t), 8.53 (3H, s); ^{13}C NMR (75.5 MHz, CD_3COCD_3) δ 20.4−20.5, 43.8, 60.1, 62.7, 66.2, 66.3, 69.6, 69.7, 98.7, 128.8, 135.5, 166.4, 170.0−170.4; MALDI-TOF-MS *m/z* 3684 $[M + Na]^+$. Anal. Calcd for $C_{153}H_{204}N_6O_{96}$: C, 50.12; H, 5.57; N, 2.29. Found: C, 49.95; H, 5.57; N, 2.34.

9-Mer Dendrimer (19). Deacetylation of **18** (0.150 g, 0.041 mmol) was carried out in 0.1 M methanolic NaOMe solution (20 mL) for 6 h, followed by workup and purification as described in the general procedure for deacylations to afford **19** (0.060 g, 68%) as a glassy solid: $[\alpha]_D$ +58.3° (*c* 1.25, H_2O); MALDI-TOF-MS *m/z* 2171 $[M + Na]^+$.

Peracetylated 18-Mer Dendrimer (20). A solution of **11** (0.50 g, 0.208 mmol) in CH_2Cl_2 (10 mL) was added dropwise to a stirred solution of **17** (0.024 g, 0.063 mmol), DCC (0.043 g, 0.208 mmol), and HOBT (0.028 g, 0.208 mmol) in CH_2Cl_2/DMF (2:1) (30 mL) at 0 °C under an N_2 blanket. The reaction mixture was left stirring at room temperature for 60 h and then worked up as described in the general procedure to obtain the acetylated dendrimer **20** (0.420 g, 88%) as a foamy powder that was purified by GPC: $[\alpha]_D$ +34.4° (*c* 0.7, $CHCl_3$); ^1H NMR (500 MHz, CD_3SOCD_3 353 K) δ 1.92, 1.98, 2.01, 2.09 (∼216H, 4 × s), 2.36 (12H, br), 3.48 (12H, br), 3.76 (18H, d, J = 10.0 Hz), 3.91−4.22 (84H, band), 4.82 (18H, app s), 5.08−5.19 (54H, band), 7.62 (6H, br), 8.50 (3H, s), 8.55−8.72 (6H, br); ^{13}C NMR (125.5 MHz, CD_3SOCD_3) δ 20.2, 20.5, 20.7, 20.9, 33.6, 42.4, 58.5, 61.6, 65.2, 65.3, 68.5, 68.6, 97.3, 128.0, 139.1, 169.3, 169.4, 169.5, 169.9, 170.4; MALDI-TOF-MS *m/z* 7580 $[M + Na]^+$. Anal. Calcd for $C_{315}H_{429}N_{15}O_{195}$: C, 50.16; H, 5.73; N, 2.79. Found: C, 50.45; H, 5.80; N, 2.81.

18-Mer Dendrimer (21). The deacetylation of **20** (0.150 g, 0.020 mmol) in $MeOH/H_2O$ (1:1) (20 mL) was performed using 1 M NaOMe in MeOH (0.8 mL) 24 h, followed by workup and purification as described in the general procedure for deacylations to afford 2**1** (0.042 g, 45%) as a glassy solid: $[\alpha]_D$ +54.7° (*c* 1.12, H_2O).

Peracetylated 36-Mer Dendrimer (22). A solution of **16** (0.450 g, 0.092 mmol) in CH_2Cl_2 (15 mL) was added dropwise to a stirred solution of **17** (0.01 g, 0.026 mmol), DCC (0.031 g, 0.082 mmol), and HOBT (0.011 g, 0.082 mmol) in CH_2Cl_2/DMF (2:1) (50 mL) at 0 °C under an N_2 blanket. The reaction mixture was left stirring at room temperature for 5 days and then worked up as described in the general procedure to obtain the *O*-acetylated dendrimer **20**, which was purified by GPC to provide **22** (0.154 g, 39%): $[\alpha]_D$ +36.4° (*c* 0.9, $CHCl_3$); ^1H NMR (500 MHz, CD_3SOCD_3, 353 K) δ 1.92, 1.97, 2.01, 2.08 (432H, 4 × s), 2.41 (24H, br), 2.68 (12H, br), 3.46 (36H, br), 3.77 (36H, d, J = 9.5 Hz), 3.95 (84H, br), 4.07 (36H, app d, J = 12.0 Hz), 4.20 (36H, dd, J = 5, 12.0 Hz), 4.82 (36H, app s), 5.08−5.18 (108H, band), 7.58 (12H, br), 8.50 (9H, br); ^{13}C NMR (125.5 MHz, CD_3SOCD_3) δ 20.2, 20.4, 21.0, 33.6, 43.4, 58.4, 61.5, 65.2, 65.6, 68.2, 68.5, 68.6, 97.4, 128.0, 139.1, 169.3, 169.4, 169.5, 169.9, 171.3; MALDI-TOF-MS *m/z* 14969 $[M + H]^+$. Anal. Calcd for $C_{627}H_{861}N_{27}O_{387}$: C, 50.31; H, 5.80; N, 2.53. Found: C, 50.38; H, 5.74; N, 2.61.

36-Mer Dendrimer (23). The deacetylation of **22** (0.10 g, 0.007 mmol) in $MeOH/H_2O$ (1:1) (10 mL) was performed using a 1 M methanolic NaOMe solution (1.0 mL) for 30 h, followed by workup and purification as described in the general procedure for deacylations, to afford **23** (0.025 g, 42%) as a glassy solid: $[\alpha]_D$ +50.2° (*c* 1.35 H_2O).

Enzyme-Linked Immuno Sorbent Assay (ELISA). Wells of Nunc Maxisorp U96 microtiter plates were coated with a 100 μL solution of antigen (Sc500, 100 ng mL^{-1} in PBS, pH 7.5). The plate was incubated for 16−20 h at 4 °C (in the refrigerator). The coating solution was removed, and the wells were blocked by the addition of 200 μL 0.5% BSA in PBS, pH 7.5, with incubation for 4 h at 4 °C. The blocking solution was removed, and serial dilutions of the dendrimers were added in 0.5% BSA in PBS, 50 μL to each well, followed by 50 μL solution of (monoclonal antibody or) serum-containing antibodies (diluted 1:200 in 0.5% BSA in PBS). The plate was incubated for 16−24 h at 4 °C, after 1 h of shaking at 20 °C. The excess of the solution was removed, and the wells were washed with 2 × 200 μL PBS, pH 7.5. The peroxidase-labeled second antibody (100 μL of anti-IgG solution diluted 1:1000 in 0.5% BSA in PBS, pH 7.5) was added to each well and the plate incubated for 4 h at 20 °C. The solutions were removed and the wells washed with 3 × 200 μL PBS, pH 7.5. A freshly prepared solution (100 μL) of *p*-nitrophenyl phosphate (1 mg mL^{-1}) (Sigma alkaline phosphatase substrate) in 0.1 M Tris, pH 10 was added to each well. The plate was incubated at 20 °C until an appropriate color was generated (30−60 min, highest value ca. 1) and the absorbance read at 405 nm using a microplate reader.

Enzyme-Linked Lectin Assay (ELLA) with Con A. Wells of Nunc Maxisorp U96 microtiter plates were coated with a 100 μL solution of *S. cerevisiae* mannan (100 ng mL^{-1} in PBS, pH 7.5). The plate was incubated for 16−20 h at 4 °C (in the refrigerator). The coating solution was removed, and the wells were blocked by the addition of 200 μL 0.5% BSA in PBS, pH 7.5, with incubation for 4 h at 4 °C. Serial dilutions of the dendrimers or methyl α-D-mannopyranoside were made up in 0.5% BSA in PBS, pH 7.5, and 50 μL added to each well, followed by 50 μL of biotin-labeled ConA (10 μg mL^{-1} in 0.5% BSA in PBS, pH 7.5, containing 1 mM $CaCl_2$ and 1 mM $MgCl_2$). The plate was again incubated for 16−24 h at 40 °C, after 1 h of shaking at 20 °C. The excess of the solution was removed, and the wells were washed with 2 × 200 μL PBS, pH 7.5. To each well was added 100 μL of antibiotin solution diluted 1:15 000 in 0.5% BSA, pH 7.5, and containing 1 mM $CaCl_2$ and 1 mM $MgCl_2$, and the plate was incubated for 4 h at room temperature (20 °C). The solutions were removed and the wells washed with 3 × 200 μL of PBS, pH 7.5. To each well was added 100 μL of a freshly prepared solution of *p*-nitrophenyl phosphate (1 mg mL^{-1} in 0.1 M Tris, pH 10). The plate was incubated until an appropriate color was generated (30−90 min) at 20 °C and the absorbance read at 405 nm on a microplate reader.

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