



Macromolecular Recognition: Effect of Multivalency in the Inhibition of Binding of Yeast Mannan to Concanavalin A and Pea Lectins by Mannosylated Dendrimers

Daniel Pagé, Diana Zanini and René Roy*

Department of Chemistry, University of Ottawa, Ottawa, ON, Canada K1N 6N5

Abstract—The synthesis and binding properties of a new family of high affinity α -D-mannopyranoside ligands are described. The synthesis of the new multivalent ligands is based on the scaffolding of multiantennary branches of L-lysine residues having electrophilic *N*-chloroacetylated end groups as core structures. An α -D-mannopyranoside with *p*-substituted aryl aglycon ending with a thiol group was prepared and covalently attached to each of the branches of the dendritic structures. The resulting glycodendrimers with 2 (**12**), 4 (**14**), 8 (**16**), and 16 (**18**) mannoside residues were tested for their relative inhibitory potency by solid-phase enzyme-linked lectin assays (ELLA) using methyl and *p*-nitrophenyl α -D-mannopyranosides as standards. Concentrations necessary for 50% inhibition (IC_{50} s) of binding of yeast mannan to Jack bean phytohemagglutinin (*Canavalia ensiformis*, concanavalin A) and to pea lectin (*Pisum sativum*) were determined. Analogous mannosylated copolyacrylamides were also prepared for comparison. The IC_{50} values were also plotted as a function of dendrimer valencies. The inhibitions showed 16-mer **18** to be approximately 600- and 2000-fold more potent than methyl α -D-mannopyranoside, and 66- and 1383-fold more potent than *p*-nitrophenyl α -D-mannopyranosides with Con A and pea lectins, respectively. Even when these numbers are expressed relative to single mannosylated residues per dendrimers, the relative potencies against the aromatic mannoside are still 4- and 86-fold better against Con A and pea lectins. These results unequivocally indicate that the optimum inhibitory binding properties of the new mannosylated dendrimers vary with both dendrimer and lectin valencies. Copyright © 1996 Elsevier Science Ltd

Introduction

Carbohydrate–protein interactions are ubiquitous at the cellular level. Such interactions have been involved in numerous pathogenic infections, inflammation processes, metastasis, and (glyco)protein regulations and trafficking.¹ It has been generally admitted that intrinsic carbohydrate–protein interactions are usually of low affinities unless carbohydrate ligands are organized as multivalent clusters ('cluster effects').² Hence, multivalent carbohydrate–protein interactions have been recognized as critical features for tight and perhaps specific binding associations responsible for the biological effects of interest. For instance, divalent α -sialoside and sialyl Lewis-X clusters have been shown to bind with only little improvement to their respective influenza virus hemagglutinin³ and selectin receptors,⁴ while polymeric α -sialosides⁵ and sialyl Lewis-X⁶ showed up to 1000-fold increases in binding properties.

Mannose-binding receptors found on macrophages,⁷ hepatic sinusoidal cells,⁸ fimbriated bacterial pathogens,⁹ serum-type mannose binding proteins (MBP),⁷ placental mannose receptors,¹⁰ and phytohemagglutinins (lectins)¹¹ appear to differ significantly in their ligand-cluster requirements. Small bi- and tri-sected oligomannose-type glycopeptide sequences can show several 100-fold increases in binding properties relative to plant and hepatic lectins when compared to monosaccharides.¹² Serum-type mannose-binding protein (MBP), an important defense molecule, recognizes monosaccharides with low affinity (K_D 0.1–1

mM), whereas high affinity ligands ($K_D \sim$ nM) can be generated when the same monosaccharides are attached to a macromolecular carrier such as bovine serum albumin.¹³ However, MBP, as opposed to asialoglycoprotein hepatic receptors, does not express significant binding enhancement toward ligands of low valency.¹⁴ As many of the above receptors have carbohydrate-recognition domains (CRDs) also organized as clusters,^{13,15} it is important to have access to well characterized multivalent mannoses for binding studies. Such glycoconjugates would be of great value for the fundamental understanding of multivalent interactions.

In some ways, neoglycoproteins¹² and carbohydrate-containing polymers ('glycopolymers')^{16,17} fulfill the criteria of multivalency. However, one of the major drawbacks of neoglycoproteins and glycopolymers is the heterogeneity of their carbohydrate contents and positions. This feature alone has major implications in quantitative inhibition experiments. Moreover, the exact locations of the carbohydrate residues are ill-defined and randomly distributed.

In order to address these drawbacks, we recently designed potent carbohydrate inhibitors. The synthesis and binding properties of this new family of multivalent and high affinity carbohydrate ligands have been reviewed.¹⁸ This new class of compounds, designed to better mimic multiantennary glycoproteins, has been synthesized using an approach commensurate to recently developed dendrimers.¹⁹ These dendritic

carbohydrate derivatives ('glycodendrimers') constitute a new family of glycoconjugates which demonstrated high inhibitory potential against influenza virus hemagglutinins.^{20,21} To further exploit the beneficial binding properties of glycodendrimers, we describe herein the synthesis of dendritic α -D-mannopyranosides having well organized and well characterized multivalencies. Their inhibition and binding properties have been evaluated using two plant lectins as models.

Results and Discussion

The mannose binding lectins from *Canavalia ensiformis* (concanavalin A, Con A) and from *Pisum sativum* (pea lectin) have been studied in detail.¹¹ The energetics of the binding interactions^{22,23} and the X-ray^{24,25} data with co-crystallized mannose and trimannoside derivatives are available for both Con A^{23,24} and for pea lectin.^{22,25} Moreover, it has been demonstrated that both lectins showed preferential binding for methyl α -D-mannopyranoside over D-mannose (α -specificity),²⁶ and that aromatic aglycons also contribute significantly to the binding interactions.^{27–30} Con A binds three times more strongly to methyl α -D-mannopyranoside than does pea lectin.²² Since both lectins are known to bind to *Saccharomyces cerevisiae* yeast mannan,¹¹ we evaluated synthetic multiantennary aryl mannosides for their binding properties relative to both lectins using inhibition experiments with yeast mannan.

Synthesis of mannosylated dendrimers

The synthesis of new mannosylated dendrimers, containing up to 16 α -D-mannopyranoside residues, was based upon the convergent assembly of pre-formed *N*-chloroacetylated dendrimers and thiolated mannoside derivatives.^{20,21} As it was also desirable to prepare analogous mannosylated polymers having strong binding properties for solid-phase enzyme linked lectin assays, the required mannoside precursor was synthesized in a form suitable for both dendrimer and polymer syntheses. To this end, readily available peracetylated *p*-nitrophenyl α -D-mannopyranoside (**1**)³¹ was reduced in 94% yield by catalytic transfer hydrogenation (10% Pd-C, NH_4HCO_3) under conditions previously described for a similar lactose derivative (Scheme 1).³² The *p*-aminophenyl intermediate **2** was then treated with acryloyl chloride to provide monomer precursor **3** in 75% yield. Conjugate addition of thioacetic acid under basic conditions (K_2CO_3 , CH_3CN) afforded stable thioacetate **4** in 81% yield. Chemoselective thioacetate deprotection³³ of **4** in the presence of other O-acetates was cleanly achieved with hydrazinium acetate in DMF under nitrogen atmosphere to give thiol **5** in 92% yield.

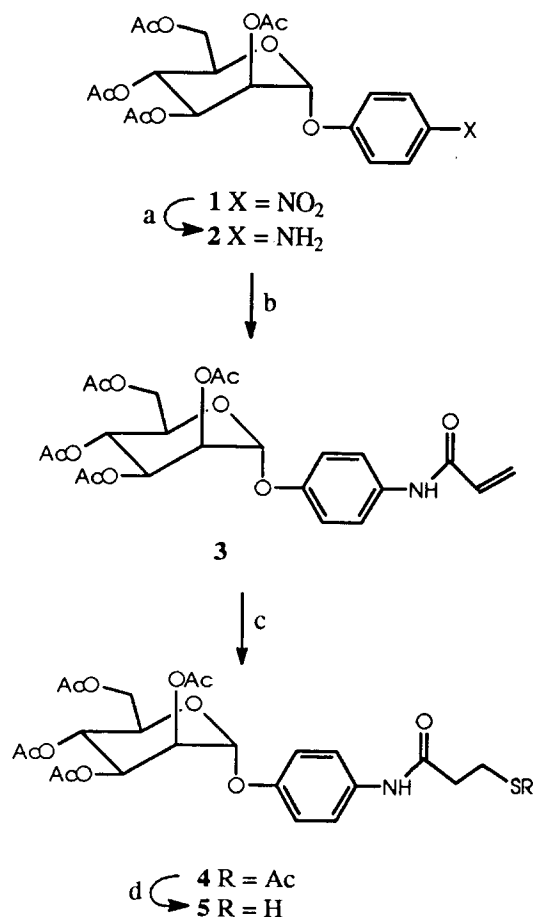
The choice of the aromatic aglycon was not just one of convenience. Indeed, it has been previously demonstrated by Iyer and Goldstein²⁷ and by Van Wauve et al.^{28–30} that the inhibitory property of *p*-nitrophenyl α -D-mannopyranoside toward concanavalin A was almost twice that of methyl α -D-mannopyranoside.

Moreover, a similar but enhanced effect (by a factor of 70) has also been observed in the inhibition of binding of yeasts and intestinal epithelial cells to the lectin of Type I fimbriated *E. coli* 025 and 0128.³⁴ This effect has also been noticed for pea lectin.^{28,30} Since the dendrimers described here have been designed as potential inhibitors of bacterial adherence, the increased binding properties set by both valency and intrinsic affinity was considered beneficial.

Attachment of thiolated mannoside **5** to *N*-chloroacetylated dendrimers having valencies of 2, 4, 8, and 16, respectively, was done as previously described for sialoside (Neu5Ac),^{20,21} lactoside,³⁵ *N*-acetylglucosaminide (GlcNAc),³⁶ and *N*-acetylgalactosaminide (GalNAc)³⁷ using a 1.5–2-fold excess of thiol **5** per *N*-chloroacetyl group (Scheme 2). Glycodendrimer precursors **6–9**, having L-lysine as multiantennary core structure, were prepared by solid-phase peptide chemistry using Wang resin and Fmoc strategy.

Synthesis of mannosylated copolymers

We^{16,17} and others³⁸ have previously demonstrated the usefulness of glycopolymers in immunochemical assays. They have been shown to possess good antigenic

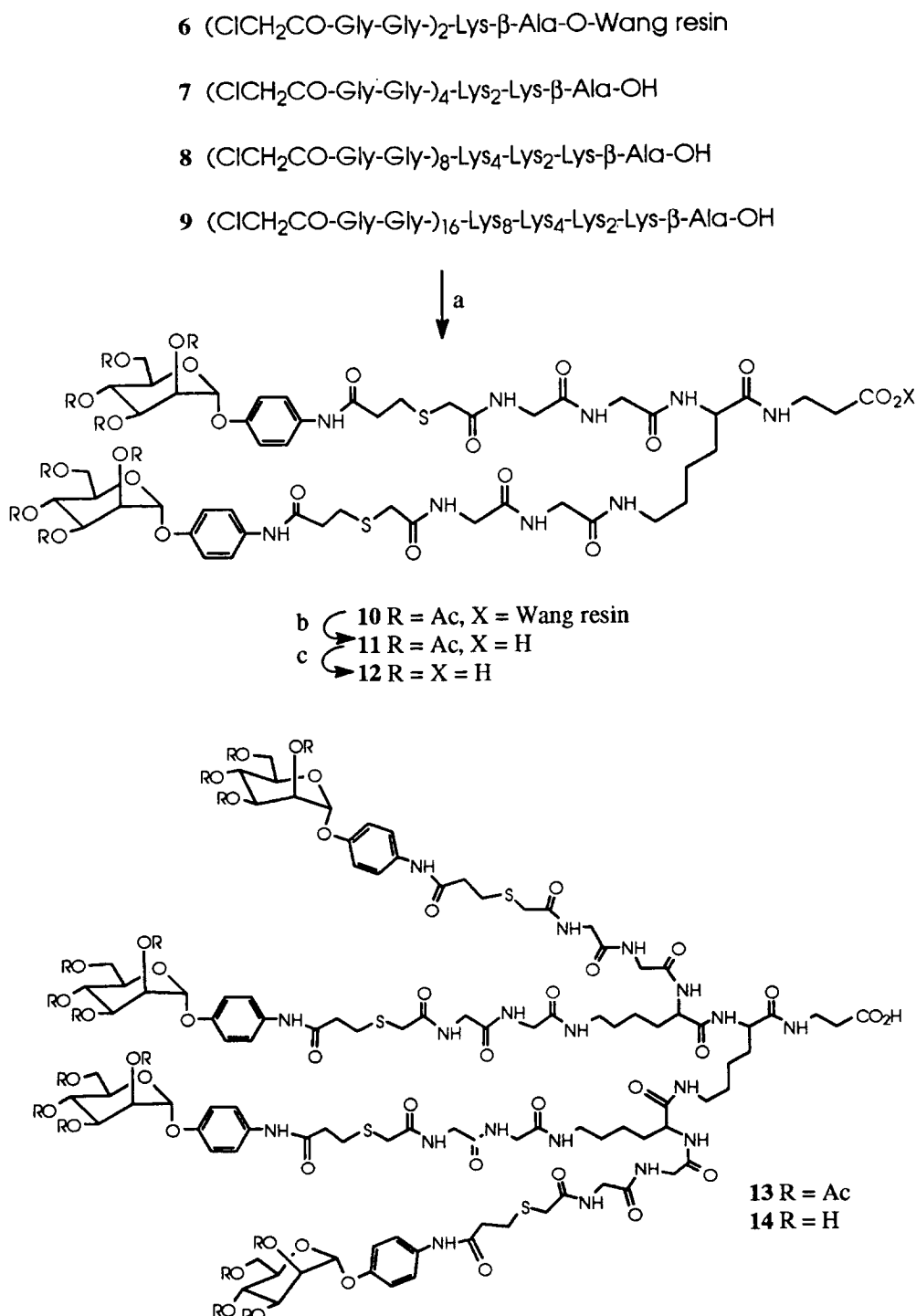


Scheme 1. Reagents and conditions: (a) 10% Pd-C, NH_4HCO_3 , MeOH, 30 min, 94%; (b) $\text{CH}_2=\text{CH}-\text{COCl}$, Et_3N , CH_2Cl_2 , 0 $\rightarrow$ 25 $^\circ\text{C}$, 45 min, 75%; (c) AcSH, K_2CO_3 , CH_3CN , 5 h, r.t., 81%; (d) $\text{H}_2\text{NNH}_2\text{-HOAc}$, DMF, N_2 , 20 min, 25 $^\circ\text{C}$, 92%.

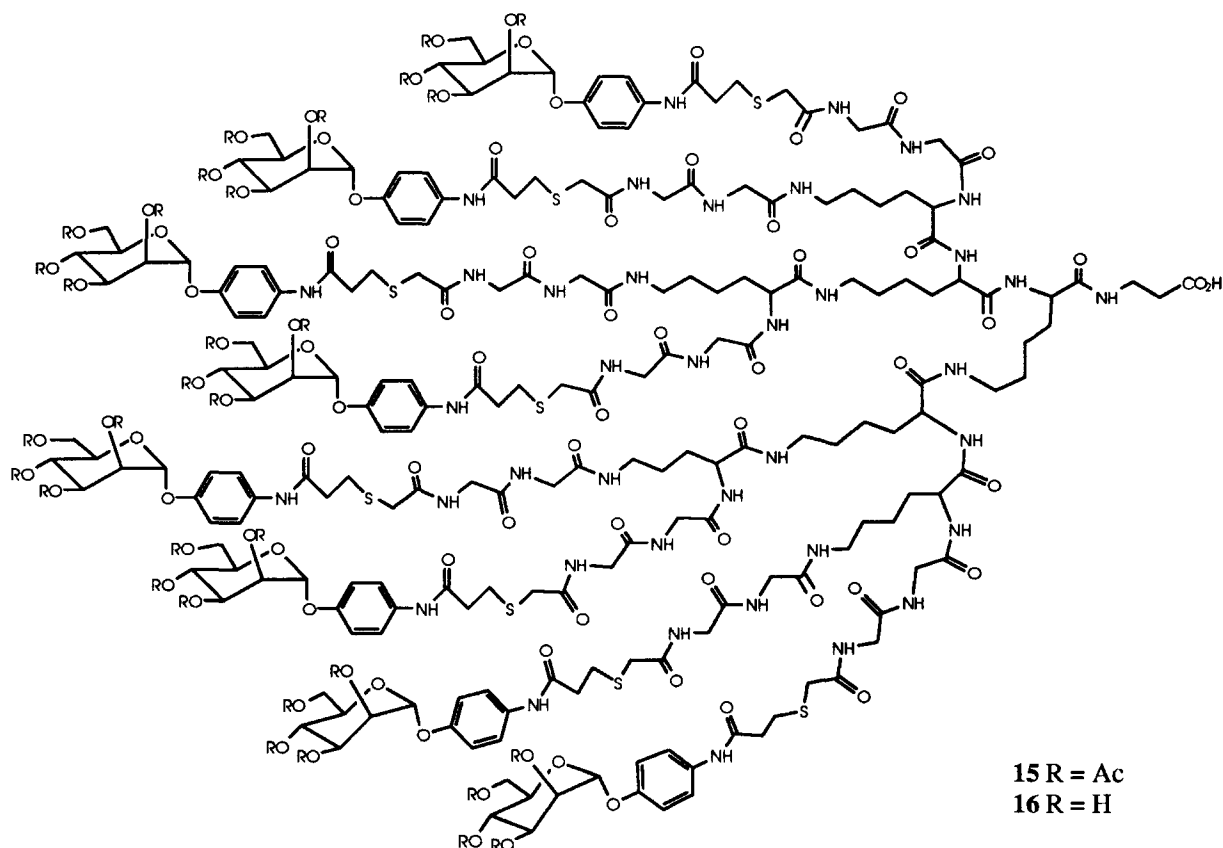
properties as shown by agar gel double immunodiffusion, quantitative precipitation, hemagglutination, and by solid-phase enzyme linked lectin assays (ELLA) and immunoassays (ELISA).³⁹ Therefore, in many respects they surpass natural glycoproteins and polysaccharides in that their carbohydrate homogeneity and molecular weight are somewhat controllable. Their use as coating antigens in ELLA and ELISA makes them appropriate ligands for inhibition experiments since their carbohydrate densities can be adjusted to suit the receptor's

affinity for the inhibitors. For the present investigation, we also prepared mannosylated copolyacrylamide to be used as positive control and to compensate for the too low overall binding affinity of pea lectin to yeast mannan in the inhibition experiments (see below).

Peracetylated *p*-acrylamidophenyl α -D-mannopyranoside monomer precursor (**3**) was de-O-acetylated under Zemplén conditions to provide **19** in essentially quantitative yield (Scheme 3). Ammonium persulfate initi-



Scheme 2. Reagents and conditions: (a) thio-mannoside **5**, 1% DIPEA, DMF; (b) 95% aq TFA, 25 °C, 1.5 h; (c) NaOMe, MeOH.



ated copolymerization (25 min, 90 °C) of **19** with different molar ratios of acrylamide in aqueous solution afforded mannosylated copolymers **20a–c** in which the molar ratios of acrylamide to mannoside residues varied from 37:1 (**20a**) to 9:1 (**20c**) as judged from the relative integration of the ^1H NMR signals of the backbone methine and methylene protons (δ 2.41 and 2.34, respectively, D_2O) relative to those of the anomeric proton at δ 5.68. Phenol–sulfuric acid analysis⁴⁰ of the copolymers showed them to contain 6% (**20a**), 14% (**20b**), and 30% (**20c**) of mannose (w/w) (Table 1). For comparison purposes (positive control in agar gel diffusion), an analogous copolyacrylamide (**21**) was prepared from allyl α -D-mannopyranoside under the conditions described above. To prove the absence of unspecific binding originating from the polyacrylamide backbone, β -D-N-acetylglucosaminide copolyacrylamide (**22**),⁴¹ known to be devoid of Con A and pea lectin binding properties, was prepared and used as a negative control.

Binding studies of mannosylated dendrimers to concanavalin A and pea lectins

The salient features of glycodendrimers are their well characterized chemical architectures and multivalencies. The tetrameric binding sites of Con A⁴² and the dimeric binding sites of pea lectin¹¹ favor their cross-linking with divalent and complex carbohydrate ligands in well organized insoluble lattices.⁴³ Therefore, similar

to multiantennary oligosaccharides and bisected hybrid type glycopeptides, glycodendrimers offer unprecedented opportunities for studying cluster effects² from the point of view of both ligands and receptors. To illustrate the potential of the new mannosylated dendrimers described above as high affinity ligands, they were used initially in direct binding assays. They were then used as inhibitors in model binding solid-phase ELLA in order to evaluate the concentrations necessary for 50% inhibition (IC_{50} values) in model lectin binding assays with Con A and pea lectin.

Evidence for direct binding of mannosylated dendrimers to lectins

The ability of mannosylated 16-mer dendrimer **18** and polymers **20c** and **21** to form stable insoluble complexes with plant lectins was first illustrated by radial agar gel diffusion (Fig. 2) using Con A and pea lectin (not shown). The position of the precipitin bands relative to the wells provided an estimate of the relative molecular weight of the dendrimer and polymers in comparison to Con A (M_w 106 kDa)¹¹ and pea lectin (M_w 50 kDa).^{11,44}

The observed reactivity of dendrimer **18** and its smaller homologues **12**, **14**, and **16** (not shown) toward Con A and pea lectin (not shown) was further substantiated by turbidimetric measurements in microtiter plates at 490 nm. The time course of formation of insoluble precipitin complexes between Con A and yeast mannan or

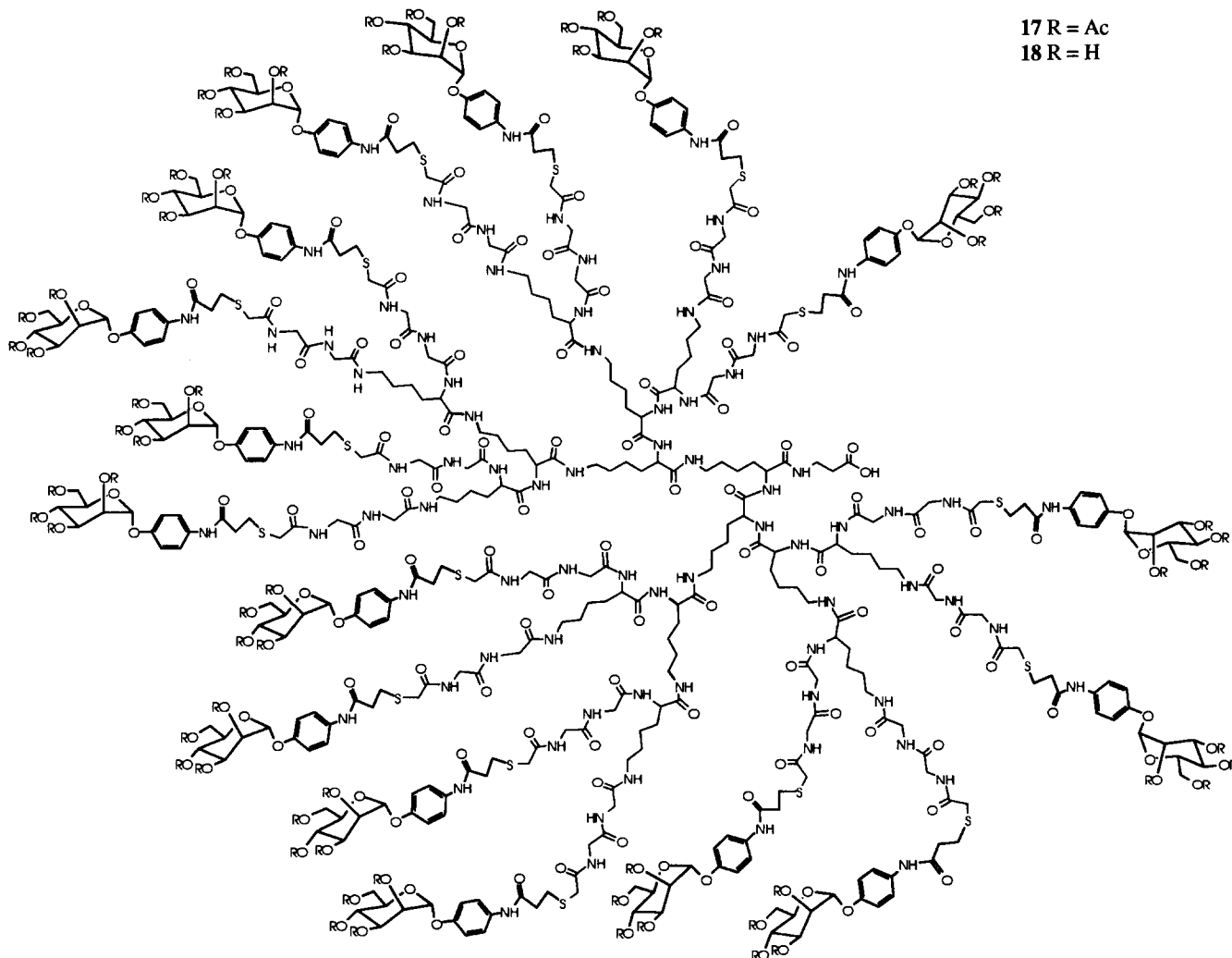
dendrimer **18** is illustrated in Figure 3. In both cases, maximum turbidity occurred after approximately 2 h. Interestingly, Con A-mannan interaction could be inhibited by *p*-nitrophenyl α -D-mannopyranoside, whereas that of Con A-dendrimer **18** could not. This result may reflect the high stability of multivalent interactions generated here.

Mannosylated dendrimers as potent inhibitors of binding

The efficiency of each of the dendrimers to inhibit the binding of yeast mannan to Con A and pea lectin was next measured by ELLA. Yeast mannan was used as a coating antigen in microtiter plates and horseradish peroxidase-labeled Con A and pea lectin were used for detection. However, in the case of pea lectin bindings to yeast mannan, the measured optical densities were too small for precise determination. In this case, low density mannosylated polymer **20a** having an acrylamide to mannoside ratio of 37:1 was used as coating capture antigen. The use of copolymers **20b–c** with higher mannoside contents resulted in impractically high concentrations of inhibitors required for IC_{50}

measurements. In typical inhibition experiments, the concentrations of yeast mannan and copolymer **20a** adsorbed on microtiter plates relative to those of peroxidase-labeled Con A or pea lectins were adjusted to those corresponding to approximately 80% of the total binding capacities based on standard dilution determinations. Mannosylated dendrimers **12**, **14**, **16**, and **18** were then added to the lectin–antigen complexes, and IC_{50} s were determined.

The results for the inhibition of binding of Con A to yeast mannan are shown in Figure 4. Methyl α -D-mannopyranoside and *p*-nitrophenyl α -D-mannopyranoside were used as monomers for comparison purposes. The results clearly confirmed previous findings^{27–30} for the better binding properties of aryl mannoside (IC_{50} 105.6 μ M) relative to that of methyl mannoside (IC_{50} 924.1 μ M). In this assay, the aryl mannoside binds 8.8 times better than the methyl glycoside (Table 2). Divalent mannodendrimer (**12**) had an IC_{50} (69.4 μ M) which was only slightly better than that of aryl mannoside with a relative inhibitory potency of 13.3 when measured on the basis of the dendrimer concentrations. Tremendous increases in inhibitory potencies were achieved with tetramer **14**



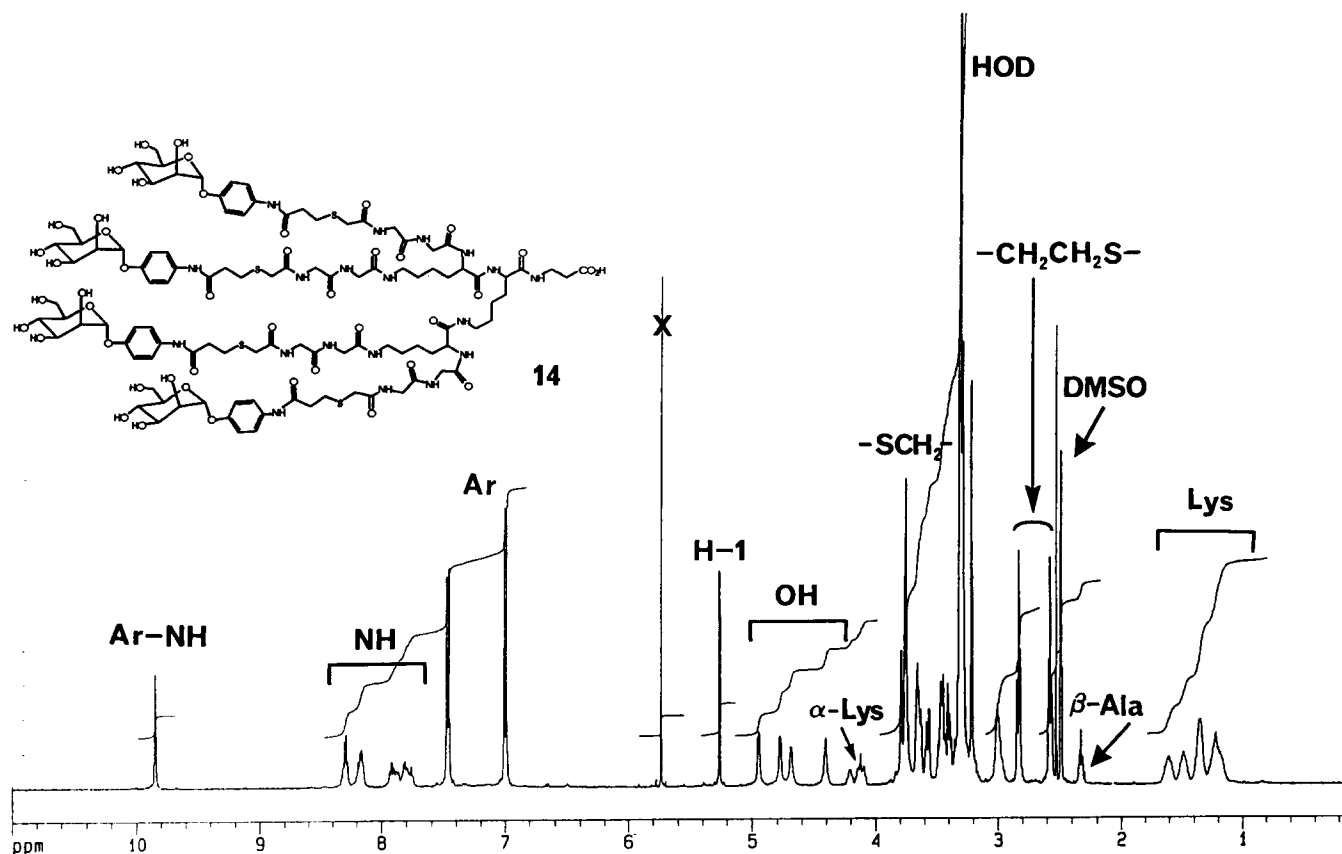


Figure 1. 500 MHz ^1H NMR spectrum ($\text{DMSO}-d_6$) of tetraivalent mannosylated dendrimer **14**.

(IC_{50} 13.2 μM), octamer **16** (IC_{50} 3.3 μM) and hexadecamer **18** (IC_{50} 1.6 μM) (Table 2). When the resulting IC_{50} s were plotted as a function of dendrimer valencies (Fig. 5), it was possible to observe a distinct plateau of inhibition at a valency of 4 (dendrimer **14**). From the tetramer and upward, the relative inhibitory potencies indicated 70 (**14**) to almost 600 (**18**) times better binding affinities. Taking into consideration the number of mannoside residues per dendrimer molecules, the valency effect accounts for 35- (**16**) to 36-fold (**18**) increased inhibitory potential relative to methyl α -D-mannopyranoside and 4 (**16**) and 4.1 (**18**) relative to Man- α -D-O-Ph-*p*- NO_2 . Since the IC_{50} values obtained for the higher dendrimers are lower than expected, it is possible to suspect that only the longest branches of the dendrimers (ϵ - NH_2 -Lysyl groups) are readily accessible for binding. For comparison, polymer **20c** at a concentration of 0.037 μM (6.5 μM on the basis of individual mannoside residues) showed more

than 90% inhibition of binding of Con A to yeast mannan. This indicates that, although better defined, glycodendrimers are not yet as effective as glycopolymers.

The results from the inhibition of pea lectin to mannosylated copolyacrylamide **20a** are shown in Figure 6. The results also illustrate the preference of pea lectin toward aryl mannoside (IC_{50} 2489 μM) over methyl α -D-mannopyranoside ligand (IC_{50} 3850 μM), albeit with a lower contribution resulting from the aromatic aglycon (only 1.5-fold increase relative to 8.8 with Con A) (Table 2). There was, however, a marked increase in inhibitory potency arising from dimer **12** (IC_{50} 85 μM) with marginal increases observed for tetramer **14** (IC_{50} 78 μM). The relative potencies were 45 and 49 times better against Man- α -D-OMe, and 29 and 31 times better against Man- α -D-O-Ph-*p*- NO_2 (8–15/Man residue). This is in sharp contrast to Con A. Alternatively, the octameric dendrimer **16** had an IC_{50} of 9.9 μM while 16-mer **18** had an IC_{50} of 1.8 μM . This represents relative potencies of 389- and 2139-fold increases over Man- α -D-OMe and 251 and 1383 against Man- α -D-O-Ph-*p*- NO_2 (31 and 86/Man residue), respectively. The 16-mer **18** was thus 5.5 times more potent than the octamer **16**. Figure 6 illustrates the effect of the valency as a function of IC_{50} s. Here again, even when the relative potencies are expressed on the basis of mannoside residues in each dendrimer, the level of inhibition still shows 49–134-fold increase for

Table 1. Results of copolymerization of mannoside **19** to acrylamide

Polymer	Monomer ratio ^a	Yield %	Polymer composition ^{a,b}	Mannoside content wt, % ^c
20a	1:30	67%	1:37	6%
20b	1:20	46%	1:15	14%
20c	1:10	43%	1:9	30%

^aMolar ratio of carbohydrate monomer **19** to acrylamide.

^bBased on ^1H NMR.

^cBased on phenol-sulfuric acid analysis.

compounds **16** and **18** relative to methyl α -D-mannopyranoside and 31 to 86 relative to Man- α -D-O-Ph-*p*-NO₂. In this case, the maximum inhibitory potency showed the plateau to occur at a valency of eight mannoside residues (Fig. 5). It is also worth mentioning that Con A, which has four binding sites per molecule, has its valency effect (beginning of the plateau) at four mannoside residues, whereas divalent pea lectin has its corresponding valency effect (Fig. 5) at the octameric level.

Conclusions

The design of dendritic multivalent carbohydrate conjugates having well defined and organized architec-

ture represents a major achievement for the understanding of multivalent interactions.¹⁸ Such synthetic multiantennary glycan mimetics can be readily prepared in various shapes, densities, and with a large variety of carbohydrate structures. The results obtained above, together with those previously observed with analogous glycodendrimer-lectin binding interactions,^{18,20,21,35-37} and sialo-dendrimer interactions with influenza virus hemagglutinin^{20,21} strongly suggest that the structural features of this new family of glycoconjugates, termed 'glycodendrimers', offers unprecedented opportunities for the design of potent ligands for carbohydrate receptors. As demonstrated above, glycodendrimers with as little as four mannoside residues can show 50- to 70-fold increases in binding capacities. These values can reach up to 600- to 2000-fold increases at the hexadecameric level when compared to their monosaccharide analogues. Even when expressed on a molar basis of mannoside residues per dendrimer, the relative inhibitory potencies are still high (36-48 for the above interactions). Thus dendritic structures as those described in this manuscript have the potential to surpass natural bi- or tri-sected oligomannose-type glycopeptides. Moreover, they can be prepared with enhanced binding elements (aromatic aglycons) and are easily amenable to large scale synthesis. These new glycodendrimers thus compare advantageously to previously described smaller clusters.^{2,45,46}

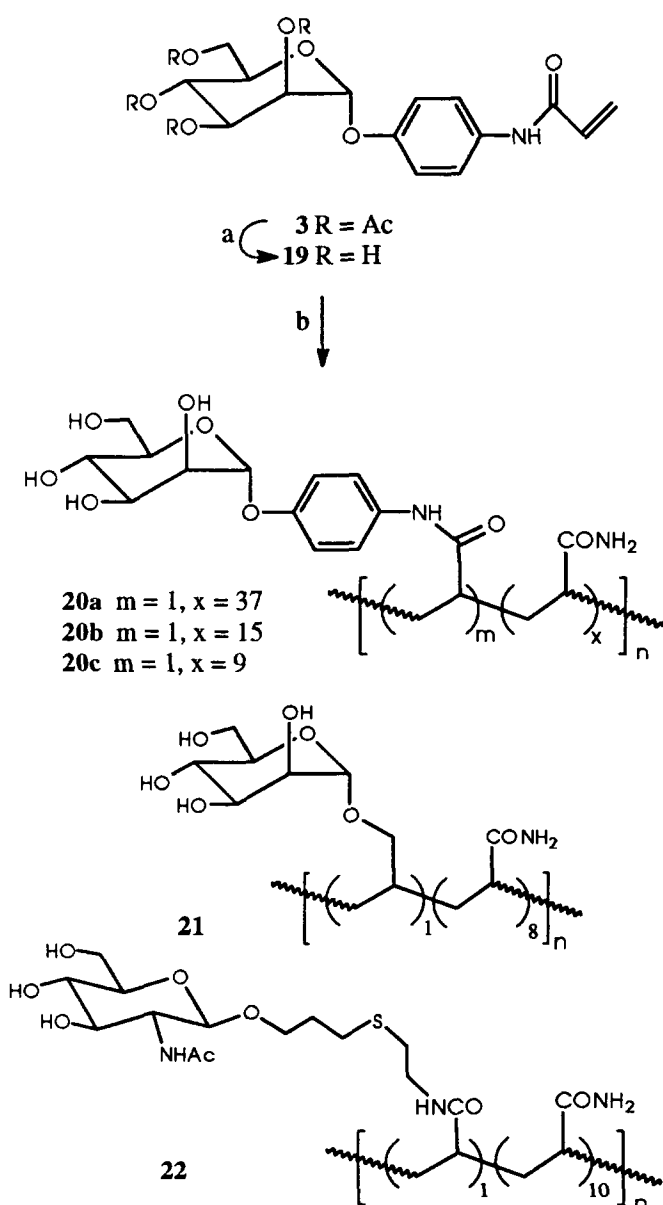
Work is now in progress to adjust the intra-mannoside distances and architecture in order to optimize binding interactions while minimizing the valency requirements. Preliminary results have also demonstrated strong binding interactions originating from α -D-Manp-(1 $\rightarrow$ 3)-[α -D-Manp-(1 $\rightarrow$ 6)]- α -D-Manp-OR trisaccharide suggesting that glycodendrimers incorporating this structure would be extremely potent ligands.

The potential applications of such structures span from inhibition of bacterial adherence to inhibition of inflammation processes and organs or cell targeting. Work is ongoing in order to reach these goals.

Experimental

General methods

Melting points were determined on a Gallenkamp apparatus and are uncorrected. The ¹H and ¹³C NMR spectra were obtained on a Brüker 500 MHz AMX NMR spectrometer. The proton chemical shifts (δ) are given relative to internal chloroform (7.24 ppm) for CDCl₃ solns, to internal DMSO (2.49 ppm) for DMSO-d₆ solns, and to internal HOD (4.65 ppm) for D₂O solns. The carbon chemical shifts are given relative to CDCl₃ (77.0 ppm) and DMSO-d₆ (39.5 ppm). The assignments were based on COSY, DEPT, and HMQC experiments. Optical rotations were measured on a Perkin-Elmer 241 polarimeter and were run at 23 °C. Mass spectra were recorded on a VG 7070-E spectrometer (CI ether) and Kratos Concept IIH for FABMS using glycerol matrix. Thin layer chromatography (TLC) was performed using



Scheme 3. Reagents and Conditions: (a) NaOMe, MeOH, 2 h, r.t., 99%; (b) CH₂=CH-CONH₂, (NH₄)₂S₂O₈, H₂O, N₂, 25 min, 90 °C, 67%.

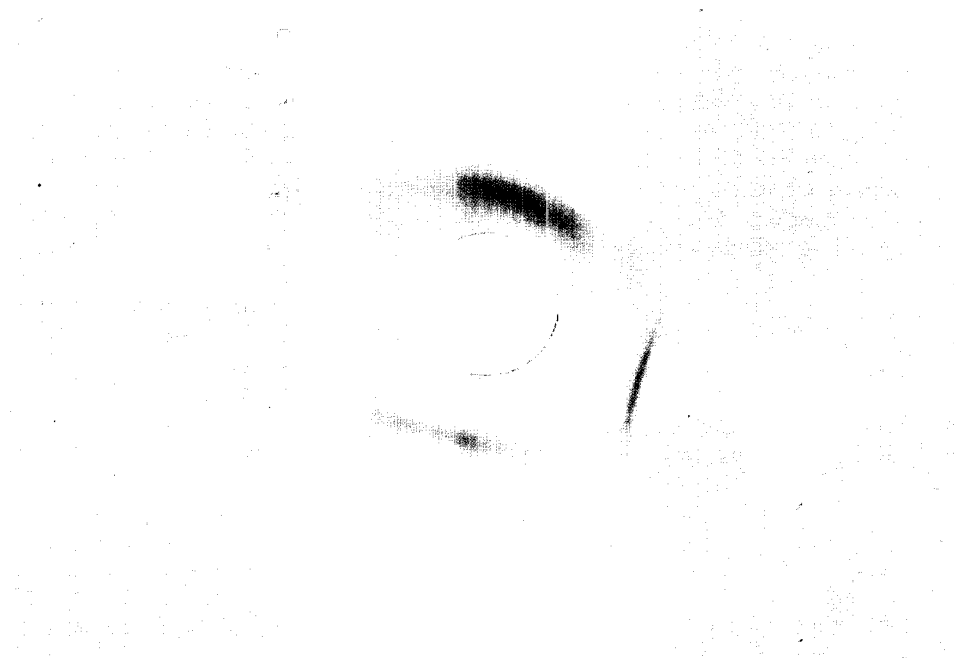


Figure 2. Agar gel diffusion of Con A (middle well) against poly(acrylamide-co-allyl α -D-mannopyranoside) (**21**), positive control (bottom well); poly(acrylamide-co- β -D-N-acetylglucosaminide) (**22**), negative control (left well); 16-mer mannosylated dendrimer **18** (top well); poly(acrylamide-co-4-acrylamidophenyl α -D-mannopyranoside) **20c** (right well).

silica gel 60 F-254 and column chromatography on silica gel 60. Optical densities (O.D.) for the ELLA tests and turbidimetric measurements were performed on a Dynatech MR 600 Microplate Reader. Methyl α -D-mannopyranoside and *p*-nitrophenyl α -D-mannopyranoside were purchased from Aldrich (WI) and Fluka, respectively. Glycopolymers **21** with a molar ratio of acrylamide to mannose of 8:1 and **22** (10:1) were prepared as previously described.⁴¹ The lectins from *Canavalia ensiformis* (concanavalin A) and concanavalin A-peroxidase labeled, along with yeast mannan from *Saccharomyces cerevisiae* were purchased from

Sigma (cat. # C 2631, L 6397 and M 7504, respectively). Crude pea lectin (*Pisum sativum*) and horseradish peroxidase labeled pea lectin were obtained from EY Laboratories (cat. # L-2700 and H-2701-1).

***p*-Aminophenyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranoside (**2**).** *p*-Nitrophenyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranoside (**1**)³¹ (2.00 g, 4.26 mmol) was dissolved in 150 mL of warm methanol containing ammonium formate (5.40 g, 85.7 mmol) and 10% Pd/C (400 mg). The soln was stirred at room temperature for 30 min. The solvent was filtered through Celite and evapd under red. pres. The oily residue was then dissolved in ethyl acetate (20 mL), washed twice with equal vols of water, dried over anhydrous Na_2SO_4 and

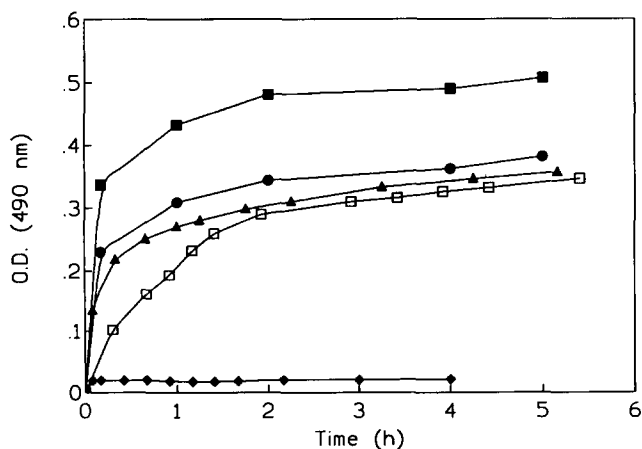


Figure 3. Turbidimetric analysis (micro-quantitative precipitation) of Con A (1 mg mL^{-1}) with yeast mannan at 0.5 mg mL^{-1} (▲) and 16-mer manno-dendrimer **18** at 0.5 mg mL^{-1} (■) and at 0.25 mg mL^{-1} (●). Inhibition effect of *p*-nitrophenyl α -D-mannopyranoside on binding of Con A to yeast mannan (◆). The measurements were done in PBS using an ELISA plate reader at 25°C .

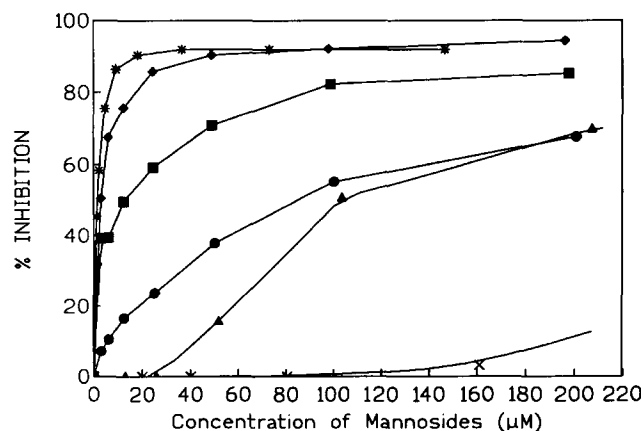


Figure 4. Inhibition of binding of Con A to yeast mannan by Man- α -OMe (X), Man- α -OPh-*p*-NO₂ (▲), and mannosylated dendrimers **12** (●), **14** (■), **16** (◆), and **18** (*).

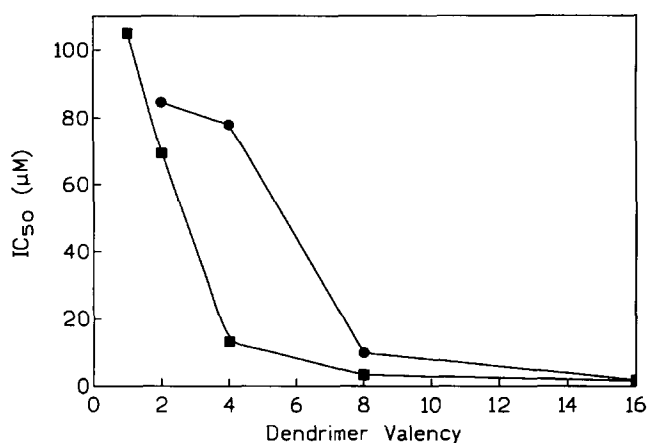


Figure 5. Effect of dendrimer valency on the inhibition of binding of Con A to yeast mannan (■) and pea lectin to mannose-co-polymer **20a** (●).

evapd under red. pres. Crystallization of the residue from hot ethanol gave pure **2** (1.75g) in 94% yield: mp 71–72 °C; $[\alpha]_D^{25} + 71.2^\circ$ (c 1.00, CHCl₃); ¹H NMR (CDCl₃): δ 6.88 (d, 2H, $J_{o,m}$ = 8.9 Hz, H-*meta*), 6.59 (d, 2H, H-*ortho*), 5.52 (dd, 1H, $J_{2,3}$ = 3.5 Hz, $J_{3,4}$ = 10.0 Hz, H-3), 5.40 (dd, 1H, $J_{1,2}$ = 1.8 Hz, H-2), 5.34 (d, 1H, H-1), 5.32 (dd, 1H, $J_{4,5}$ = 10.1 Hz, H-4), 4.26 (dd, 1H, $J_{5,6}$ = 5.4 Hz, $J_{6,6'}$ = 12.2 Hz, H-6), 4.13 (ddd, 1H,

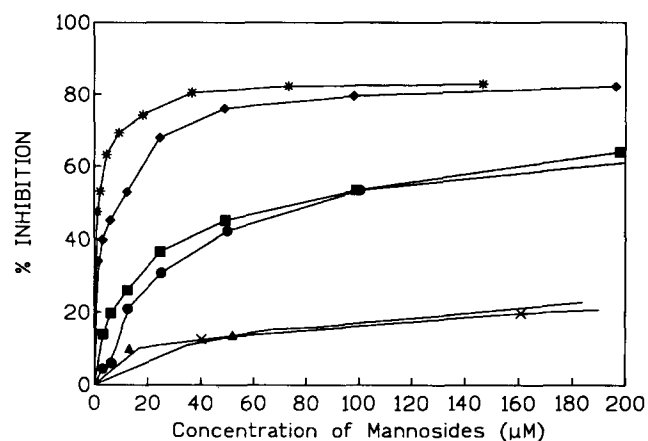


Figure 6. Inhibition of binding of pea lectin to mannose-co-polymer **20a** by Man-α-OMe (X), Man-α-OPh-*p*-NO₂ (▲), and mannosylated dendrimers **12** (●), **14** (■), **16** (◆), and **18** (*).

Table 2. Inhibition of binding of yeast mannan and mannose-co-polymer **20a** to concanavalin A and pea lectin, respectively, by α-mannosides and mannosylated dendrimers **12**, **14**, **16**, and **18**

Inhibitor	M_r	Con A IC ₅₀ (μM)	Relative Potency ^a	Pea lectin IC ₅₀ (μM)	Relative Potency
Man-α-OMe	194.2	924.1	1.0 (0.11)	3850 ^b	1.0 (0.65)
Man-α-OPh- <i>p</i> -NO ₂	301.3	105.6	8.8 (1.0)	2489 ^b	1.5 (1.0)
12 (dimer)	1244.8	69.4	13.3 (1.5/0.75)	85.0	45.3 (29/14.5)
14 (tetramer)	2528.7	13.2	70 (8/2)	78.0	49.4 (32/8)
16 (octamer)	5096.5	3.3	280 (32/4)	9.9	389 (251/31)
18 (16-mer)	10,231.8	1.6	578 (66/4.1)	1.8	2139 (1383/86)

^aValues in parentheses are expressed relative to Man-α-OPh-*p*-NO₂ and per mannoside residues in each dendrimer.

^bValues extrapolated from Figure 5.

$J_{5,6'} = 2.3$ Hz, H-5), 4.07 (dd, 1H, H-6'), 3.58 (bs, 2H, NH₂), 2.17, 2.03 (2 ×), 2.00 (4s, 12H, OAc); ¹³C NMR (CDCl₃): δ 170.0, 169.9 (2C), 169.7 (C=O), 148.2 (C-*ipso*), 142.0 (C-*para*), 117.9 (C-*meta*), 116.0 (C-*ortho*), 96.8 (C-1), 69.5 (C-2), 68.9 (C-5), 68.9 (C-3), 62.2 (C-6), 20.7 (3C), 20.9 (Ac); mass spectrum (CI) (rel. int.) m/z 439.9 (M⁺, 35%), 330.8 (M⁺ - aglycon, 100%). Anal. calcd for C₂₀H₂₅NO₁₀: C, 54.67; H, 5.73; N, 3.19%; found: C, 54.27; H, 5.73; N, 3.10%.

p-Acrylamidophenyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (3). To a soln of **2** (1.50 g, 3.41 mmol) and triethylamine (2.00 mL, 4.5 eq) in dichloromethane (150 mL) stirred at 0 °C, was added dropwise a solution of acryloyl chloride (345 μL, 1.25 eq) in CH₂Cl₂ (10 mL) over a period of 30 min. The reaction mixture was further stirred at room temperature for another 45 min. Water (50 mL) was added and the organic phase successively washed with equal vols of 0.5 M HCl, satd NaHCO₃ soln, and again with water. The organic phase was dried over anhydrous Na₂SO₄ and evapd under red. pres. The residue was purified by silica gel column chromatography using EtOAc-CHCl₃ (1:1, v:v) as eluent. Recrystallization from hot ethanol gave pure **3** (1.17g) in 75% yield: mp 169–170 °C; $[\alpha]_D^{25} + 74.4^\circ$ (c 1.00; CHCl₃); ¹H NMR (CDCl₃): δ 7.50 (d, 2H, $J_{o,m}$ = 9.0 Hz, H-*meta*), 7.36 (bs, 1 H, NH), 7.03 (d, 2H, H-*ortho*), 6.39 (d, 1H, J_{trans} = 16.8 Hz, J_{gem} = 0.85 Hz, H-*trans*), 6.21 (dd, 1H, J_{cis} = 10.3 Hz, CH=CH₂), 5.73 (dd, 1H, H-*cis*), 5.52 (dd, 1H, $J_{2,3}$ = 3.5 Hz, $J_{3,4}$ = 10.0 Hz, H-3), 5.46 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 5.41 (dd, 1H, H-2), 5.33 (dd, 1H, $J_{4,5}$ = 10.1 Hz, H-4), 4.25 (dd, 1H, $J_{5,6}$ = 5.5 Hz, $J_{6,6'}$ = 12.4 Hz, H-6), 4.08 (ddd, 1H, $J_{5,6'} = 2.3$ Hz, H-5), 4.05 (dd, 1H, H-6'), 2.17, 2.03, 2.02, 2.01 (4s, 12 H, OAc); ¹³C NMR (CDCl₃): δ 170.5, 170.0, 169.9, 169.7, 163.4 (C=O), 152.3 (C-*ipso*), 133.0 (C-*para*), 131.0 (CH=), 127.7 (CH₂=), 121.5 (C-*meta*), 117.1 (C-*ortho*), 96.1 (C-1), 69.4 (C-2), 69.2 (C-5), 68.9 (C-3), 66.0 (C-4), 62.1 (C-6), 20.8, 20.7 (3C) (Ac); mass spectrum (CI) (rel. int.) m/z 493.9 (M⁺, 22%), 330.8 (M⁺ - aglycon, 100%). Anal. calcd for C₂₃H₂₇NO₁₁: C, 55.98; H, 5.51; N, 2.84%; found: C, 56.10; H, 5.49; N, 2.84%.

p-(3-Thioacetyl)-propionamidophenyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (4). Compound **3** (1.00 g, 2.03 mmol) was dissolved in acetonitrile (10 mL) containing a catalytic amount of K₂CO₃. Thioacetic

acid (385 mg, 2.5 eq) was added and the soln stirred at room temperature for 5 h. The solvent was evapd under red. pres and the residue obtained dissolved in EtOAc and successively washed with equal vol of satd NaHCO₃ soln, water, and satd NaCl soln. The organic phase was dried over anhydrous Na₂SO₄ and evapd under red. pres. Compound **4** was obtained in 81% yield (0.94 g) after crystallization from hot ethanol: mp 127–128 °C; [α]_D +69.9° (*c* 1.00; CHCl₃); ¹H NMR (CDCl₃): δ 7.41 (d, 2H, *J*_{o,m} = 9.0 Hz, H-*meta*), 7.39 (bs, 1H, NH), 7.01 (d, 2H, H-*ortho*), 5.51 (dd, 1H, *J*_{2,3} = 3.5 Hz, *J*_{3,4} = 10.0 Hz, H-3), 5.44 (d, 1H, *J*_{1,2} = 1.8 Hz, H-1), 5.40 (dd, 1H, H-2), 5.33 (dd, 1H, *J*_{4,5} = 10.1 Hz, H-4), 4.25 (dd, 1H, *J*_{5,6} = 5.5 Hz, *J*_{6,6'} = 12.1 Hz, H-6), 4.06 (ddd, 1H, *J*_{5,6'} = 2.3 Hz, H-5), 4.05 (dd, 1H, H-6'), 3.18 (t, 2H, CH₂—SAC), 2.63 (t, 2H, NHC(O)CH₂), 2.32 (s, 3H, SC(O)CH₃), 2.17, 2.03, 2.02, 2.01 (4s, 12H, Ac); ¹³C NMR (CDCl₃): δ 196.4 (SC=O), 170.5, 170.0, 169.9, 169.7 (C=O), 168.7 (NHC=O), 152.3 (C-*ipso*), 133.0 (C-*para*), 121.4 (C-*meta*), 117.1 (C-*ortho*), 96.1 (C-1), 69.4 (C-2), 69.2 (C-5), 68.9 (C-3), 66.0 (C-4), 62.1 (C-6), 37.3 (NHC(O)CH₂), 30.6 (SC(O)CH₃), 24.8 (CH₂SAC), 20.8, 20.7 (2C), 20.6 (Ac); mass spectrum (CI) (rel. int.) *m/z* 569.9 (M⁺, 99.2%), 330.8 (M⁺ – aglycon, 100%). Anal. calcd. for C₂₅H₃₁NO₁₂S: C, 52.72; H, 5.49; N, 2.46%; found: C, 52.71; H, 5.48, N, 2.47%.

***p*-(3-Thio)-propionamidophenyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranoside (**5**).** Compound **4** (200 mg, 0.352 mmol) was dissolved in deoxygenated DMF (2 mL) obtained by multiple freeze–thaw cycles and subsequent bubbling with nitrogen. A freshly prepared deoxygenated solution of 2.5 M hydrazinium acetate (420 μ L) was added and the soln stirred at room temperature under nitrogen for 20 min. The reaction mixture was dild in degassed EtOAc and washed successively with equal vols of satd NaHCO₃ soln, water, and satd NaCl solution. The organic phase was dried over anhydrous Na₂SO₄ and evapd under red. pres. Compound **5** was obtained in 92% yield (170 mg) from evapn of the solvent and kept under nitrogen. ¹H NMR (CDCl₃): δ 7.74 (bs, 1H, NH) 7.41 (d, 2H, *J*_{o,m} = 9.0 Hz, H-*meta*), 6.98 (d, 2H, H-*ortho*), 5.49 (dd, 1H, *J*_{2,3} ND, *J*_{3,4} = 10.0 Hz, H-3), 5.41 (d, 1H, *J*_{1,2} = 1.9 Hz, H-1), 5.38 (dd, 1H, H-2), 5.31 (dd, 1H, *J*_{4,5} = 10.0 Hz, H-4), 4.22 (dd, 1H, *J*_{5,6} = 5.3 Hz, *J*_{6,6'} = 12.0 Hz, H-6), 4.06 (ddd, 1H, *J*_{5,6'} = 2.5 Hz, H-5), 4.05 (dd, 1H, H-6'), 2.82 (dd, 2H, CH₂SH), 2.61 (t, 2H, NHC(O)CH₂), 2.15, 2.01, 2.00 (2 $\times$) (Ac); ¹³C NMR (CDCl₃): δ 170.5, 169.9 (2C), 169.0 (C=O), 152.2 (C-*ipso*), 133.1 (C-*para*), 121.4 (C-*meta*), 116.9 (C-*ortho*), 96.0 (C-1), 69.3 (C-2), 69.0 (C-5), 68.8 (C-3), 65.8 (C-4), 62.0 (C-6), 41.0 (NHC(O)CH₂), 20.8, 20.6 (3C) (Ac), 20.3 (CH₂SH). Compound **5** was used for the following step without any further characterization.

Synthesis of peracetylated mannoside dendrimers (**11**, **13**, **15**, **17**)

N-Chloroacetylated dendrimer backbones **6** to **9** (Scheme 2) were synthesized as previously

described.^{20,21} Coupling of the thiomannosyl derivative **5** with divalent **6** was done on solid phase (procedure 1), whereas coupling with the tetra- (**7**), octa- (**8**) and hexadeca-valent (**9**) *N*-chloroacetylated dendrimers was done in solution (procedure 2), starting with previously cleaved dendrimers (**7–9**) (95% aq TFA, 25 °C, 1.5 h). The two general procedures used are described below.

Procedure 1. Polymer-supported dendrimer **6** with a degree of substitution of 0.58 mmol g^{−1} of Wang resin was placed in 1% DIPEA–DMF (v:v) (2 mL) containing compound **5** (175 mg, 2 eq per *N*-chloroacetyl group). Nitrogen was bubbled through the solution for 16 h. Before the bulk of the solution was released from the polymeric support, aliquots of **10** were withdrawn and hydrolysed (95% aq TFA, 1.5 h). The completeness of the coupling was estimated from the ¹H NMR spectrum of the glycosylated dendrimer which showed characteristic signal for any residual *N*-chloroacetyl methylene groups at δ 4.12 (DMSO-*d*₆). When required, the coupling was repeated. Once the coupling was completed, **10** was hydrolysed from its solid support, rinsed with DMF and dried overnight under vacuum. The divalent peracetylated glycodendrimer **11** was obtained in 74% yield by precipitation from cold ether.

Procedure 2. Tetravalent *N*-chloroacetylated dendrimer **7** (45 mg, 36.4 μ mol) was dissolved in deoxygenated 1% DIPEA–DMSO soln (v:v) (2 mL) obtained through multiple freeze–thaw cycles. Compound **5** (120 mg, 6 eq) was added to the soln and the reaction mixture stirred under nitrogen for 3 h. The solvent was lyophilized and the residue obtained purified by silica gel column chromatography, initially using CH₂Cl₂–MeOH (9:1, v:v) as eluent to remove any residual thiol and/or disulfide formed, followed by CH₂Cl₂–MeOH–H₂O (10:6:1, v:v:v). The same procedure and workup were followed for the synthesis of octa- and hexadeca-valent peracetylated mannoside dendrimers. The tetra- (**13**), octa- (**15**) and hexadeca-valent (**17**) glycodendrimers were obtained in 80, 70 and 60% yields, respectively, by precipitation from cold ether. All new compounds gave consistent ¹H and ¹³C NMR data. Owing to the repetitive structure of the dendrimers, selected data are reported. Compound **13**: ¹H NMR (DMSO-*d*₆): δ 1.22 (m, 6 H, lysyl γ -CH₂), 1.35 (m, 6H, lysyl δ -CH₂), 1.49 and 1.61 (2m, 6H, lysyl β -CH₂), 1.91, 1.96, 2.02 and 2.11 (4s, 48H, Ac), 2.34 (t, 2H, *J* = 6.9 Hz, β -Ala α -CH₂), 2.58 (t, 8H, *J* = 7.0 Hz, aryl α -CH₂), 2.83 (t, 8H, aryl β -CH₂), 3.01 (m, 6H, lysyl ϵ -CH₂), 3.21 (m, 2H, β -Ala β -CH₂), 3.22 (s, 8H, SCH₃), 3.66 and 3.76 (2s, 16H, *J* = 5.4 Hz, glycol CH₂s), 3.96 (dd, 4H, *J*_{5,6'} = 2.2 Hz, *J*_{6,6'} = 12.1 Hz, H-6'), 4.07 (ddd, 4H, H-5), 4.12 and 4.20 (2m, 3H, lysyl α -CH), 4.14 (dd, 4H, *J*_{5,6} = 5.5 Hz, H-6), 5.16 (t, 4H, *J*_{3,4} = 9.9 Hz, *J*_{4,5} = 9.8 Hz, H-4), 5.31 (m, 8H, H-2 and H-3), 5.62 (bs, 4H, H-1), 7.07 (d, 8H, *J*_{o,m} = 9.0 Hz, H-*ortho*), 7.52, (d, 8H, H-*meta*), 7.75 (m, 3H, lysyl ϵ -NH), 7.82 (m, 3H, lysyl α -NH), 8.15 and 8.29 (2m, 8H, glycol NH), 9.90 (s, 4H, aryl NH); ¹³C NMR (DMSO-*d*₆): δ 20.4 (3C) and 20.6 (Ac), 22.8 (lysyl γ -C), 27.7 (aryl β -C), 28.7 (lysyl δ -C),

31.6 (lysyl β -C), 33.8 (β -Ala α -C), 34.4 (SCH₂), 34.8 (β -Ala β -C), 36.2 (aryl α -C), 38.4 (lysyl ϵ -C), 42.0 and 42.4 (glycyl C), 52.6 (lysyl α -C), 61.7 (C-6), 65.3 (C-4), 68.4 (C-3), 68.5 (C-2), 68.7 (C-5), 95.7 (C-1), 117.4 (C-ortho), 120.4 (C-meta), 134.5 (C-para), 150.6 (C-*ipso*), 168.4 to 172.4 (C=Os). Compounds **11**, **15**, and **17** exhibited identical chemical shifts with corresponding integration values in ¹H and ¹³C NMR. Compound **11**: FAB-MS (pos) calcd for C₆₇H₈₉N₉O₃₁S₂ 1580.6; found 1581.3 (M⁺ + 1, 1.1% base peak).

De-O-acetylated mannoside dendrimers (12, 14, 16, 18). Tetravalent peracetylated dendrimer **13** (49 mg, 15.3 μ mol) was dissolved in warm MeOH (5.0 mL) containing 1.0 M NaOMe (pH $\approx$ 8.5, to avoid β -elimination) and was stirred at room temperature for 3 h, while continuously maintaining the pH by adding 1 M NaOMe. The solvent was then evapd under red. pres. and the residue dissolved in H₂O–DMSO (1:1, v:v) and neutralized with Amberlite IR-120(H⁺) ion exchange resin. The solution was filtered through cotton wool and lyophilized, giving tetravalent **14** (35 mg) in 94% yield. The same procedure was repeated for di-, octa- and hexadeca-valent glycodendrimers giving de-O-acetylated products **12**, **16** and **18** in 95–100% yields. Again, due to the repetitive structure of the dendrimers, selected data are reported. Compound **14** (Fig. 1): ¹H NMR (DMSO-*d*₆): δ 1.22 (m, 6H, lysyl γ -CH₂), 1.35 (m, 6H, lysyl δ -CH₂), 1.49 and 1.60 (2m, 6H, lysyl β -CH₂), 2.33 (t, 2H, J = 6.7 Hz, β -Ala α -CH₂), 2.58 (t, 8 H, J = 7.1 Hz, aryl α -CH₂), 2.83 (t, 8H, aryl β -CH₂), 3.01 (m, 6H, lysyl ϵ -CH₂), 3.21 (m, 2H, β -Ala β -CH₂), 3.22 (m, 10H, β -Ala β -CH₂ and SCH₂), 3.35–3.50 (bm, 12H, H-4, H-5 and H-6'), 3.57 (bd, 4H, H-6), 3.63 and 3.76 (m, 16H, glycyl CH₂), 3.66 (bs, 4H, H-3), 3.79 (bs, 4H, H-2), 4.12 and 4.20 (2m, 3H, lysyl α -CH), 4.40 (bt, 4H, OH-6), 4.69 (d, 4H, OH-3), 4.75 (d, 4H, OH-4), 4.95 (bd, 4H, OH-2), 5.26 (d, 4H, $J_{1,2}$ = 1.3 Hz, H-1), 7.00 (d, 8H, $J_{o,m}$ = 9.0 Hz, H-ortho), 7.47 (d, 8H, H-meta), 7.80 (m, 3H, lysyl ϵ -NH), 7.90 (m, 3H, lysyl α -NH), 8.17 and 8.30 (2m, 8H, glycyl NH), 9.85 (s, 4H, aglycon NH); ¹³C NMR (DMSO-*d*₆): δ 22.8 (lysyl γ -C), 27.8 (aryl β -C), 28.7 (lysyl δ -C), 31.6 (lysyl β -C), 33.9 (β -Ala α -C), 34.4 (SCH₂), 34.8 (β -Ala β -C), 36.2 (lysyl α -C), 38.4 (lysyl ϵ -C), 42.0 and 42.3 (glycyl C), 52.6 (lysyl α -C), 61.1 (C-6), 66.8 (C-4), 70.1 (C-2), 70.7 (C-3), 74.8 (C-5), 99.3 (C-1), 117.2 (C-ortho), 120.4 (C-meta), 133.6 (C-para), 152.2 (C-*ipso*), 168.4 to 172.9 (C=Os). Compound **12**: FAB-MS (pos.) calcd. for C₅₁H₇₂N₉O₂₃S₂ 1243.3; found 1244.4 (M⁺ + 1, 1.1% base peak).

***p*-Acrylamidophenyl α -D-mannopyranoside (19).** Tetra-O-acetylated mannopyranoside **3** (Scheme 3) (100 mg, 0.203 mmol) was dissolved in MeOH (5 mL) containing 1 M NaOMe (pH $\leq$ 8.5). The solution was stirred at room temperature for 2 h. The reaction mixture was neutralized, filtered and the solvent evapd under red. pres. Compound **19** was obtained in 99% yield (65 mg) after freeze-drying the methanolic residue. The product failed recrystallization:

$[\alpha]_D + 80.0^\circ$ (c 0.10; H₂O); ¹H NMR (D₂O): δ 7.49 (d, 2H, $J_{o,m}$ = 8.9 Hz, H-*meta*), 7.21 (d, 2H, H-*ortho*), 6.44 (dd, 1H, J_{cis} = 10.1 Hz, CH=CH₂), 6.38 (d, 1H, J_{trans} = 17.0 Hz, J_{gem} nd, H-*trans*), 5.92 (d, 1H, H-*cis*), 5.63 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 4.22 (dd, 1H, $J_{2,3}$ = 3.3 Hz, H-2), 4.10 (dd, 1H, $J_{3,4}$ = 9.1 Hz, H-3), 3.79–3.87 (m, 4H, H-4, H-5, H-6, H-6'); ¹³C NMR (D₂O): δ 166.3 (C=O), 152.4 (C-*ipso*), 131.2 (C-*para*), 129.8 (CH=CH₂), 127.7 (CH=CH₂), 122.9 (C-*meta*), 117.1 (C-*ortho*), 98.0 (C-1), 72.9 (C-5), 69.9 (C-3), 69.4 (C-2), 66.1 (C-4), 60.2 (C-6); mass spectrum (CI) (rel. intensity) m/z 325.5 (M⁺, 11.9%), 163.8 (M⁺ - aglycon, 100%).

Poly(acrylamide-co-*p*-acrylamidophenyl- α -D-mannopyranoside) copolymers (20a–c). Copolymers **20a–c** were synthesized following the general procedure described below. The ratio sugar/acrylamide added each time was varied accordingly to obtain the desired sugar content.

Procedure: compound **19** (7 mg, 0.0215 mmol) and acrylamide (44 mg, 0.620 mmol, 30 eq) were dissolved in deoxygenated distilled-deionized H₂O (750 μ L). Ammonium persulfate (10 μ L of a fresh 10 mg/mL stock solution) was then added and the reaction mixture was stirred under nitrogen at 90 $^\circ$ C for 25 min or until TLC showed complete disappearance of **19**. The cooled reaction mixture was diluted with water (10 mL) and dialysed (12,000 MW cutoff) exhaustively against water (4 $\times$ 2 L). The aq soln of the polymer was lyophilized to afford mannosyl copolymer **20a** (34 mg) in 67% yield. Similarly, polymers with an initial molar ratio of 1:20 (**19**: acrylamide) for **20b** and 1:10 for **20c** (Table 1) were obtained in 46 and 43% yields. The mannoside contents of the copolymers were determined by the phenol–sulfuric acid method of Dubois et al.⁴⁰ using free mannose as standard. Owing to the repetitive structure of the polymers, only selected data are reported. Copolymer **20a**: $[\alpha]_D + 10.4^\circ$ (c 0.50; H₂O); ¹H NMR (D₂O): δ 7.47 (bd, 2H, H-*meta*), 7.26 (bd, 2H, H-*ortho*), 5.68 (bs, 1H, H-1), 4.25 (bs, 1H, H-2), 4.11 (bd, 1H, H-3), 3.89–3.81 (m, 4H, H-4, H-5, H-6, H-6'), 2.41 and 2.34 (2 bs, 38H, polymer backbone), 1.85 and 1.73 (2 bs, 74H, polymer backbone). The sugar content of the polymers, obtained from the ratio of the integration value of the anomeric proton signal (δ 5.68) relative to that of the acrylamide backbone signals (2 bs, δ 2.41 and 2.34), was determined to be 1:37 (sugar:acrylamide) for **20a**, 1:15 for **20b** and 1:9 for **20c**.

Enzyme linked lectin assay (ELLA)

Linbro (Titertek) microtitration plates were coated with yeast mannan or copolymer **20a** at 100 μ L/well diluted from a stock solution of 10 μ g mL⁻¹ in 0.01 M phosphate buffer saline (PBS, pH 7.3) at room temperature overnight. The wells were then washed three times with 300 μ L/well of washing buffer [PBS containing 0.05% (v/v) Tween 20] (PBST). This washing procedure was repeated after each incubation throughout the assay. The wells were then blocked with

150 μL /well of 1% BSA/PBS for 1 h at 37 °C. After washing, the wells were filled with 100 μL /well of serial dilutions of concanavalin A–peroxidase labeled (Con A-HRP) or *Pisum sativum* lectin–peroxidase labeled (PSA-HRP) from 10^{-1} to 10^{-5} mg mL $^{-1}$ in PBS and incubated at 37 °C for 1 h. The plates were washed and 50 μL /well of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (1 mg 4 mL $^{-1}$) in citrate–phosphate buffer (0.2 M, pH 4.0 with 0.015% H $_2$ O $_2$) was added. The reaction was stopped after 20 min by adding 50 μL /well of 1 M H $_2$ SO $_4$ and the optical density (O.D.) was measured at 410 nm relative to 570 nm. Blank wells contained citrate–phosphate buffer. The concentration of each lectin–enzyme conjugate that read an O.D. between 0.8 and 1.0 was used for inhibition experiments.

Inhibition experiments

The microtiter plates were coated overnight at room temperature with yeast mannan or copolymer **20a** (100 μL of 10 μg mL $^{-1}$ soln). The plates were then washed and blocked with BSA as described previously. The following inhibitors were used as stock solutions varying from 1 to 3 mg mL $^{-1}$ of PBS: *p*-nitrophenyl α -D-mannopyranoside (**1**) as reference monovalent compound, di- (**12**), tetra- (**14**), octa- (**16**) and hexadeca-valent (**18**) mannose dendrimers. Each inhibitor was added in serial two-fold dilutions (60 μL /well) in PBS with 60 μL of the desired lectin–enzyme conjugate concn on Nunclon (Delta) microtiter plates and incubated at 37 °C for 1 h. The above solutions (100 μL) were then transferred to the antigen-coated plates which were incubated for another hour at 37 °C. The plates were washed as described above and the ABTS substrate was added (50 μL /well). Color development was stopped after 20 min and the O.D. was measured at 410 nm relative to that at 570 nm. The data were plotted and analysed using Graphpad Inplot Software, v. 4.03. The percent inhibitions were calculated as follows:

$$\% \text{ Inhibition} = (A_{(\text{no inhibitor})} - A_{(\text{with inhibitor})}) / A_{(\text{no inhibitor})} \times 100.$$

IC $_{50}$ s were reported as the concentration required for 50% inhibition of the coating antigen.

Agar gel diffusion

Agar gel diffusion experiments were performed in 1% agarose containing 2% poly(ethyleneglycol) (M_w 8000) in PBS buffer following a procedure described elsewhere.³² The lectin (Con A) and compounds tested (**18** and **20c**) were used at a concentration of 1 mg mL $^{-1}$ in PBS. The precipitin bands were allowed to form overnight at 4 °C and were stained with 0.025% Coomassie Brilliant Blue solution.

Turbidimetric analysis

Turbidimetry experiments were performed on Linbro (Titertek) microtitration plates where 50 μL /well of stock lectin solutions prepared from Con A (2 mg mL $^{-1}$ PBS), wheat germ agglutinin (2 mg mL $^{-1}$), and

crude pea lectin (10 mg mL $^{-1}$) were mixed with 50 μL /well of hexadecavalent mannosylated dendrimer solution (**18**) (1 and 0.5 mg mL $^{-1}$ PBS) and incubated at room temperature for 2–5 h (Fig. 1). The turbidity of the solutions was monitored by reading the optical density (O. D.) at 490 nm at regular time intervals until no noticeable changes could be observed. Each test was done in triplicate.

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