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Novel multivalent mannose compounds and their inhibition of the adhesion of type 1 fimbriated uropathogenic *E. coli*

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Abstract—A series of multivalent mannose containing compounds were prepared varying in size from small divalent, to 16-valent glycodenrimers and 21-valent glycopolymers. The molecules were approached via a common mannose building block. As scaffolds dendrimers and dendrons based on the 3,5-di-(2-aminoethoxy)-benzoic acid branching unit were used along with commercially available PAMAM dendrimers. To include larger structures, linear glycopolymers with varying amounts of mannose were prepared via radical polymerization. The compounds were tested for their biological activity using a newly developed ELISA based inhibition assay, for their ability to inhibit the binding of recombinant type I fimbriated *E. coli* to a monolayer of T24 cell line derived from human urinary bladder epithelium. All compounds showed enhanced affinity as compared to mannose with IC_{50}'s down to the low micromolar range.

1. Introduction

Bacterial adhesion, a prelude to many infections, is commonly mediated by protein–carbohydrate interactions where the bacteria contain lectin-like adhesion proteins that bind to specific surface exposed carbohydrates of glycolipids or glycoproteins on tissue cells.[1–3] Inhibition of the adhesion would be an attractive method of preventing and treating infections. The emergence of antibiotic resistance is becoming a substantial problem in medicine,[4] creating an urgency in the design of additional anti-infectious approaches.[5] As the bacterial adhesion involves carbohydrate–protein interactions, interference in this process provides a logical strategy. Urinary tract infections (UTIs) rank amongst the most frequently occurring bacterial diseases in humans.[6] The vast majority of these UTIs are caused by uropathogenic *Escherichia coli* (UPEC).[6b] In addition to virulence factors such as O-antigens, capsules, toxins and serum resistance, UPEC strains express fimbrial adhesins with carbohydrate binding specificities.[7] The most common fimbrial type is known to be the mannose specific type 1, which contains the FimH adhesion protein[8] and is a common adhesive organelle found in many species of the enterobacterial family. Epidemiological studies indicated the prevalence of type 1 fimbriae in UTIs, since about half of the *E. coli* strains from patients with pyelonephritis and the majority of strains from patients with cystitis are described to express type 1 fimbriae.[9] While bacterial adhesion is often a prerequisite to infection[10] the fluid sheer conditions prevalent in the human bladder particularly necessitate such interactions. For type I fimbriae the FimH adhesin binds to mannose residues present on the bladder epithelial cell surface of members of the glycoprotein family of the uroplakins.[11] The ability of type 1 fimbriated strains to bind mannose strongly differs between strains, yet is the crucial factor for pathogenicity.[21,12] Binding to mannotriose, an ability common to type 1 fimbriated *E. coli* does not correlate to UTI causing abilities. The residues in the mannose binding pocket appeared to be highly conserved, being present in over 200 strains of UPEC. The UTI-infection leads to exfoliation of bladder epithelial cells, a host defence mechanism, while bacteria escape this by invading into deeper tissue, a mechanism likely responsible for recurrent infections.[13]

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For the generation of effective anti-adhesion compounds a major hurdle is the fact that, in general, monosaccharides display low affinities for their target, typically in the millimolar range.[10] The problem may be overcome by the use of multivalent ligands.[14] Synthetic multivalent carbohydrates have been shown highly effective for well-defined targets such as the AB_5 toxins.[15] For interference with bacterial adhesion only a few studies involving multivalent carbohydrates have been reported. A particularly effective inhibition was observed in two studies involving the pig pathogen that can also cause meningitis in humans, *Streptococcus suis*.[16] The adhesion of the Gram-positive pathogen was effectively inhibited by multivalent galabiose (Galα1-4Gal) compounds, where an octavalent compound inhibited haemagglutination with an MIC of 0.3 nM, to the best of our knowledge the first example of sub-nanomolar inhibition of bacterial binding by a synthetic multivalent compound.[16b] We previously reported on multivalent GalNAcβ1-4Gal- and GalNAcβ1-4Galβ1-4Glc-containing glycodendrimers as inhibitors for F1C fimbriated *E. coli* and for *Pseudomonas aeruginosa*.[17] A combination of spacer and multivalency effects led to a 38-fold increase in the potency of a divalent inhibitor as compared to the parent sugar. However, the most prominently studied target has been type 1 fimbriated uropathogenic *E. coli*. Lindhorst et al. prepared a number of multivalent mannose compounds of varying efficacy against the type 1 *E. coli*.[18] Their most effective system was a trivalent system with the mannose residues linked via C-6,[18a,d] which was on a per sugar basis more active than *p*-nitrophenyl α-D-mannoside, a previously observed[19] active ligand. Lee and co-workers[20] prepared larger glycondendrimers as well as neoglycoproteins. These studies showed a significant effect of the aglycon part and also multivalency effects of one order of magnitude. Similar aglycon effects have previously been observed[19] and can be understood when examining the crystal structure of the adhesin, which has hydrophobic areas just outside the mannose binding pocket.[21] The multivalency effect was attributed to bridging between separate fimbrial tips.

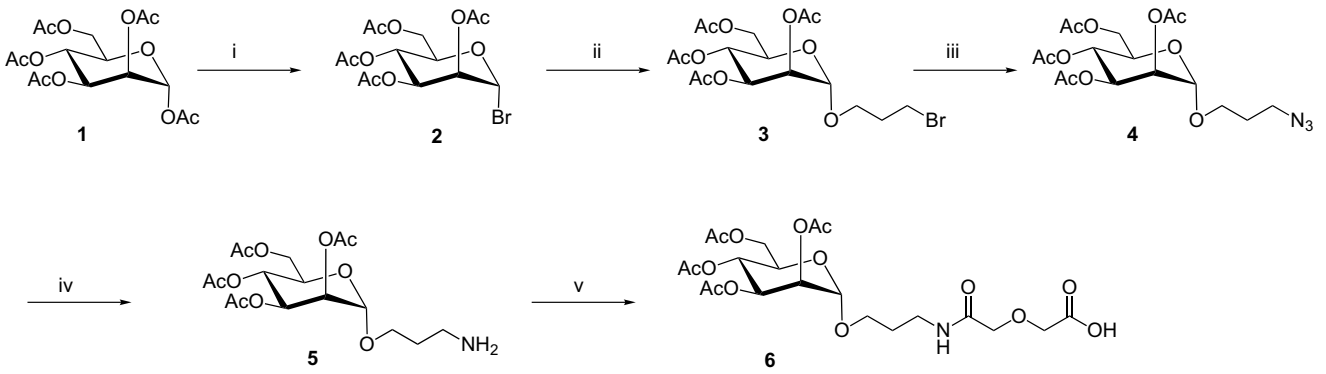
In this study we describe our efforts for the preparation and evaluation of a significant variety of multivalent mannosides, including small divalent systems, glycodendrimers and glycopolymers, as inhibitors of type 1 fimbriated uropathogenic *E. coli*. They were evaluated in a novel highly biorelevant assay involving a bladder cell-line. Multivalency effects have previously been observed in the peeling of bacteria from a mannose containing self-assembled monolayer.[22] The observed effects were attributed to individual fimbriae binding to and releasing from the surface. While the FimH adhesins are known to be located at the tip of the fimbriae,[23] others located in the fimbrial shaft are present as well but deemed only active upon fragmentation and exposure of the binding site.[24] However, previous observations were made where glyconjugates were seen to actively bind along the shaft of the fimbriae.[8] The latter arrangement would imply that the binding sites were more closely spaced and thus would make the binding more susceptible to multivalency effects. Furthermore observations were made to suggest the presence of multiple copies of FimH at the fimbrial tip,[23b] but also data consistent with a single copy exists.[25] Based on all of this information the preparation of a variety of mannose containing systems seemed appropriate. To this end glycopolymers were prepared that represent a linear arrangement of mannose moieties covering relatively long distances. Members of this class were not previously evaluated as inhibitors for type 1 fimbriated *E. coli*, although previous work suggested their potential in this case.[20] In other systems, for example, in viral adhesion inhibition, glycopolymers have proven highly effective.[26] Furthermore, glycodendrimers were made with the potential to simultaneously block several binding sites on the fimbrial shaft. Moreover, especially the smaller ones, could act as mimics of mannotriose, a 20-fold stronger binding moiety than mannose,[20] whose binding site encompasses the mannose binding site.[21] Possible amplification of this effect with an additional 135-fold[20] benefit of hydrophobic aglycon interactions further motivated our studies.

In order to evaluate compounds for their potential the biological context should approximate the actual situation as much as possible. Haemagglutination experiments are often used, although they are complicated by the diversity of displayed carbohydrates on red blood cells and also reproducibility can be problematic. Roy and co-workers[20] used a new assay based on the inhibition of mannosides on the bacterial binding to a radiolabelled glycoprotein in solution. This assay gave much lower IC_{50} values that observed by haemagglutination. Whitesides et al.[27] have developed an assay based on the association of fluorescently labelled bacteria to mannose displaying self-assembled monolayers generated on gold coated 96-well plates. We chose for an assay that is close to the in vivo situation and used a solid phase assay of the ELISA type using a bladder cell line in the well. IC_{50}'s resulting from the assay were not in the nanomolar range for *p*-nitrophenyl α-D-mannoside as in the assay of Lee et al., and neither was there a complete absence of background binding as in the assay of Whitesides, yet in our view the conditions used were realistic. Nonspecific binding of bacteria to cell surfaces is a real occurrence and caused by hydrophobic, Van der Waals and/or electrostatic interaction. In the reported studies here the background binding, which could amount to about 30% of the tota observed binding, was determined by experiments with nonfimbriated bacteria and subtracted consistently.

2. Results and discussion

2.1. Synthesis of general mannose building block

An appropriate mannose building block had to be synthesized for coupling to the various scaffolds. As the scaffolds contain an amine functionality, a carboxylic acid moiety was introduced on the carbohydrate, enabling peptide coupling conditions. Hereto, 1,2,3,4,6-penta-*O*-acetyl-α-D-mannopyranose **1** was converted to



Scheme 1. Reagents and conditions: (i) HBr, AcOH, CH_2Cl_2, rt (98%); (ii) IBr, $HO(CH_2)_3Br$, CH_2Cl_2, CH_3CN, 0 °C to rt (55%); (iii) NaN_3, DMF (83%); (iv) H_2, Pd–C, EtOAc, Et_3N (98%); (v) diglycolic anhydride, pyridine, dioxane (64%).

bromide **2** using HBr, after which an anomeric 3-bromopropanol spacer was introduced using IBr yielding **3** (Scheme 1).[28] After introduction of an azido function using NaN_3 in DMF to give **4**, the compound was converted to amine **5** by hydrogenation. Finally, elaboration with diglycolic anhydride resulted in key building block **6**.

2.2. Synthesis of glycodendrimers

Building block **6** was coupled to various scaffolds generating compounds containing 1–16 mannose molecules (Fig. 1). Dendrimers based on the 3,5-di-(2-aminoethoxy)-benzoic acid branching unit[29] were used as they were previously shown to be successful in generating multivalent carbohydrates.[17,29d,30] Mannose **6** was coupled to the amine containing scaffolds using BOP or TBTU as the coupling reagent and DIPEA as the base in good yields (Table 1). Deacetylation using sodium methoxide in MeOH and subsequent neutralization with Dowex H^+ resulted in mono-, di-, tetra- and octavalent end products **9**, **12**, **27** and **30**. Mono- and divalent compounds **21** and **24**, containing a significantly longer spacer, were synthesized to examine the effect of the spacer on binding. All compounds were fully characterized by NMR and high-resolution mass spectrometry, confirming their identity. Two other divalent compounds **15** and **18** were prepared on the bis-3-amino-prop-1-ynyl benzene scaffold[16b] introducing some pre-orientation of the carbohydrates. Finally, PAMAM dendrimers were used as an alternative dendritic scaffolds, as others[31] have achieved good results with this type of dendrimer. Acetylated compounds **32** and **35** were synthesized in DMSO rather than DCM due solubility problems and purified by size exclusion chromatography (LH_{20}). The structures were confirmed by NMR and MALDI mass spectrometry.

2.3. Synthesis of glycopolymers

Linear glycopolymers were also synthesized with variable numbers of mannose molecules attached to it. The use of carbohydrate containing polymers has been successful,[26,32–34] especially for the inhibition of selectins, and the influenza virus. The polyacrylamide polymers were prepared by radical polymerization using ammonium persulfate and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) in water,[35] containing various amounts of mannose, ranging from 0 (control) to 40 mol%. *N*-(2-Hydroxylpropyl) methacrylamide (HPMA) was chosen as a co-monomer for its good water solubility and excellent biocompatibility.[36] Mannose monomer **38** was prepared, from amine **5**, which was converted to acrylamide **37** by the action of acryloyl chloride (Scheme 2). Subsequently, this compound was deacetylated with NaOMe in MeOH, yielding unprotected monomer **38**, which was used immediately for the polymerization reaction to prevent unwanted premature polymerization of the monomer itself. Hereto, **38** was dissolved in water together with HPMA and TMEDA and polymerized by ammonium persulfate. The polymer was dialyzed against water and lyophilized. The chemical identity of the glycopolymers was analyzed via proton NMR to determine the ratio of each monomer, which was consistent with the input. GPC was used for the determination of the molecular weight ($M_w \sim 10.000$) and polydispersity ($1.3 < M_w/M_n < 1.8$).

2.4. Adhesion inhibition

A biologically relevant testing system was developed: compounds were assayed in an ELISA-based assay for their ability to inhibit the binding of mannose binding type I fimbriated *E. coli* to a monolayer of T24 cell line derived from human urinary bladder epithelium. The inhibitory potencies of the glycodendrimers and polymers are summarized in Table 2 and exemplary inhibition curves are shown in Figure 2. Mannose itself was a poor inhibitor of *E. coli* binding with an IC_{50} of 7.6 mM. Potencies improved with the attachment of scaffolds, as was seen for **9**, which showed an IC_{50} of 337 μM, that is, a relative potency of 23 when compared to mannose. Increasing the number of mannoses to two and even four, improved this affinity both in absolute value (IC_{50} of **27** = 51 μM) and relative when divided by the number of attached saccharides (rel. pot. per sugar 37-fold). For the octavalent **30**, the activity was slightly decreased again. The partially rigidified **15** and **18** displayed inhibitory potencies similar to the flexible divalent **9**. The compounds with elongated spacers,

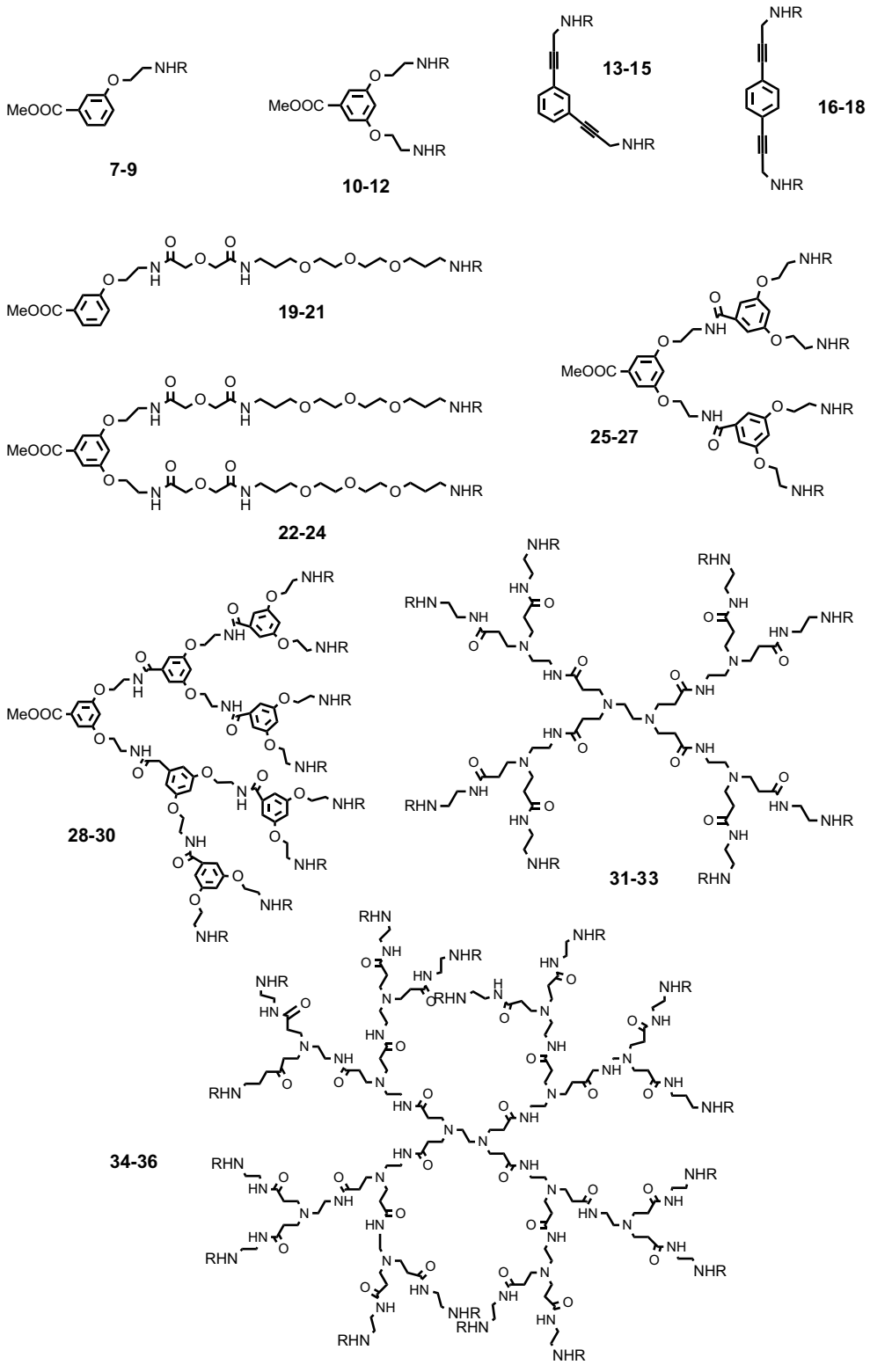
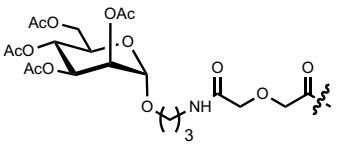
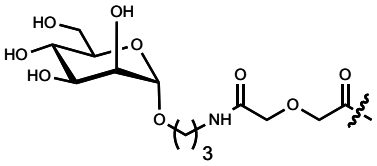
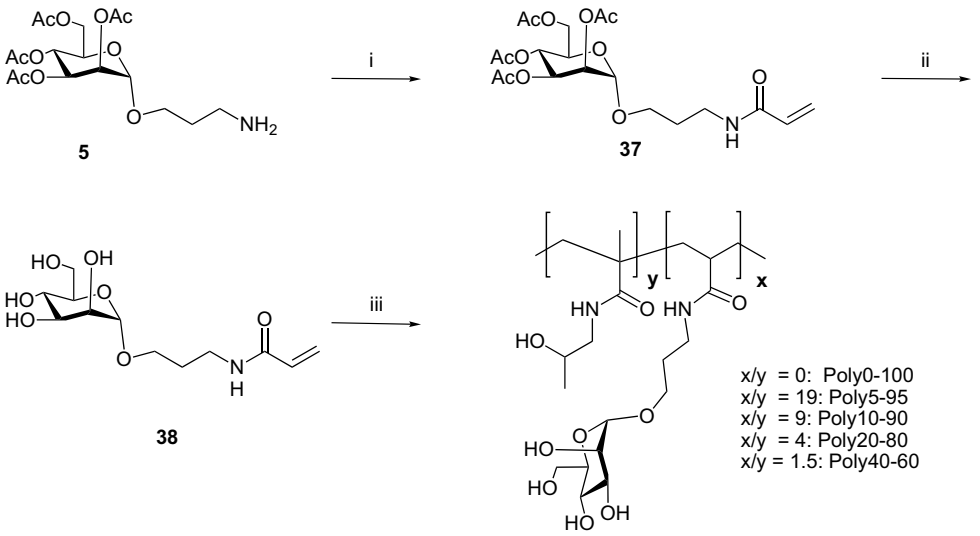


Figure 1. Chemical structures of the prepared (glyco)dendrimers.

monovalent **21** and divalent **24**, showed a 3–8-fold increased activity compared to their short spacered counterparts. In fact the divalent **24** showed the highest relative potency per sugar of 141-fold enhancement versus mannose. The PAMAM glycodendrimer **36** displayed the highest affinity towards the target, although their relative potency per mannose was decreased. The glycopolymers (with 3–21 mannose units per polymer) showed enhanced activity with increasing mannose substitution to an IC_{50} as low as 12 μM. The relative potency per mannose for the polymer series was relatively constant at around 30–40. No binding of the con-

Table 1.

R = H.TFA or H_2		R = 		R = 
7		**8**		**9**
10		**11**		**12**
13		**14**		**15**
6 + 16		**17**		**18**
19	⟶	**20**	⟶	**21**
22	BOP, DIPEA, DCM	**23**		**24**
25	or	**26**	NaOMe, MeOH	**27**
28	TBTU, DIPEA, DMSO	**29**		**30**
31		**32**		**33**
34		**35**		**36**



Scheme 2. Reagents and conditions: (i) acryloyl chloride, dioxane, water, $NaHCO_3$, 0 °C to rt (76%); (ii) NaOMe, MeOH, rt (quant.); (iii) HPMA, APS, TMEDA, H_2O, 40 °C (40–65%).

trol polymer, containing no carbohydrate, was observed in our assay.

The results indicate that a major multivalency effect, as seen with *S. suis* using essentially the same scaffold systems,[16b] did not occur. Affinity enhancements were observed, but the main part of the effect can be attributed to the beneficial effect of a lipophilic aglycon part. Nonetheless the divalent **24**, combined these effects with a moderate multivalency effect leading to a compound with a 281-fold overall affinity improvement over mannose itself. The linear orientation of the glycopolymers did not yield additional benefits over glycodendrimers of similar size and valency, as PAMAM derivative **36** displayed a similar IC_{50} to **Poly40–60**, both in the low micromolar range.

3. Conclusions

In this study we successfully prepared a series of multivalent mannose compounds, that can be divided into the categories: small divalent systems, larger glycodendrimers and linear glycopolymers. They were evaluated as inhibitors in a novel ELISA-type assay that reports on the adhesion of type 1 fimbriated uropathogenic *E. coli* to a relevant cell line. Multivalency in the binding to type 1 fimbriae could in principle occur at different levels. Multiple binding sites on a single FimH molecule are present, considering the enhanced affinity of mannotriose over mannose itself.[21] Multiple copies of FimH have been suggested to be present at fimbrial tips.[23b] Multiple binding occurrences of a glycoconjugate to the fimbrial shaft have been observed at short and longer distance

Table 2.

Compound	Valency	$pIC_{50} \pm SD$	IC_{50} (μM)	Relative potency to mannose (per mannose)
Mannose	1		7600	1
9	1	−3.47 ± 0.13	337	23 (23)
12	2	−3.69 ± 0.043	204	37 (19)
15	2	−3.43 ± 0.035	376	20 (10)
18	2	−3.80 ± 0.038	159	48 (24)
21	1	−3.88 ± 0.050	131	58 (58)
24	2	−4.56 ± 0.055	27	281 (141)
27	4	−4.29 ± 0.10	51	149 (37)
30	8	−4.14 ± 0.032	72	106 (13)
33	8	−4.43 ± 0.033	37	205 (26)
36	16	−4.71 ± 0.047	19	400 (25)
Poly0–100	0		n.d.[a]	—
Poly5–95	3	−4.19 ± 0.038	65	117 (39)
Poly10–90	7	−4.48 ± 0.032	33	230 (33)
Poly20–80	12	−4.70 ± 0.050	20	380 (32)
Poly40–60	21	−4.92 ± 0.050	12	633 (30)

[a] n.d. = no detectable inhibition (less than 10% at 0.5 mM).

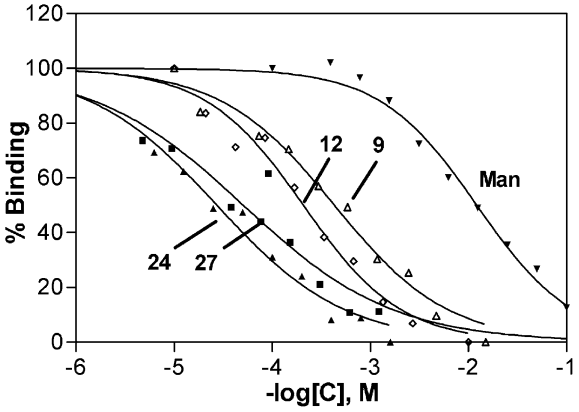


Figure 2. Competition of type I fimbriated *E. coli* binding to human bladder cells by mannose (▼), **9** (△), **12** (◊), **24** (▲) and **27** (■). Human bladder cells were incubated with type I fimbriated *E. coli* with T24 human bladder cells for 15 min at room temperature in the presence of various inhibitors. Data points are means from 3 independent experiments of 9 data points.

separations.[8] Finally, multivalency as the bacteria use it effectively in tissue binding is the simultaneous attachment of multiple fimbriae. To inhibit the latter interactions truly large systems would be required, larger than we here prepared and larger than previously made glycoconjugated proteins.[20] It seems that at the smaller end of the spectrum more can be gained, and in fact was gained judging from the mentioned 146-fold affinity gain by the divalent **24** and also the trivalent C6 linked mannoside reported by Lindhorst and co-workers.[18a,d] At which level these gains are made is still unclear. Overall further affinity improvement may also come from the design of improved monovalent systems based on the FimH X-ray structure,[21] harnessing the full potential of interactions, lipophilic and other, between ligand and protein. A recent example of such an approach indicates its potential.[37]

4. Experimental

4.1. General methods

Chemicals were obtained from commercial sources and used without further purification unless stated otherwise. Reactions were monitored by TLC on Silica gel 60 F_{254} (Merck, Amsterdam, the Netherlands). After examination under UV light, compounds were visualized by heating with 10% (v/v) methanolic H_2SO_4 or with ninhydrine (3 mg mL^{-1}). ^{1}H NMR spectra were recorded at 300 K with a Varian Gemini-300 (300 MHz) or a Varian Unity UNOVA 500 (500 MHz spectrometer); δ_H values are given in ppm relative to the signal of internal Me_4Si ($\delta_H = 0$, $CDCl_3$ or CD_3OD). ^{13}C NMR spectra were recorded at 300 K with a Varian Gemini-300 (75 MHz); δ_C values are given in ppm relative to the signal of $CDCl_3$ ($\delta_C = 77.0$) or CD_3OD ($\delta_C = 49.0$). Exact mass spectra were measured by nanoelectrospray time-of-flight mass spectrometry using a Micromass LCToF mass spectrometer at a resolution of 5000 FWHM. Dowex 50 × 8 (H^+ form; 20–50 mesh, Fluka) was used for neutralization. Organic layers were dried with Na_2SO_4. Amines **7**, **10**, **13**, **16**, **19**, **22**, **25** and **28** were prepared according to the literature procedures.[16b,17,29] Compounds **31** and **34** were obtained from Sigma–Aldrich (Zwijndrecht, the Netherlands).

4.2. Synthesis of 1-bromo-2,3,4,6-*O*-acetyl-α-D-mannopyranoside 2

Peracetylated mannose (**1**, 2.12 g, 5.43 mmol) was dissolved in dry DCM (100 mL), cooled to 0 °C, after which HBr–AcOH (4.64 mL, 33% solution) was added dropwise. After overnight stirring (TLC, EtOAc–hexane, 1:1), the solution was diluted with DCM and ice water, and neutralized with $NaHCO_3$. The organic layer was dried and concentrated, yielding bromide **2** as a oil (2.19 g, 98% yield), which was used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.01, 2.09, 2.11, 2.18 (4 × s, 12H, $COCH_3$), 4.13 (dd, 1H, *J* 2.1, 12.4 Hz, H-6b), 4.20–4.26 (m, 1H, H-5), 4.34 (dd, 1H, *J* 4.7, 12.4 Hz, H-6a), 5.38 (t, 1H, *J* 10.9 Hz, H-4), 5.44 (dd, 1H, *J* 1.6, 3.3 Hz, H-2), 5.70 (dd, 1H, *J* 3.3, 10.1 Hz, H-3), 6.33 (d, 1H, *J* 1.1 Hz, H-1).

4.3. Synthesis of 3-bromopropyl-2,3,4,6-*O*-acetyl-α-D-mannopyranoside 3

To a cooled solution of bromide **2** (1.87 g, 4.55 mmol) in dry CH_3CN (30 mL) was added IBr (11.4 mL, 11.4 mmol). After stirring for 3 h (TLC, EtOAc–hexane, 1:1), the solution was diluted with DCM, washed with an 5% aqueous $Na_2S_2O_5$ solution, dried and concentrated. Column chromatography (20–50% EtOAc–hexane) yielded compound **3** (1.08 g, 55% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.94–1.99 (m, 2H, OCH_2CH_2-CH_2Br), 2.00, 2.06, 2.11, 2.17 (4 × s, 12H, $COCH_3$), 3.51–3.58 (m, 3H, $OCHHCH_2CH_2Br$), 3.88–3.95 (m, 1H, $OCHHCH_2CH_2Br$), 3.95–4.07 (m, 1H, H-5), 4.11 (dd, 1H, *J* 2.2, 12.4 Hz, H-6b), 4.28 (dd, 1H, *J* 4.7, 12.4 Hz, H-6a), 4.84 (d, 1H, *J* 1.4 Hz, H-1), 5.21–5.29 (m, 3H, H-2, H-3, H-4). ^{13}C NMR (75 MHz, $CDCl_3$):

δ 20.0 ($CO\mathit{C}H_3$), 29.6 ($OCH_2CH_2\mathit{C}H_2Br$), 31.4 ($OCH_2\mathit{C}H_2CH_2Br$), 61.7 ($O\mathit{C}H_2CH_2CH_2Br$), 64.8, 65.3, 68.0, 68.4, 68.7 (C-2, C-3, C-4, C-5, C-6), 96.9 (C-1), 169.0, 169.1, 169.2, 169.8 ($\mathit{C}OCH_3$). MS for $C_{17}H_{25}O_{10}Br$ (M, 468.06): [M+Na^+] calcd 491.05, found: 491.20.

4.4. Synthesis of 3-azidopropyl 2,3,4,6-*O*-acetyl-α-D-mannopyranoside 4

To a solution of **3** (7.18 g, 15.3 mmol) in dry DMF (75 mL) was added NaN_3 (4.67 g, 71.9 mmol). After overnight stirring at 100 °C (TLC, toluene–EtOAc, 1:1), the solution was filtered over Hyflo and co-concentrated with toluene. The residue was diluted with DCM and washed with brine, dried and concentrated. Column chromatography (toluene–EtOAc, 15/1 → 10/1) yielded **4** (5.47 g, 83% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.89–1.96 (m, 2H, $OCH_2C\mathit{H}_2CH_2N_3$), 2.00, 2.06, 2.11, 2.17 (4 × s, 12H, $COC\mathit{H}_3$), 3.45 (t, 2H, *J* 6.6 Hz, $OCH_2CH_2C\mathit{H}_2N_3$), 3.50–3.57 (m, 1H, $OC\mathit{H}HCH_2CH_2N_3$), 3.79–3.86 (m, 1H, $OCH\mathit{H}CH_2CH_2N_3$), 3.95–4.00 (m, 1H, H-5), 4.12 (dd, 1H, *J* 2.5, 12.1 Hz, H-6b), 4.29 (dd, 1H, *J* 5.4, 12.1 Hz, H-6a), 4.83 (d, *J* 1.4 Hz, H-1), 5.23–5.35 (m, 3H, H-2, H-3, H-4). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.9 ($CO\mathit{C}H_3$), 28.6 ($OCH_2\mathit{C}H_2CH_2N_3$), 48.0 ($OCH_2\mathit{C}H_2CH_2N_3$), 62.4, 64.8 ($O\mathit{C}H_2CH_2CH_2N_3$, C-6), 66.0, 68.6, 69.0, 69.4 (C-2, C-3, C-4, C-5), 97.6 (C-1), 169.7, 169.9, 170.1, 170.6 ($\mathit{C}OCH_3$). MS for $C_{17}H_{25}N_3O_{10}$ (M, 431.15): [M+Na^+] calcd 454.14, found: 454.40.

4.5. Synthesis of 3-aminopropyl 2,3,4,6-*O*-acetyl-α-D-mannopyranoside 5

Azido **4** (712 mg, 1.65 mmol) was dissolved in dry EtOAc, after which Pd–C (142 mg) and a few drops of Et_3N were added. After stirring for 3 h under H_2 (TLC, DCM–MeOH, 2:1) the reaction mixture was filtered off over Hyflo and concentrated, yielding **5** as a slightly yellow oil (656 mg, 98% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.94–1.98 (m, 2H, $OCH_2C\mathit{H}_2CH_2NH_2$), 2.00, 2.06, 2.11, 2.17 (4 × s, 12H, $COC\mathit{H}_3$), 2.97–3.08 (m, 2H, $OCH_2CH_2C\mathit{H}_2NH_2$), 3.40–3.47 (m, 2H, $OC\mathit{H}_2CH_2CH_2NH_2$), 4.29 (dd, 1H, *J* 4.7, 12.4 Hz, H-6a), 4.83 (d, 1H, *J* < 1 Hz, H-1), 5.23–5.28 (m, 3H, H-2, H-3, H-4). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.6 ($CO\mathit{C}H_3$), 37.3 ($OCH_2\mathit{C}H_2CH_2NH_2$), 46.2 ($OCH_2CH_2\mathit{C}H_2NH_2$), 62.2, 64.8 ($O\mathit{C}H_2CH_2CH_2NH_2$, C-6), 65.6, 66.0, 70.2, 71.0 (C-2, C-3, C-4, C-5), 97.3 (C-1), 169.6, 170.0, 170.1, 170.6 ($\mathit{C}OCH_3$). MS for $C_{17}H_{27}NO_{10}$ (M, 405.16): [M+H^+] calcd 406.17, found 406.25.

4.6. Synthesis of general building block 6

Amine **5** (86 mg, 0.21 mmol) was dissolved in pyridine (6 mL)/dioxane (3 mL) and glycolic anhydride (24.5 mg, 0.21 mmol) was added. After stirring overnight under N_2 at 90 °C (TLC, DCM–MeOH, 2:1) the solution was concentrated and co-evaporated with toluene. The residue was diluted with DCM, washed with 1 N $KHSO_4$ and brine, dried and concentrated, yielding **6** (70 mg, 64% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.87–1.93 (m, 2H, $OCH_2C\mathit{H}_2CH_2NH$), 2.00, 2.06, 2.11, 2.17 (4 × s, 12H, $COC\mathit{H}_3$), 3.42–3.58 (m, 3H, $OC\mathit{H}HCH_2C\mathit{H}_2NH$), 3.75–3.85 (m, 1H, $OCH\mathit{H}CH_2CH_2NH$), 3.97–4.02 (m, 1H, H-5), 4.07–4.11 (m, H-6b), 4.15 and 4.21 (2 × s, 4H, $COC\mathit{H}_2OC\mathit{H}_2CO$), 4.28 (dd, 1H, *J* 4.9, 12.4 Hz, H-6a), 4.83 (d, 1H, *J* < 1 Hz, H-1), 5.23–5.34 (m, 3H, H-2, H-3, H-4). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.7 ($CO\mathit{C}H_3$), 28.7 ($OCH_2\mathit{C}H_2CH_2NH$), 36.6 ($OCH_2CH_2\mathit{C}H_2NH$), 62.5 ($O\mathit{C}H_2CH_2CH_2NH$), 66.1, 66.3, 68.4, 69.1, 69.4, 69.5, 71.3 (C-2, C-3, C-4, C-5, C-6, $CO\mathit{C}H_2O\mathit{C}H_2CO$), 97.7 (C-1), 169.8 170.1, 172.3, 172.9 ($\mathit{C}OCH_3$). HR-MS for $C_{21}H_{31}NO_{14}$ (M, 521.175): [M+Na^+] calcd 522.182, found 522.189.

4.7. General procedure for synthesis of protected glycodendrimers

Amine containing scaffolds (as free amine or TFA salt) were dissolved in dry DCM and general building block **6** (1.2 equiv per amine), BOP (1.2 equiv per amine) and DIPEA (6 equiv per amine) were added. After stirring for 4–16 h at rt the solution was diluted with DCM and the organic layer washed with 1 N $KHSO_4$, 1 N NaOH, water and brine. After drying and concentration, acetylated glycodendrimers were purified by column chromatography (0–50% MeOH–DCM). For larger dendrimers (⩾8 amine groups), DMSO instead of DCM and TBTU (1.2 equiv per amine) instead of BOP was used. Purifications of these large glycodendrimers was performed based on size exclusion (LH_{20}, eluents DCM–MeOH, 1:1).

4.7.1. Monovalent *O*-acetyl protected mannose short spacer, compound 8. Compound **8** was prepared according to general procedure 4.7 from **7**. 98 mg, 83% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.78–1.95 (m, 2H, $OCH_2C\mathit{H}_2CH_2NH$), 1.99, 2.06, 2.11, 2.18 (4 × s, 12H, $COC\mathit{H}_3$), 3.40–3.58 (m, 3H, $OC\mathit{H}HCH_2C\mathit{H}_2NH$), 3.74–3.78 (m, 3H, $OCH\mathit{H}CH_2CH_2NH$, $OCH_2C\mathit{H}_2NH$), 3.91 (s, 3H, $OC\mathit{H}_3$), 3.95–4.00 (m, 1H, H-5), 4.07 and 4.10 (2 × s, 4H, $COC\mathit{H}_2OC\mathit{H}_2CO$), 4.11–4.15 (m, 1H, H-6b), 4.28 (dd, 1H, *J* 4.2, 12.4 Hz, H-6a), 4.80 (d, 1H, *J* 1.2 Hz, H-1), 5.24–5.29 (m, 3H, H-2, H-3, H-4), 7.20 (t, 1H, *J* 5.5 Hz, $C\mathit{H}_{arom}$), 7.36 (t, 1H, *J* 8.0 Hz, $C\mathit{H}_{arom}$), 7.54 (s, 1H, CH_{arom}), 7.64 (d, 1H, *J* 7.6 Hz, $C\mathit{H}_{arom}$). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.6 ($CO\mathit{C}H_3$), 28.9 ($OCH_2\mathit{C}H_2CH_2NH$), 36.7 ($OCH_2CH_2\mathit{C}H_2NH$), 38.3 ($OCH_2\mathit{C}H_2NH$), 52.1 ($O\mathit{C}H_3$), 62.3 ($O\mathit{C}H_2CH_2CH_2NH$), 65.9, 66.4, 66.5, 68.3, 69.0, 69.2, 71.0 (C-2, C-3, C-4, C-5, C-6, $CO\mathit{C}H_2O\mathit{C}H_2CO$, $O\mathit{C}H_2CH_2NH$), 97.5 (C-1), 114.7, 119.5, 122.3, 129.4 ($\mathit{C}H_{arom}$), 131.4 ($C_{q\,arom}COOMe$), 158.2 ($C_{q\,arom}$), 166.6 ($\mathit{C}OOMe$), 168.6, 168.9, 169.6, 169.9, 170.0, 170.6 ($\mathit{C}OCH_3$, $\mathit{C}OCH_2OCH_2\mathit{C}O$). HR-MS for $C_{31}H_{42}N_2O_{16}$ (M, 698.2534), calcd [M+H^+] 699.2612, found 699.2673, [M+Na^+] calcd 721.2432, found 721.2371.

4.7.2. Divalent *O*-acetyl protected mannose-short spacer, compound 11. Compound **11** was prepared according to general procedure 4.7 from **10**. 37.4 mg, 52% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.82–1.92 (m, 4H, $OCH_2C\mathit{H}_2CH_2NH$), 1.99–2.16 (4 × s, 24H, $C\mathit{H}_3$), 3.40–3.45 (m, 4H, $OCH_2CH_2C\mathit{H}_2NH$), 3.50–3.55 (m, 2H,

OC*H*HCH$_2$CH$_2$NH), 3.72 (dd, 4H, *J* 5.5, 10.6 Hz, OCH$_2$C*H*$_2$NH), 3.77–3.82 (m, 2H, OCH*H*CH$_2$-CH$_2$NH), 3.90 (s, 3H, OC*H*$_3$), 3.92–3.99 (m, 2H, H-5), 4.06 and 4.10 (2 × s, 8H, COC*H*$_2$OC*H*$_2$CO), 4.11–4.15 (m, 2H, H-6b), 4.28 (dd, 2H, *J* 4.1, 12.2 Hz, H-6a), 4.81 (d, 2H, *J* 0.9 Hz, H-1), 5.24–5.31 (m, 6H, H-2, H-3, H-4), 6.92 (s, 1H, C*H*$_{arom}$), 7.18 (s, 2H, C*H*$_{arom}$). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.7 (CO*C*H$_3$), 29.0 (OCH$_2$*C*H$_2$CH$_2$NH), 36.9 (OCH$_2$CH$_2$*C*H$_2$NH), 38.4 (OCH$_2$*C*H$_2$NH), 52.3 (O*C*H$_3$), 62.3 (O*C*H$_2$CH$_2$-CH$_2$NH), 66.0, 66.6, 66.8, 68.4, 69.1, 69.3, 71.1 (C-2, C-3, C-4, C-5, C-6, CO*C*H$_2$O*C*H$_2$CO, O*C*H$_2$CH$_2$NH), 97.6 (C-1), 106.6, 108.1 (*C*H$_{arom}$), 132.2 (C$_{q\,arom}$-COOMe), 159.5 (C$_{q\,arom}$), 168.7, 169.1, 169.7, 170.1, 170.7 (*C*OCH$_3$, *C*OCH$_2$OCH$_2$*C*O). HR-MS calcd for $C_{54}H_{76}N_4O_{30}$: 1260.4544, found 1261.4323 [M+H$^+$], 1283.4461 [M+Na$^+$].

4.7.3. Divalent *O*-acetyl protected mannose 1–3 rigid short spacer, compound 14. Compound **14** was prepared according to general procedure 4.7 from **13**. 138 mg, 90% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.81–1.92 (m, 4H, OCH$_2$C*H*$_2$CH$_2$NH), 2.00, 2.05, 2.10, 2.15 (4 × s, 24H, COC*H*$_3$), 3.34–3.51 (m, 6H, OC*H*HCH$_2$C*H*$_2$NH), 3.74–3.79 (m, 2H, OCH*H*CH$_2$-CH$_2$NH), 3.95–3.98 (m, 2H, H-5), 4.08–4.19 (m, 10H, COC*H*$_2$OC*H*$_2$CO, H-6b), 4.23–4.36 (m, 6H, C≡C*H*$_2$, H-6a), 4.80 (d, 2H, *J* < 1 Hz, H-1), 5.23–5.32 (m, 6H, H-2, H-3, H-4), 7.20–7.39 (m, 4H, C*H*$_{arom}$). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.5 (CO*C*H$_3$), 28.7, 29.1, 36.6 (*C*H$_2$C≡, OCH$_2$*C*H$_2$CH$_2$NH, OCH$_2$CH$_2$*C*H$_2$NH), 62.3 (O*C*H$_2$CH$_2$CH$_2$NH), 65.8, 66.3, 68.2, 69.0, 69.1, 70.7 (C-2, C-3, C-4, C-5, C-6, CO*C*H$_2$O*C*H$_2$CO), 81.8 (≡*C*CH$_2$), 85.3 (≡*C*CH), 97.4 (C-1), 122.5 (*C*$_{q\,arom}$), 131.3 (*C*H$_{arom}$), 168.8, 168.9, 169.5, 170.1, 170.6 (*C*OCH$_3$, CONH, *C*OCH$_2$OCH$_2$*C*O). HR-MS for $C_{54}H_{70}N_4O_{26}$ (M, 1190.4278), [M+H$^+$] calcd 1191.4356, found: 1191.2833.

4.7.4. Divalent *O*-acetyl protected mannose 1–4 rigid short spacer, compound 17. Compound **17** was prepared according to general procedure 4.7 from **16**. 84 mg, 89% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.81–1.93 (m, 4H, OCH$_2$C*H*$_2$CH$_2$NH), 2.00, 2.05, 2.10, 2.15 (4 × s, 24H, COC*H*$_3$), 3.40–3.55 (m, 6H, OC*H*HCH$_2$C*H*$_2$NH), 3.74–3.81 (m, 2H, OCH*H*CH$_2$-CH$_2$NH), 3.94–4.02 (m, 1H, H-5), 4.06–4.19 (m, 10H, COC*H*$_2$OC*H*$_2$CO, H-6b), 4.23–4.34 (m, 6H, C≡C*H*$_2$, H-6a), 4.80 (d, 2H, *J* < 1 Hz, H-1), 5.24–5.33 (m, 6H, H-2, H-3, H-4), 7.27–7.41 (m, 4H, C*H*$_{arom}$). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.5 (CO*C*H$_3$), 28.6, 29.1, 36.3 (*C*H$_2$C≡, OCH$_2$*C*H$_2$CH$_2$NH, OCH$_2$CH$_2$*C*H$_2$NH), 62.2 (O*C*H$_2$CH$_2$CH$_2$NH), 65.7, 66.3, 68.2, 68.9, 69.1, 70.7 (C-2, C-3, C-4, C-5, C-6, CO*C*H$_2$O*C*H$_2$CO), 82.1 (≡*C*CH$_2$), 86.3 (≡*C*CH), 97.3 (C-1), 122.2 (*C*$_{q\,arom}$), 131.3 (*C*H$_{arom}$), 168.7, 168.9, 169.5, 170.1, 170.6 (*C*OCH$_3$, *C*ONH, *C*OCH$_2$OCH$_2$*C*O). HR-MS for $C_{54}H_{70}N_4O_{26}$ (M, 1190.4278), [M+H$^+$] calcd 1191.4356, found: 1191.5167.

4.7.5. Monovalent *O*-acetyl protected mannose-long spacer, compound 20. Compound **20** was prepared according to general procedure 4.7 from **19**. 44 mg, 39% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.61–1.82 (m, 6H, OCH$_2$C*H*$_2$CH$_2$NH), 1.97, 2.03, 2.09, 2.14 (4 × s, 12H, COC*H*$_3$), 3.14–3.77 (m, 22H, OC*H*$_2$CH$_2$-C*H*$_2$NH, OCH$_2$C*H*$_2$NH, OC*H*$_2$C*H*$_2$O), 3.88 (s, 3H, OC*H*$_3$), 3.90–4.14 (m, 13H, COC*H*$_2$OC*H*$_2$CO, OC*H*$_2$-CH$_2$NH, H-5, H-6a, H-6b), 4.77 (d, 1H, *J* < 1 Hz, H-1), 5.21–5.31 (m, 3H, H-2, H-3, H-4), 7.34 (s, 1H, C*H*$_{arom}$), 7.27–7.32 (m, 3H, C*H*$_{arom}$). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.5 (CO*C*H$_3$), 28.5 (OCH$_2$-*C*H$_2$CH$_2$NH), 36.9 (OCH$_2$CH$_2$*C*H$_2$NH), 38.5 (OCH$_2$-*C*H$_2$NH), 52.1 (O*C*H$_3$), 62.3 (OC*H*$_2$CH$_2$CH$_2$NH), 65.9, 68.3, 69.2, 69.3, 69.8, 70.0 (C-2, C-3, C-4, C-5, C-6, CO*C*H$_2$O*C*H$_2$CO, *C*H$_2$O*C*H$_2$*C*H$_2$O, O*C*H$_2$-CH$_2$NH), 97.1 (C-1), 114.7, 119.5, 122.1, 129.4 (*C*H$_{arom}$), 131.2, 158.4 (C$_{q\,arom}$), 166.8 (COOMe), 169.6, 169.8, 170.2, 170.3, 170.7 (*C*OCH3, *C*O-CH$_2$OCH$_2$*C*O). HR-MS for $C_{45}H_{68}N_4O_{22}$ (M, 1016.4325), [M+H$^+$] calcd 1017.4404, found: 1017.4350.

4.7.6. Divalent *O*-acetyl protected mannose-long spacer, compound 23. Compound **23** was prepared according to general procedure 4.7 from **22**. 60 mg, 65% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.58–1.82 (m, 12H, OCH$_2$C*H*$_2$CH$_2$NH), 1.98, 2.04, 2.09, 2.15 (4 × s, 24H, COC*H*$_3$), 3.11 (dd, 12H, OCH$_2$CH$_2$C*H*$_2$NH), 3.15–3.87 (m, 32H, OC*H*$_2$CH$_2$CH$_2$NH, OCH$_2$C*H*$_2$NH, OC*H*$_2$C*H*$_2$O), 3.87 (s, 3H, COC*H*$_3$), 3.91–4.22 (m, 26H, COC*H*$_2$OC*H*$_2$CO, OC*H*$_2$CH$_2$NH, H-5, H-6a, H-6b), 4.79 (d, 2H, *J* < 1 Hz, H-1), 5.18–5.36 (m, 3H, H-2, H-3, H-4), 7.06–7.25 (2 × s, 3H, C*H*$_{arom}$). HR-MS for $C_{82}H_{128}N_8O_{42}$ (M, 1896.8126), calcd [M−2Ac+Na$^+$] 1835.781, found 1835.813.

4.7.7. Tetravalent *O*-acetyl protected mannose-short spacer, compound 26. Compound **26** was prepared according to general procedure 4.7 from **25**. 50.4 mg, 66% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.78–1.92 (m, 8H, OCH$_2$C*H*$_2$CH$_2$NH), 1.99, 2.05, 2.10, 2.15 (4 × s, 48H, COC*H*$_3$), 3.16–3.83 (m, 28H, OC*H*$_2$CH$_2$C*H*$_2$NH, OCH$_2$C*H*$_2$NH), 3.89 (s, 3H, OC*H*$_3$), 4.03 and 4.07 (2 × s, 16H, COC*H*$_2$OC*H*$_2$CO), 4.08–4.16 (m, 12H, OC*H*$_2$CH$_2$NH), 4.17–4.31 (m, 12H, H-5, H-6a, H-6b), 4.79 (d, 4H, *J* < 1 Hz, H-1), 5.23–5.29 (m, 12H, H-2, H-3, H-4), 6.85–6.96 (m, 3H, C*H*$_{arom}$), 7.13–7.20 (m, 6H, C*H*$_{arom}$). HR-MS for $C_{118}H_{162}N_{10}O_{62}$ (M, 2712.6), [M+Na$^+$] calcd average 2735.6, found 2735.3.

4.7.8. Octavalent *O*-acetyl protected mannose-short spacer, compound 29. Compound **29** was prepared according to general procedure 4.7 from **28**. 26.8 mg, 54% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.77–1.97 (m, 16H, OCH$_2$C*H*$_2$CH$_2$NH), 1.98, 2.04, 2.09, 2.14 (4 × s, 96H, COC*H*$_3$), 3.16–4.32 (m, 147H, CO-C*H*$_2$OC*H*$_2$CO, OC*H*$_2$CH$_2$C*H*$_2$NH, OCH$_2$C*H*$_2$NH, OC*H*$_3$, H-5, H-6a, H-6b), 4.79 (d, 8H, *J* < 1 Hz, H-1), 5.22–5.26 (m, 12H, H-2, H-3, H-4), 6.21–6.58 (m, 7H, C*H*$_{arom}$), 6.73–7.13 (m, 14H, C*H*$_{arom}$). HR-MS for $C_{246}H_{334}N_{22}O_{126}$ (M, 5612.0), [M+H]$^+$ average calcd 5616.4, found 5619.4

4.7.9. Octavalent *O*-acetyl protected PAMAM based mannose-short spacer, compound 32. Compound **32**

was prepared according to general procedure 4.7 from **31**. 17.6 mg, 48% yield. ^{1}H NMR (300 MHz, MeOD): δ 1.87–1.94 (m, 16H, $OCH_2CH_2CH_2NH$), 1.95, 2.04, 2.06, 2.13 (4 × s, 96H, $COCH_3$), 2.34–2.42 (m, 24H, NCH_2CH_2NHCO), 2.46–2.64 (m, 12H, NCH_2CH_2N, $CONHCH_2CH_2N$), 2.72–2.90 (m, 24H, NCH_2CH_2-NHCO), 3.20–3.42 (m, 64H, $OCHHCH_2CH_2NH$, $CONHCH_2CH_2N$, $NHCH_2CH_2NH$), 3.47–3.58 (m, 8H, $OCHHCH_2CH_2NH$), 3.77–3.84 (m, 8H, H-5), 3.97–4.17 (m, 40H, $COCH_2OCH_2CO$, H-6a), 4.25 (dd, 8H, *J* 4.8, 12.4 Hz, H-6b), 4.83 (8H, H-1), 5.19–5.23 (m, 24H, H-2, H-3, H-4). MALDI-MS calcd for $C_{230}H_{360}N_{34}O_{116}$: 5457.5, found 5456.8.

4.7.10. Hexadecavalent *O*-acetyl protected PAMAM based mannose-short spacer, compound 35. Compound **35** was prepared according to general procedure 4.7 from **32**. 27.4 mg, 43% yield. ^{1}H NMR (300 MHz, MeOD): δ 1.87–1.94 (m, 32H, $OCH_2CH_2CH_2NH$), 1.96, 2.05, 2.07, 2.15 (4 × s, 96H, $COCH_3$), 2.34–2.42 (m, 56H, NCH_2CH_2NHCO), 2.48–2.63 (m, 28H, NCH_2CH_2N, $CONHCH_2CH_2N$), 2.76–2.91 (m, 56H, NCH_2CH_2NHCO), 3.20–3.42 (m, 156H, $OCHHCH_2$-CH_2NH, $CONHCH_2CH_2N$, $NHCH_2CH_2NH$), 3.49–3.62 (m, 16H, $OCHHCH_2CH_2NH$), 3.76–3.87 (m, 16H, H-5), 3.98–4.19 (m, 60H, $OCH_2CH_2CH_2NH$, H-6a), 4.21–4.27 (m, 16H, H-6b), 4.83 (16H, H-1), 5.21–5.38 (m, 48H, H-2, H-3, H-4).

4.8. General procedure for preparation of unprotected glycodendrimers

The protected glycodendrimers were dissolved in dry MeOH and sodium methoxide (30% w/w, 10× diluted) was added. TLC analysis (MeOH–CH_2Cl_2, 1:9) was used to determine if the reaction was completed. If product precipitated from the solution, a few drops of water were added and stirring was continued for 1 h. The reaction mixture was neutralized with Dowex H^+ and concentrated in vacuo. Products were purified using a Seppack column with water/ACN as eluents and lyophilized.

4.8.1. Monovalent mannose-short spacer, compound 9. Compound **9** was prepared according to general procedure 4.8 from **8**. 41.1 mg, 55% yield. ^{1}H NMR (300 MHz, D_2O): δ 1.58–1.62 (m, 2H, OCH_2CH_2-CH_2NH), 3.03–3.76 (m, 12H, $OCH_2CH_2CH_2NH$, OCH_2CH_2NH, H-2, H-3, H-4, H-5, H-6a, H-6b), 3.73 (s, 3H, $COCH_3$), 3.83 and 3.89 (2 × s, 4H, $COCH_2OCH_2CO$), 3.95–4.11 (m, 2H, OCH_2CH_2NH), 4.60 (1H, H-1), 6.96 (d, 1H, CH_{arom}), 7.16–7.23 (m, 2H, CH_{arom}), 7.34 (d, 1H, CH_{arom}). HR-MS for $C_{23}H_{34}N_2O_{12}$ (M, 530.2113), [M+H^+] calcd 531.2190, found: 531.2032, [M+Na^+] calcd 553.2010, found 553.2054.

4.8.2. Divalent mannose-short spacer, compound 12. Compound **12** was prepared according to general procedure 4.8 from **11**. 14.1 mg, 51% yield. ^{1}H NMR (300 MHz, D_2O): δ 1.58–1.62 (m, 4H, OCH_2CH_2-CH_2NH), 3.08–3.77 (m, 21H, $OCH_2CH_2CH_2NH$, OCH_2CH_2NH, H-2, H-3, H-4, H-5, H-6a, H-6b), 3.71 (s, 3H, $COCH_3$), 3.84 and 3.91 (2 × s, 4H, $COCH_2OCH_2CO$), 3.93–4.03 (m, 4H, OCH_2CH_2NH), 4.59 (d, 2H, *J* 2.0 Hz, H-1), 6.58 (s, 1H, CH_{arom}), 7.35 (s, 2H, CH_{arom}). HR-MS calcd for $C_{38}H_{60}N_4O_{22}$ (M, 924.3699) found: [M+H^+] calcd 925.3777, found 925.3823, [M+Na^+] calcd 947.3597, found 947.3153.

4.8.3. Divalent mannose 1–3 rigid-short spacer, compound 15. Compound **15** was prepared according to general procedure 4.8 from **14**. 43.9 mg, 73% yield. ^{1}H NMR (300 MHz, D_2O): δ 1.58–1.65 (m, 4H, OCH_2CH_2-CH_2NH), 3.12–3.22 (m, 4H, $OCH_2CH_2CH_2NH$), 3.25–3.78 (m, 16H, $OCH_2CH_2CH_2NH$, H-2, H-3, H-4, H-5, H-6a, H-6b), 3.97 and 4.05 (2 × s, 4H, $COCH_2OCH_2$-CO), 4.11 (s, 4H, CCH_2), 4.63 (d, 2H, H-1), 7.13–7.19 (m, 3H, CH_{arom}), 7.29 (s, 1H, CH_{arom}). HR-MS for $C_{38}H_{54}N_4O_{18}$ (M, 854.3433), [M+H^+] calcd 855.3511, found: 855.6205, [M+Na^+] calcd 877.3331, found 877.5468.

4.8.4. Divalent mannose 1–4 rigid-short spacer, compound 18. Compound **18** was prepared according to general procedure 4.8 from **17**. 76.1 mg, 77% yield. ^{1}H NMR (300 MHz, D_2O): δ 1.58–1.67 (m, 4H, OCH_2CH_2-CH_2NH), 3.13–3.22 (m, 4H, $OCH_2CH_2CH_2NH$), 3.24–3.77 (m, 16H, $OCH_2CH_2CH_2NH$, H-2, H-3, H-4, H-5, H-6a, H-6b), 3.95 and 4.07 (2 × s, 4H, $COCH_2OCH_2$-CO), 4.12 (s, 2H, CCH_2), 4.63 (d, 2H, H-1), 7.32–7.45 (m, 4H, CH_{arom}). HR-MS for $C_{38}H_{54}N_4O_{18}$ (M, 854.3433), [M+H^+] calcd 855.3511, found: 855.4216, [M+Na^+] calcd 877.3331, found 877.2683.

4.8.5. Monovalent mannose-long spacer, compound 21. Compound **21** was prepared according to general procedure 4.8 from **20**. 12 mg, 31% yield. ^{1}H NMR (300 MHz, D_2O): δ 1.68–1.81 (m, 6H, OCH_2CH_2-CH_2NH), 3.20–3.31 (m, 6H, $OCH_2CH_2CH_2NH$), 3.45–3.52 (m, 6H, $OCH_2CH_2CH_2NH$), 3.57–3.80 (m, 16H, H-2, H-3, H-4, H-5, H-6a, H-6b, OCH_2CH_2O, OCH_2CH_2NH), 3.86 (s, 3H, $COCH_3$), 3.98 4.04 (2 × s, 8H, $COCH_2OCH_2CO$), 4.14–4.18 (t, 2H, OCH_2-CH_2NH), 4.63 (d, 1H, H-1), 7.14–7.18 (m, 1H, CH_{arom}), 7.37 (t, 1H, CH_{arom}), 7.45 (s, 1H, CH_{arom}), 7.53–7.57 (m, 1H CH_{arom}). MS for $C_{37}H_{60}N_4O_{18}$ (M, 848.390), [M+H^+] calcd 849.398, found: 849.375.

4.8.6. Divalent mannose-long spacer, compound 24. Compound **24** was prepared according to general procedure 4.8 from **23**. 32 mg, 71% yield. ^{1}H NMR (300 MHz, D_2O): δ 1.67–1.78 (m, 12H, OCH_2CH_2-CH_2NH), 3.19–3.25 (m, 12H, $OCH_2CH_2CH_2NH$), 3.37–3.81 (m, 44H, $OCH_2CH_2CH_2NH$, H-2, H-3, H-4, H-5, H-6a, H-6b, OCH_2CH_2O, OCH_2CH_2NH), 3.81 (s, 3H, $COCH_3$), 3.98 4.04 (2 × s, 16H, $COCH_2OCH_2$-CO), 4.14–4.18 (t, 4H, OCH_2CH_2NH), 4.69 (d, 2H, H-1), 6.68 (s, 1H, CH_{arom}), 7.05 (s, 3H, CH_{arom}). HR-MS for $C_{66}H_{112}N_8O_{34}$ (M, 1560.728), calcd [M+H^+] 1561.736, found: 1561.831, [M+Na^+] calcd 1583.718, found 1583.551.

4.8.7. Tetravalent mannose-short spacer, compound 27. Compound **27** was prepared according to general procedure 4.8 from **26**. 37.8 mg, 98% yield. ^{1}H NMR

(300 MHz, D_2O): δ 1.53–1.63 (m, 8H, OCH_2CH_2-CH_2NH), 3.79–4.11 (m, 31H, $COCH_2OCH_2CO$, OCH_2CH_2NH, $COCH_3$), 4.58 (4H, H-1), 6.59 (s, 3H, CH_{arom}), 7.31 (s, 6H, CH_{arom}). HR-MS for $C_{86}H_{130}N_{10}O_{46}$ (M, 2038.8), [M+Na^+] average 2063.0, calcd found: 2063.0.

4.8.8. Octavalent mannose-short spacer, compound 30. Compound **30** was prepared according to general procedure 4.8 from **29**. 19.3 mg, 87% yield. ^{1}H NMR (300 MHz, D_2O): δ 1.51–1.71 (m, 16H, OCH_2CH_2-CH_2NH), 3.79–4.11 (m, 31H, $COCH_2OCH_2CO$, OCH_2CH_2NH, $COCH_3$), 6.31 (s, 7H, CH_{arom}), 6.60 (s, 14H, CH_{arom}). HR-MS for $C_{184}H_{272}N_{22}O_{95}$ (M, 4309.7), $[M+H]^{2+}$ average calcd 2157.7, found 2158.3.

4.8.9. Octavalent PAMAM based mannose-short spacer, compound 33. Compound **33** was prepared according to general procedure 4.8 from **32**. 11.7 mg, 51% yield. ^{1}H NMR (300 MHz, MeOD): δ 1.52–1.67 (m, 16H, $OCH_2CH_2CH_2NH$), 2.10–2.21 (m, 24H, NCH_2CH_2-$NHCO$), 2.34–2.44 (m, 12H, NCH_2CH_2N, $CONHCH_2CH_2N$), 2.52–2.63 (m, 24H, NCH_2CH_2NHCO), 3.02–3.22 (m, 64H, $OCHHCH_2CH_2NH$, $CONHCH_2CH_2N$, $NHCH_2CH_2NH$), 3.19–3.67 (m, 56H, $OCHHCH_2CH_2NH$, H-2, H-3, H-4, H-5, H-6a, H-6b), 3.85 (s, 32H, $COCH_2OCH_2CO$). MALDI-MS for $C_{166}H_{296}N_{34}O_{84}$ (M, 4110.0), found: 4114.0.

4.8.10. Hexadecavalent PAMAM based mannose-short spacer, compound 36. Compound **36** was prepared according to general procedure 4.8 from **35**. 18.3 mg, 55% yield. ^{1}H NMR (300 MHz, MeOD): δ 1.51–1.65 (m, 32H, $OCH_2CH_2CH_2NH$), 2.08–2.19 (m, 56H, NCH_2CH_2NHCO), 2.32–2.41 (m, 28H, NCH_2CH_2N, $CONHCH_2CH_2N$), 2.50–2.61 (m, 56H, NCH_2CH_2-$NHCO$), 2.97–3.17 (m, 156H, $OCHHCH_2CH_2NH$, $CONHCH_2CH_2N$, $NHCH_2CH_2NH$), 3.20–3.63 (m, 112H, $OCHHCH_2CH_2NH$, H-2, H-3, H-4, H-5, H-6a, H-6b), 3.84 (s, 64H, $COCH_2OCH_2CO$).

4.9. Synthesis of mannose monomer 38

To amine **5** (4.90 g, 12.1 mmol) in dioxane (120 mL) was added $NaHCO_3$ (1.32 g, 15.7 mmol) in water (120 mL). This mixture was cooled to 0 °C and acryloyl chloride (1.48 mL, 18.2 mmol) was added slowly, after which the mixture was allowed to warm up. After 2 h, the mixture was diluted with EtOAc and the organic layer washed with water, dried and concentrated. Compound **37** was purified by column chromatography (0–5% MeOH–DCM). 422 mg, 76% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.85–1.98 (m, 2H, OCH_2CH_2-CH_2NH), 2.01, 2.06, 2.11, 2.17 (4 × s, 12H, $COCH_3$), 3.43–3.58 (m, 3H, $OCHHCH_2CH_2NH$), 3.78–3.85 (m, 1H, $OCHHCH_2CH_2NH$), 3.97–4.04 (m, 1H-, H-5), 4.11 (dd, 1H, J 2.5, 12.1 Hz, H-6b), 4.29 (dd, 1H, J 5.2, 12.1 Hz, H-6a), 4.82 (d, 1H, $J < 1$ Hz, H-1), 5.25–5.30 (m, 3H, H-2, H-3, H-4), 5.66 (dd, 1H, J 1.5, 9.9 Hz, $CH=CHH$), 6.13–6.33 (m, 2H, $CH=CHH$), 6.66 (br s, 1H, NH). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.6 ($COCH_3$), 28.8 ($OCH_2CH_2CH_2NH$), 37.1 ($OCH_2CH_2CH_2NH$), 62.5, 66.5 ($OCH_2CH_2CH_2NH$, C-6), 65.7, 65.9, 68.9, 69.2 (C-2, C-3, C-4, C-5), 97.5 (C-1), 126.5 ($C=CH_2$), 130.6 ($C=CH$), 165.7, 169.6, 170.0 (CO, $COCH3$). MS for $C_{20}H_{29}NO_{11}$ (M, 459.17), [M+Na^+] calcd 482.43, found 482.35.

Compound **37** was deacetylated as according to general procedure 4.8, yielding monomer **38** in quantitative yield. ^{1}H NMR (300 MHz, D_2O): δ 1.61–1.71 (m, 2H, $OCH_2CH_2CH_2NH$), 3.29 (m, 2H, OCH_2-CH_2CH_2NH), 3.52–3.92 (m, 8H), 4.79 (d, 1H, H-1), 5.73 (m, 1H, $=CH_2$), 6.18 (m, 2H, $HC=CH_2$). ^{13}C NMR (75.5 MHz, D_2O/MeOD): δ 28.1 (OCH_2CH_2), 36.6 (CH_2NH), 61.0, 65.2 (OCH_2, C-6), 66.8, 70.1, 70.6, 72.8 (C-2, C-3, C-4, C-5), 99.9 (C-1), 127.2 ($CH=CH_2$), 130.0 ($CH=CH_2$), 168.6 (C=O). MS for $C_{12}H_{21}NO_7$ (M, 291.13), [M+Na^+] calcd 314.12, found 314.25.

4.10. General procedure for preparation of glycopolymers

Monomer **38** and *N*-(2-hydroxypropyl) methacrylamide monomer (HPMA) were dissolved in water in various ratios. After degassing of the solution by addition of N_2 for 30 min *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA, 0.3 equiv) and ammonium persulfate (APS, 0.05 equiv) were added after which N_2 was bubbled through the solution for another 10 min. After stirring overnight at rt, the solution was dialyzed (MWCO 3500) against water for 2 days and lyophilized to afford white fluffy powder in 40–65% yield. The co-monomer ratio in the copolymer was determined from the ratio of the integral from the CH_3CH (δ = 1.00) from HPMA to the integral from the H-1 of the mannose monomer (δ = 4.88), being consistent with the input. GPC was used to determine the molecular weight ($6760 < M_w < 11{,}160$) and polydispersity ($1.3 < M_w/M_n < 1.8$). For calibration of the column polystyrene standards of known molecular weights and narrow molecular weight distribution were used.

4.11. Bacterial strains, growth conditions and plasmids

The *E. coli* K-12 strain HB 101 used in this study was transformed with the plasmid pPKL4 (pBR322 cloning vector) carrying the complete *fim* gene cluster cloned from the uropathogenic *E. coli* strain.[38] Recombinant bacteria expressing type 1 fimbriae were cultivated with shaking at 37 °C overnight in Luria–Bertani (LB) broth supplemented with the appropriate antibiotic (i.e., 100 μg of ampicillin per mL). The antibiotics were purchased from Sigma (Deisenhofen, Germany).

4.12. Cell lines, media and culture conditions

The cell line (T24) derived from human bladder was purchased from the American Type Culture Collection (Manassas, Va.). Cell line was grown in medium (McCoy's 5A medium supplemented with 2 mM glutamine, nonessential amino acids, and 10% fetal calf serum) without antibiotic at 37 °C in a 5% CO_2–95% air atmosphere with 90% humidity and were split twice a week at a ratio of 1:5. Cell culture media was purchased

from C.C. Pro (Neustadt, Germany) and supplements were from Gibco (Gaithersburg, Md.).

4.13. Adherence assay

To quantify the bacterial adhesion a cell line (T24) of epithelial-like morphology and of human origin was used as a model to eukaryotic cells. After harvesting cells from confluent monolayers, epithelial cells were seeded in 96-well flat-bottom cell culture plates (Greiner) in 200 μL aliquots for 24–30 h. The culture medium was removed and after washing monolayers once with sterile phosphate-buffered saline (PBS) they were fixed by addition of 100 μL of glutaraldehyde (1.25% in PBS) at room temperature for 30 min. After washing three times with PBS, monolayers were blocked with 3% BSA–PBS (pH 7.4) for 2 h at 37 °C. A serial dilution of type 1 fimbriated recombinant *E. coli* HB101(pPKL4), nonfimbriated control strain HB101(pBR322) was first tested to determine the numbers of bacteria required to obtain 50% binding to this cell line. The number of fimbriated and nonfimbriated control strains was determined by measuring absorbance at a wavelength of 550 nm, with a standardized chart correlating absorbance with viable counts.[39] After removal of blocking solution and washing, 100 μL of serially diluted bacterial suspensions (4×10^8 bacteria in the first well) in PBS with 1% BSA was added to each well, and plates were incubated for 2 h at 37 °C. To determine the binding of bacteria to the monolayers, wells were washed as mentioned above and incubated with rabbit anti-*E. coli* polyclonal antibody (Biodesign International, Kennebunk, Maine) in PBS–1% BSA (1:1700) for 1.5 h at 37 °C (100 μL/well). Following another washing step, peroxidase-conjugated (1:1700) goat anti-rabbit immunoglobulin G (Dako, Hamburg, Germany) was added and incubated for 1 h at 37 °C (100 μL/well). Finally, the wells were washed as above and the bound enzyme was detected by the addition of 100 μL of substrate (Pierce ImmunoPure TMB substrate Kit) in each well for 5–30 min. To stop the reaction 100 μL of 2 M H_2SO_4 was added to each well and the absorbance was measured at a wavelength of 450 nm with an enzyme-linked immunosorbant assay (ELISA) reader. The control wells were treated in the same manner except that blank control wells have no bacteria.

4.14. Binding inhibition assay

The binding inhibition assay was performed essentially as described by Khan et al.[39] Briefly, the type 1 fimbriated and nonfimbriated bacteria at the number (13×10^7/well), which gave 50% binding were preincubated with twofold serially diluted relevant dendrimers for 15 min at room temperature. After preincubation the whole mixture was transformed to the cell culture plates carrying fixed monolayers of T24 cell line. To determine the bacterial binding the above-described method was used. Percentage of inhibition was calculated as described by Khan et al.[40] The bacterial strain was tested in three independent experiments, and in each experiment, 30 determinations of bacterial adherence was performed in parallel.

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