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Diazo Transfer and Click Chemistry in the Solid Phase Syntheses of Lysine-Based Glycodendrimers as Antagonists against *Escherichia coli* FimH

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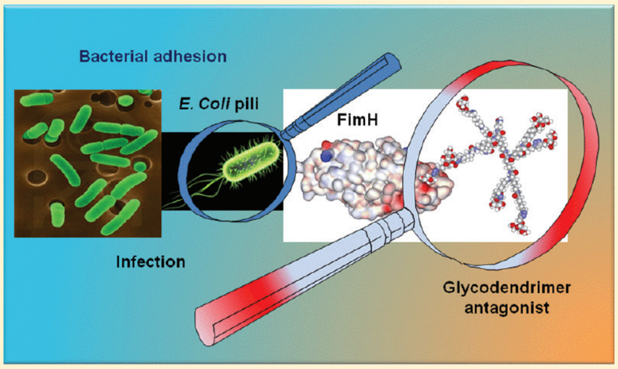

ABSTRACT: Uropathogenic *Escherichia coli* infections, ultimately leading to cystitis and pyelonephritis, are initially mediated by the adhesion of the bacterial FimH to the transmembrane glycoprotein uroplakin-1a present at the surface of urothelial cells. The adhesion is based on the recognition and high avidity binding between the high-mannose glycans of the uroplakin and the FimH, a mannose-specific lectin located at the tip of type 1 fimbriae. We found that synthetic multiantennary mannopyranosides glycodendrons, harboring triazole functionality at the anomeric position, were potent hemagglutination inhibitors of guinea pig erythrocytes and *E. coli*. A mannosylated dendrimer exposing up to sixteen sugar residues showed an HAI titer of 1 μM and was thus 500-fold more potent than the corresponding monovalent methyl α-D-mannopyranoside. The synthesis of the glycodendrons involved highly efficient solid-phase synthesis of branched L-lysine scaffolds, diazo transfer reaction on the terminal amine residues, and 1,3-dipolar copper-catalyzed azide−alkyne cycloaddition using propargyl α-D-mannopyranoside.

KEYWORDS: *E. coli, antiadhesin, mannopyranoside, glycodendrimer, triazole, lysine*

■ INTRODUCTION

Escherichia coli are familiar residents of the digestive tracts of humans and animals wherein several types or strains live innocuously. Paradoxically, some strains are responsible for serious nosocomial infections as they are the most widespread cause of Gram-negative cystitis and pyelonephritis. It is the major causative agent (60%) of urinary tract infections (UTIs).[1] Bacterial infections are becoming more problematic worldwide due to increased resistance to antibiotics. The occurrence of *E. coli* resistant strains is steadily increasing, and traditional first-line antimicrobial agents are essentially obsolete.[2,3]

Hence, there is a pressing need for new antibacterial strategies, among which preventive vaccination has been successfully added to the growing list.[4,5] It is also becoming widely accepted that early inhibition of bacterial adhesion might also constitute promising alternatives.[6,7] Urothelial cells expose a wide array of complex glycoconjugates. These carbohydrate structures play essential roles in several key cellular events.[8] Thus, glycoproteins exposing multiantennary glycans constitute privileged lines of contact against pathogens bearing the corresponding carbohydrate recognition domains (CRD).[9] In spite of these ubiquitous phenomena, the basic carbohydrate structures recognized by carbohydrate-binding proteins are surprisingly simple, with at best tri- or tetrasaccharide moieties being involved in the protein's active sites.[9] Several such proteins represent families that exploit closely related carbohydrate ligands, thus further raising the issues of selectivity.[10,11]

Fimbriated *E. coli* possess proteins at the tip of their pili (FimH) that recognize and strongly bind to the high-mannose glycans of uroplakin-1a, a transmembrane glycoprotein present at the surface of urothelial cells of host human tissues as the premise for bacterial infections.[12] Surprisingly however, classical mannoside analoguess bind to FimH with low affinity, thus representing mediocre drug candidates. The crystal structures of the uropathogenic *E. coli* FimH bound to methyl α-D-mannopyranoside (MeαMan)[13] as well as with its butylated mannoside[14] have been solved and confirmed prior investigations[15] pointing at the improved binding affinities of hydrophobic derivatives. Recent studies are similarly pointing toward mimetics possessing aromatic aglycons.[16−19]

In addition, multivalent glycoarchitectures (glycodendrimers),[20] particularly those harboring mannopyranoside residues, are also emerging as potent ligands against FimH.[10,11,21] Glycodendrimers can be assembled in a wide variety of chemical architectures and number of surface carbohydrate residues.[22−24] They may thus constitute an arsenal of novel principles for the treatment of infectious agents by the inhibition of attachment

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of the infecting pathogens to the host's cell surfaces.[12] Since the chemical structure of adhesion inhibitors must be similar to the natural ligands used by the pathogenic agents, it is unlikely that resistance (mutations) would give them the capability to overcome the inhibitory effect of the antiadhesive carbohydrate drugs without impairing their own ability to adhere to host cell glycans. Glycodendrimers can therefore be suitably designed for inhibition of bacterial adhesions to host tissues.

In this study, we present an improved family of mannosylated glycodendrimers based on successful premises combining both the multivalent principles achieved through glycocluster design together with monosaccharide improvement following QSARs.[21] The synthesis of the glycodendrons described herein involved highly efficient solid-phase synthesis of branched L-lysine scaffolds, diazo transfer reaction on the terminal amine residues, and 1,3-dipolar-copper catalyzed azide−alkyne cycloaddition using propargyl α-D-mannopyranoside. Notably, the transformations were successfully achieved directly on the solid phase using Rink amide resin.

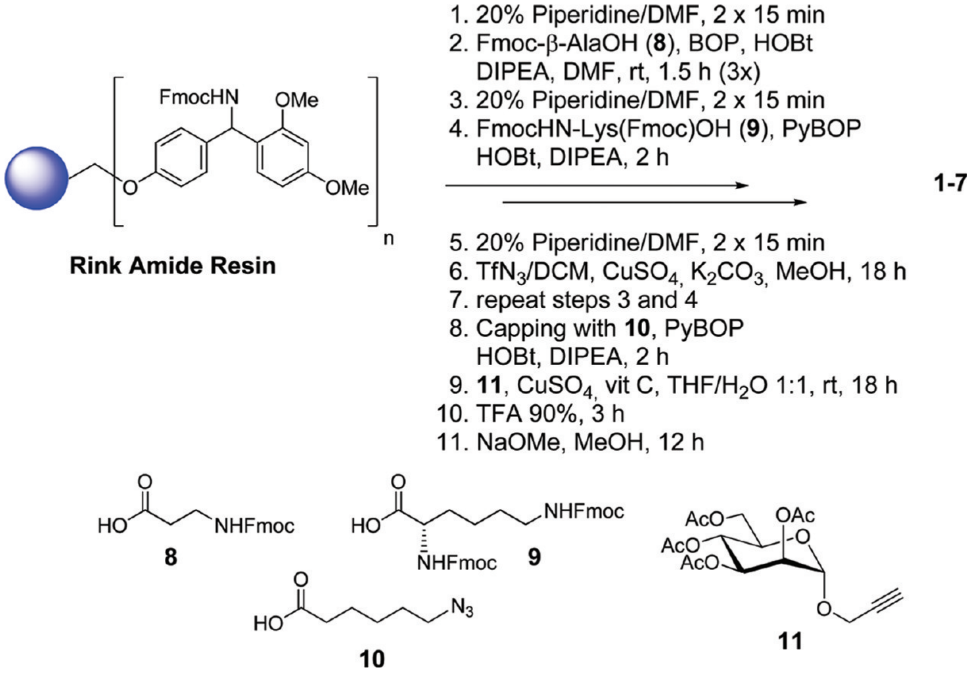
■ MATERIALS AND METHODS

Synthetic Procedures. *General Methods.* The reactions were carried out in organic media under nitrogen atmosphere using freshly distilled solvents (dichloromethane was freshly distilled over P_2O_5; DMF was distilled on ninhydrin and then stored over 4 Å molecular sieves). All reagents were used as supplied without prior purification unless otherwise stated and obtained from Acros Organics Ltd. and Sigma-Aldrich Chemical Co. Ltd. Protected amino acids and resin were obtained from NovaBiochem. After workup, the organic layers were dried over anhydrous Na_2SO_4. Evolution of reactions was monitored by analytical thin-layer chromatography using silica gel 60 F_{254} precoated plates (E. Merck), and compounds were visualized with a solution of $(NH_4)_6Mo_7O_{24}H_2O$ (10 g) and $Ce(SO_4)_2)$ (25 g) (100 mL of H_2SO_4, 900 mL of H_2O) and subsequent development by gentle warming with a heat gun. Purification by flash column chromatography was performed using silica gel from Silicycle (60 Å, 40−63 μm) with the indicated eluent. The solvents employed for the chromatography were of ACS or HPLC quality and were not distilled before use. The solvents were evaporated under reduced pressure (rotary evaporator under vacuum generated by a system of glass filter pump). Water was nanopure grade, purified through Barnstead NANOPure II Filter with Barnstead MegOhm-CM Sybron meter. Lyophilization was performed with a Freeze Mobile 24 (Virtis). Dialysis was performed with benzoylated (Avg. Flat width 9 mm, cut off ≤1200 g/mol, Sigma Aldrich). IR spectra was performed on a Bomem Hartmann&Braun MB-series FTIR and analyzed with a Grams/AI program. Compounds were analyzed as thin films on a NaCl disk or as KBr pellets. The absorptions are given in wavenumbers (cm^{-1}). Optical rotations were measured with a JASCO P-1010 polarimeter. Melting points were measured on a Fisher Jones apparatus and are uncorrected. NMR spectra were recorded on Varian Gemini-2000 (300, 75 MHz) or Varian Inova AS600 (600, 150 MHz) spectrometers. Proton and carbon chemical shifts (δ) are reported in ppm downfield from internal reference of residual solvents. Coupling constants (J) are reported in hertz (Hz), and the following abbreviations are used: singlet (s), doublet (d), doublet of doublets (dd), triplet (t), multiplet (m), broad singlet (bs). Analysis and assignments were made using COSY and HETCOR experiments. Accurate mass measurements (HRMS) were carried out on a HPLC Agilent Technologies 1200 series + Agilent Technologies 6210 MS TOF in positive electrospray mode. Low-resolution mass spectra were performed on the same apparatus or on a LCQ Advantage ion trap instrument from Thermo Fisher Scientific in positive electrospray mode (Mass Spectrometry Laboratory, University of Montreal, QC, Canada), or at Université du Québec à Montréal, Québec, Canada. Either protonated molecular ions $[M + nH]^{n+}$ or sodium adducts $[M + Na]^+$ were used for empirical formula confirmation.

Solid-Phase Synthesis of Poly-L-lysine-glycodendrons. *Protocol A.* The solid-phase mannosylated poly-L-lysine dendron syntheses were carried out by standard Fmoc SPPS chemistry using Rink Amide resin (100−200 mesh, loading = 0.54 mmol/g). The reactions were conducted by rotating agitation in Econo-Pac disposable columns 1.5 × 14 cm (20 mL) (Bio-Rad Laboratories, ON, Canada). The resin was swollen in CH_2Cl_2 during 1 h, then filtered and reconditioned in DMF during 1 h. The Fmoc- protecting groups of the commercial resin or of amino acids were removed with a solution of 20% piperidine in DMF (5 mL, 2 × 5 min then 1 × 10 min). The solvents and reagents were removed by filtration, and the resin was washed with DMF, CH_2Cl_2 and MeOH (3× with each solvent). The presence of free amino groups was verified by a Kaiser test (protocol D) or TNBS (2,4,6-trinitrobenzenesulfonic acid) test (protocol E). The free amines on the resin were then treated with a solution of preactivated Fmoc- (**9**) or azido- (**10**)[25] ending amino acids: 3 equiv of amino acids, 3 equiv of BOP (benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate) and a catalytic amount of HOBt (1-hydroxybenzotriazole) in DMF at 4 °C (10 min). DIPEA (diisopropylamine, 9 equiv) was then added into the mixture and stirred at room temperature for 1 h 30 min. Completion of the coupling was determined using a Kaiser or a TNBS colorimetric test. After filtration, the resin was washed and the Fmoc removal procedure was again repeated. The diazo transfer (protocol B) and the 1,3-dipolar cycloaddition ("click reactions") (protocol C) were carried out *in situ* on the SPPS or the sequence amidation-diazo transfer-click reactions were carried on for the next generations. At the end of the synthetic sequences, the solutions were drained off, the resin was dried under vacuum and the cleavage was carried out using trifluoroacetic acid/water (9/1) for 3 h. The resulting glycodendrons were precipitated with methyl *tert*-butyl ether and isolated from the resin beads by centrifugation (20 min, 2000 rpm, 3×). The precipitates were dried carefully with a stream of air jet. The per-*O*-acetylated glycodendrons were extracted with dichloromethane and washed twice with a saturated solution of NH_4Cl to remove the last copper traces. After trans-esterification under Zemplén conditions (protocol F), the final mannosylated L-lysine-glycodendrons were exhaustively dialyzed in nanopure water for 12 h and freeze-dried without any further purification. The purity of the final mannodendrimers **1**−**7** was evaluated by HPLC on Varian equipment (ProStar Model 3.10) at 1.3 mL/min (Varian Polaris 5 C18-A, 150 × 4.6 mm) with monitoring at 214 nm using a linear A−B gradient (solvent A, water containing 0.09% TFA; solvent B, acetonitrile containing 0.09% TFA and 9.91% H_2O). The purity of all compounds was >95%, and they were used as such in the characterization and in the biological assays.

Protocol B. Diazo Transfer on SPPS. $CuSO_4$ (0.01 equiv per amine) in H_2O, K_2CO_3 (3.0 equiv per amine) and a solution of triflic azide (TfN_3)[26] in dichloromethane (0.37 M) (1.5 equiv per amino group) were added into the SPPS reactor filled

Scheme 1. Solid-Phase Peptide Syntheses (SPPS) of Mannosylated Dendrons 1−7 and Precursors Using Diazo Transfer and "Click Chemistry" Directly onto the Solid Support



with resin containing free amino L-lysine groups. MeOH was then added into the suspension, and the mixture was stirred at room temperature for 18 h. The solvents were removed by filtration, and the resin was extensively washed with a mixture of 5% EDTA, water, dichloromethane, MeOH, and DMF.

Protocol C. "Click Chemistry" onto SPPS. To the resin having polyazido dendrons (1.0 equiv) suspended into a mixture of H_2O/THF (1:1) were added propargyl α-D-mannopyranoside **11**[16,27] (1.25 equiv per azide function), $CuSO_4 \cdot 5H_2O$ (0.3 equiv per azide function) and sodium ascorbate (0.3 equiv per azide function). The suspension was vigorously stirred at room temperature for 18 h. The resin was then washed successively with 5% aqueous solutions of EDTA, MeOH, dichloromethane, and DMF (several times).

Protocol D. Kaiser Test. Preparation of the solutions: (1) 5 g of ninhydrin in 100 mL of ethanol; (2) 80 g of liquified phenol in 20 mL of ethanol; (3) add 2 mL of a 0.001 M aqueous solution of potassium cyanide to 98 mL of pyridine. A few resin beads were washed several times with ethanol, and 2 drops of each solution were added above. The mixture was heated to 120 °C for 4−6 min. The blue resin beads provided indication of positive free amino groups. This operation was particularly useful to check complete amide coupling or diazo transfer.

Protocol E. TNBS Test. Preparation of the solutions: (1) 10% of DIPEA in DMF; (2) 1% of 2,4,6-trinitrobenzenesulfonic acid (TNBS) in DMF. A few resin beads were washed several times with DMF. One drop of each solution was added to a suspension of the resin beads in DMF and left for 5 min. The resin beads were washed with DMF to remove the red solution. The presence of red resin beads gave the indication of free amino groups, otherwise the beads remained transparent.

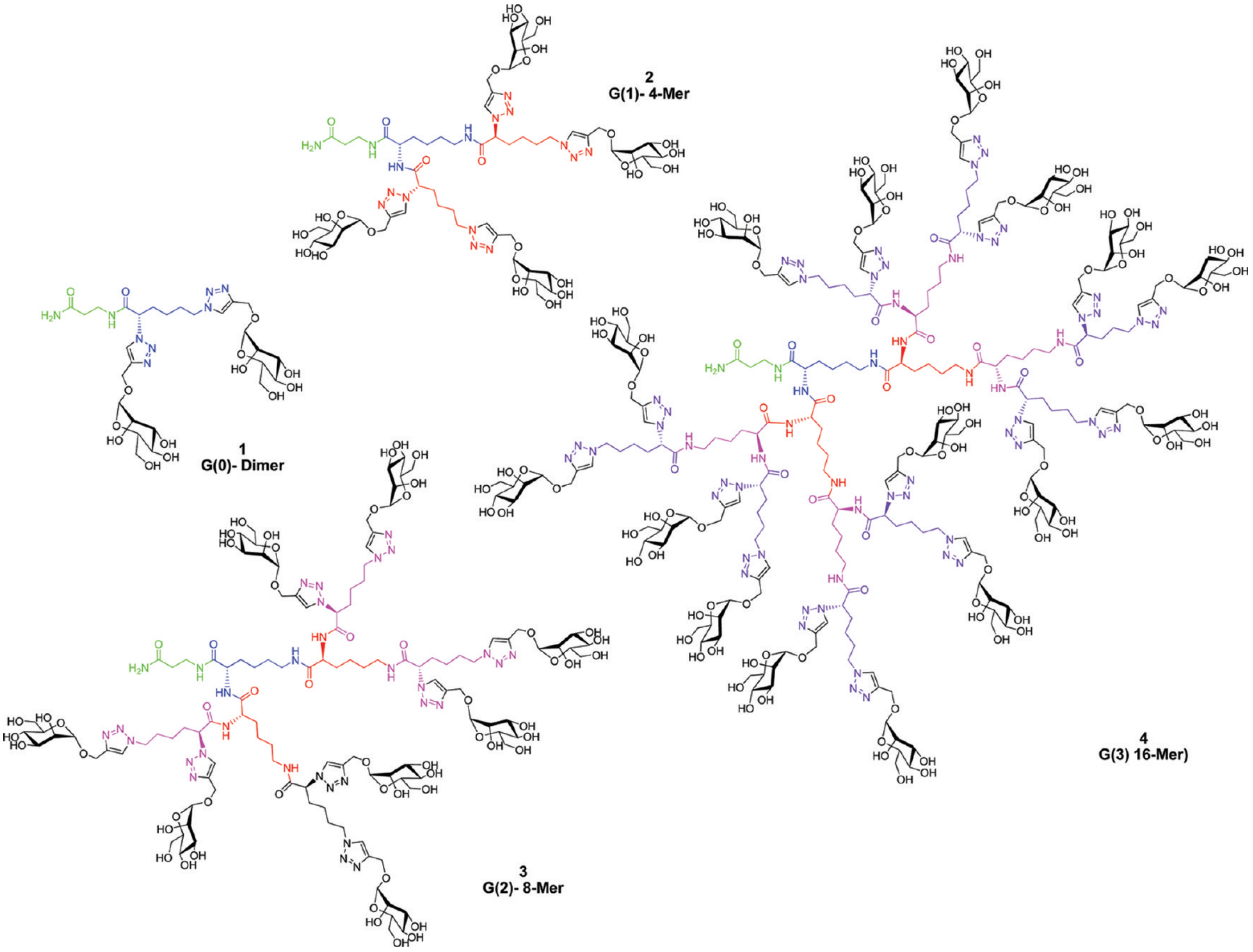
Protocol F. Zemplén Reaction (Sugar De-O-acetylation). A solution of acetylated mannodendrons in 1 M sodium methoxide in methanol at pH 9 was stirred at room temperature until consumption of starting material. The solution was then neutralized by addition of ion-exchange resin (Amberlite IR 120, H^+), filtered, and washed with MeOH, and the solvent was removed under pressure.

General Protocol for the Bacterial Culture. The two strains of nonpathogenic mutants *E. coli* K-12 (BW25113; $FimH^+$ and $FimH^-$)[28] bacterium were obtained from INRS-Institute Armand-Frappier (Dr. Charle M. Dozois). The two stocks of bacteria were inoculated on agar Petri dishes (Oxoid LB Agar; tryptone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L, agar 15 g/L) and incubated at 37 °C during 24 h, starting from stocks preserved in glycerol at −80 °C. Only one colony was taken in 5 mL of LB Miller broth (tryptone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L) that was incubated at 37 °C during 24 h with agitation. From this stock, 500 μL was transferred into an Erlenmeyer flask containing 50 mL of LB Miller broth, and the bacteria were incubated at 37 °C during 48 h without agitation to allow the expression of the fimbriae. Another 48 h static incubation was carried out three times to allow for the maximum expression of the fimbriae. The bacterial density was determined by a standard curve of growth established beforehand on a Genesis 20 Thermospectronic spectrophotometer with an optical density of 600 nm. The established bacterial concentration was of 2×10^8 units forming colony (UFC) per mL for a DO_{600nm} of 0.6. For the test, the bacteria were diluted in PBS for a concentration of 1×10^7 UFC in 40 μL for each well.

Guinea Pig Erythrocytes. The blood from guinea pigs was obtained from QueBact Laboratories (Montreal, QC, Canada) and was preserved in an Alsever solution (20.5 g/L of glucose, 8.0 g/L of trisodium citrate, 4.2 g/L NaCl and 0.55 g/L citric acid in distilled water). A suspension of 3% erythrocytes in PBS (v/v) was prepared from the above Alsever mixture (3 mL), obtained by centrifugation at 4 °C (900 rpm, 20 min, 3 times) and suspended in 50 mL of PBS.

Inhibition of Hemagglutination. The inhibition of of hemagglutination was carried out in 96 U-shaped well plates (BD Falcon). Mannosylated glycodendrimers **1−7** and the

Scheme 2. Structures of Mannosylated L-Lysine Dendrons 1−4 Having "Standard" α-Amino-L-lysine Scaffolds Showing the Sterically Hindered Mannopyranoside Residues in the α-Branches



monosaccharide standard (MeαMan) tested were weighed and diluted in PBS to provide stocks of 10 mM solutions. In the first well, 40 μL of inhibitors was deposited with 40 μL of blood suspensions and 40 μL of bacterium for a total of 120 μL. The following wells contained 20 μL of PBS, 20 μL of blood suspension and 20 μL of bacteria to keep the concentration of erythrocytes and bacteria constant. A quantity of 60 μL was transferred from the first wells toward the second and so on to make 2-fold dilutions in series and to keep the same volume. After one hour at room temperature, the plates were examined with the naked eye to observe the inhibition of hemagglutination. The test for each compound was carried out in triplicate to provide the HIA titers.

Trifluomethasulfonyl Azide (TfN_3). The reagent was prepared as previously described.[26] Trifluoromethanesulfonic anhydride (3.33 g, 11.79 mmol, 1.0 equiv) was added dropwise into a solution of sodium azide (10.80 g, 166.12 mmol, 14.0 equiv) in DCM at 4 °C with vigorous stirring. The suspension was stirred at room temperature for 2 h. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 × 22.5 mL). The combined organic layers were washed with a saturated solution of Na_2CO_3. The stock solution of TfN_3 was kept in dichloromethane (0.37 M). IR (NaCl, ν cm^{-1}): 2108 (N_3), 1327, 1198, 1069, 1033, 814, 642.

Table 1. Relative Potencies of the Two Series of Mannosylated Glycodendrimers Measured by the Inhibition of Hemagglutination Titer (HAI) against Guinea Pig Erythrocytes and Fimbriated *E. coli* BW25113[28]

compd	no. of steps (% yield)	no. (valency)	HAI titer (μM)	rel potency	rel potency/ mannoside
MeαMan		1	500	1	1
1 G(0)	9 (63)	2	78.1	6	3
2 G(1)	11 (45)	4	62.5	8	2
3 G(2)	13 (16)	8	15.6	32	4
4 G(3)	15 (15)	16	2.0	256	16
5 G(1)	11 (51)	4	62.5	8	2
6 G(2)	13 (24)	8	7.8	64	8
7 G(3)	15 (17)	16	1.0	512	32

*G(0) Dimer (**1**).* Starting with Rink Amide resin (400 mg, 0.216 mmol, 0.54 mmol/g), the compound **1** was isolated according to protocol A as a white powder (96 mg, 0.136 mmol, 63% yield over 9 steps): R_f = 0.16, CH_3CN/H_2O 7:3. ^1H NMR (300 MHz, D_2O, 24 °C): δ = 8.15 (s, 1H, C=C*H*(α)), 8.01 (s, 1H, C=C*H*(ε)), 5.29 (t, 1H, *J* = 7.5 Hz, *H*-α), 4.95 (s, 2H, *H*-1), 4.87−4.66 (m, 4H, OC*H*$_2$C=CH), 4.40 (t, 2H, *J* = 6.1 Hz, *H*-ε), 3.93 (s, 2H, *H*-2) 3.86−3.63 (m, 10H, *H*-3, *H*-4, *H*-5 and *H*-6), 3.46 (m, 2H, COCH$_2$C*H*$_2$NH), 2.45 (t, 2H, *J* = 6.2 Hz,

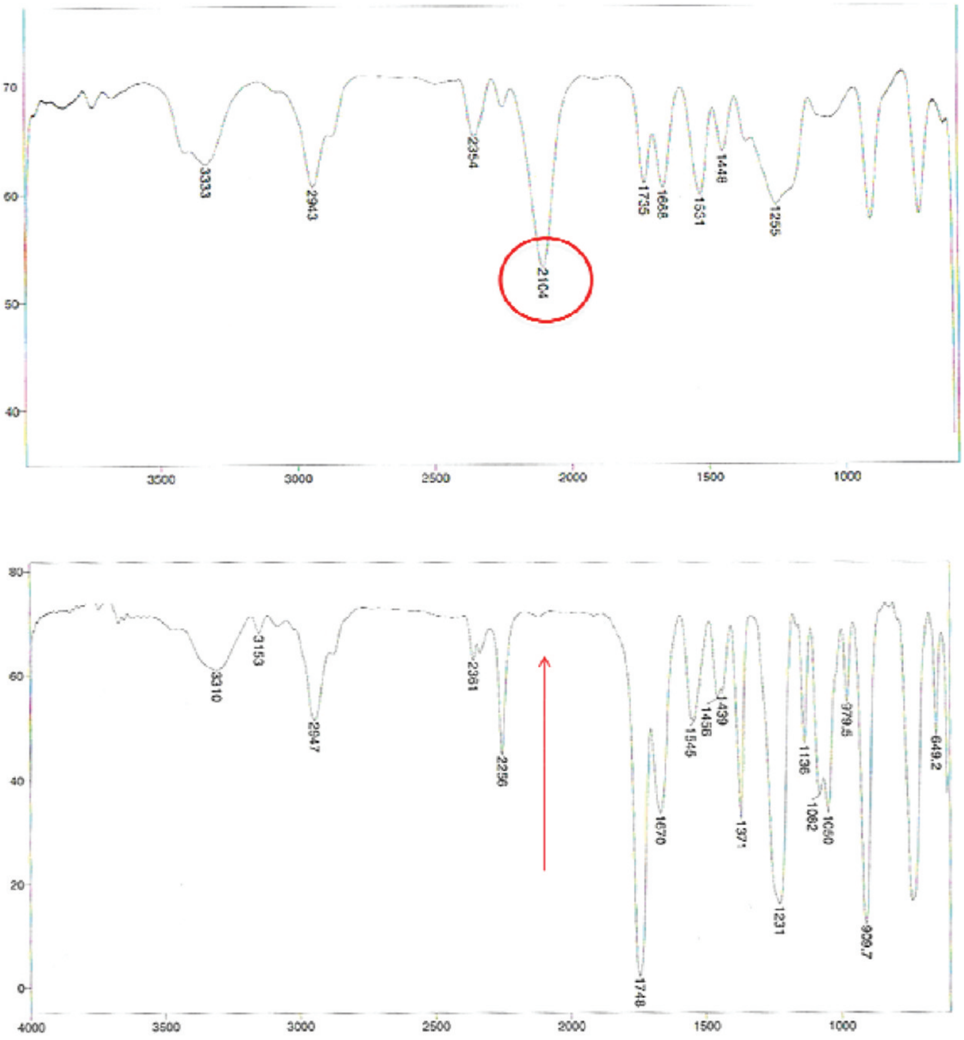


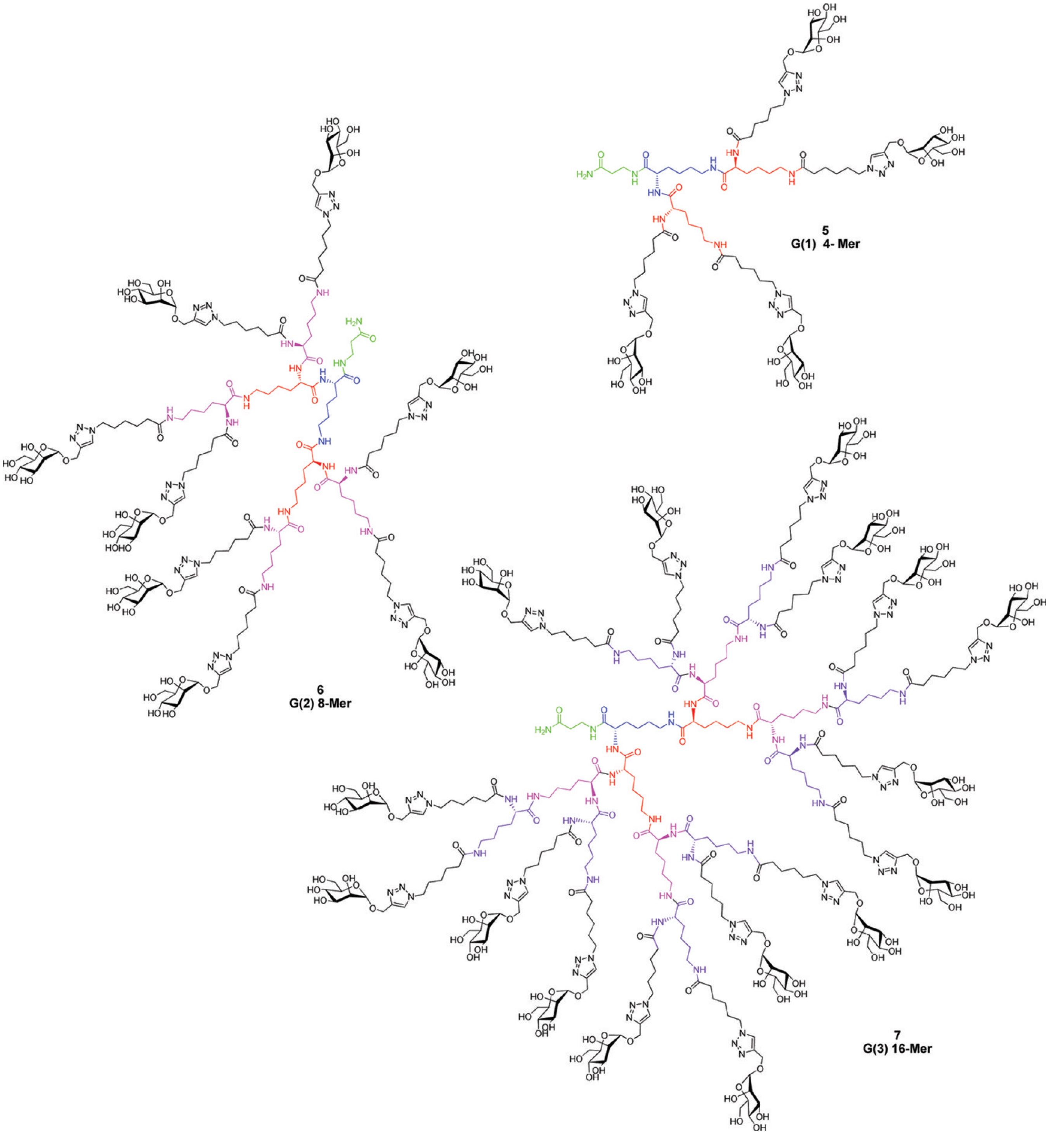
Figure 1. IR spectra of the Rink amide resin after diazo transfer (top) and alkyne−azide cycloaddition with sugar **11** (bottom).

COC*H*$_2$CH$_2$NH), 2.20 (dd, 2H, $J_{\beta-\alpha}$ = 7.5 Hz, $J_{\beta-\gamma}$ = 15.1 Hz, *H*-β), 1.92 (m, 2H, *H*-δ), 1.16 (m, 2H, *H*-γ). ^{13}C NMR (75 MHz, D$_2$O): δ = 176.0, 169.2 (CO), 143.2, 143.0 (*C*=CH (α and ε)), 124.6, 124.0 (C=*C*H (α and ε)), 99.0, 98.9 (*C*-1 and C1′), 72.5 (*C*-5), 70.0, (*C*-3), 69.5 (*C*-2), 66.2 (*C*-4), 63.0 (*C*- α), 60.4 (*C*-6), 59.2 (OCH$_2$), 49.4 (*C*-ε), 35.5 (COCH$_2$CH$_2$NH), 33.8 (COCH$_2$CH$_2$NH), 30.1 (*C*-δ), 28.0 (*C*-β), 21.2 (*C*-γ). ESI$^+$-HRMS: *m*/*z* calcd for $C_{27}H_{44}N_8O_{14}$ [M + H]$^+$, 704.2977; found, 704.2993.

*G(1) Tetramer (**2**).* Starting with Rink Amide resin (200 mg, 0.108 mmol), the glycodendron **2** was isolated according to protocol A as a white powder (70 mg, 0.049 mmol, 45% yield over 11 steps): R_f = 0.14, CH$_3$CN/H$_2$O 7:3. ^{1}H NMR (300 MHz, D$_2$O, 24 °C): δ = 8.16 (bs, 2H, C=C*H*(α)), 8.00 (bs, 2H, C=C*H*(ε)), 5.38 (t, 1H, *J* = 7.5 Hz, *H*-α_α-G1), 5.28 (1H, m, *H*-α_ε-G1), 4.94 (s, 4H, *H*-1), 4.81−4.69 (m, 8H, OC*H*$_2$C=CH), 4.40 (bs, 8H, *H*-ε-G1), 4.16 (t, 1H, *H*-α-G0), 3.91−3.63 (m, 24H, *H*-2, *H*-3, *H*-4, *H*-5 and *H*-6), 3.41 (m, 2H, COCH$_2$C*H*$_2$NH), 3.19 (m, 2H, *H*-ε-G0), 2.46 (t, 1H, COC*H*$_2$CH$_2$NH), 2.39 (t, 1H, COC*H*$_2$CH$_2$NH), 2.19 (bs, 4H, *H*-β-G1), 1.90 (bs, 4H, *H*-δ-G1), 1.69 (m, 2H, *H*-β-G0), 1.48−1.16 (m, 8H, *H*-δ-G0, *H*-γ-G0 and G1). ^{13}C NMR (75 MHz, D$_2$O): δ = 176.1, 172.9, 169.2, 169.0 (CO), 143.2, 143.0 (*C*=CH (α and ε)), 124.6, 123.9 (C=*C*H (α and ε)), 99.1, 99.0, 98.9 (*C*-1), 72.5 (*C*-5), 70.0 (*C*-3), 69.5 (*C*-2), 66.2 (*C*-4), 63.1 (*C*-α-G1), 62.7 (*C*-α-G1), 60.4 (*C*-6), 59.2 (OCH$_2$), 53.8 53.7 (*C*-α-G0), 49.4 (*C*-ε-G1), 38.7(*C*-ε-G0), 35.2 (COCH$_2$CH$_2$NH), 34.0 (COCH$_2$CH$_2$NH), 30.0, 29.5, 29.2, 28.0, 27.2, 27.1, 21.9, 21.7, 21.3 (*C*-β, *C*-δ, *C*-γ of G0 and G1). ESI$^+$-HRMS: *m*/*z* calcd for the doubly protonated ion of $C_{57}H_{92}N_{16}O_{28}$ [M + 2H]$^{2+}$, 725.3206; found, 725.3178.

*G(2) Octamer (**3**).* From Rink Amide resin (200 mg, 0.108 mmol), according to protocol A, G(2) octamer **3** was isolated as a white powder (50 mg, 0.017 mmol, 16% yield over 13 steps): R_f = 0.11, CH$_3$CN/H$_2$O 7:3. ^{1}H NMR (300 MHz, D$_2$O, 23 °C): δ = 8.16 (bs, 4H, C=C*H*(α)), 7.98 (bs, 4H, C=C*H*(ε)), 5.37 (t, 2H, *H*-α_α-G2), 5.27 (bs, 2H, *H*-α_ε-G2), 4.93 (s, 8H, *H*-1), 4.83−4.64 (m, 16H, OC*H*$_2$C=CH)), 4.37 (bs, 8H, *H*-ε-G2), 4.13 (m, 3H, *H*-α -G1 and G0), 3.90−3.63 (m, 48H, *H*-2, *H*-3, *H*-4, *H*-5 and *H*-6), 3.47−3.35 (m, 2H, COCH$_2$C*H*$_2$NH), 3.15 (m, 6H, *H*-ε-G0 and *H*-ε-G1), 2.45 (t, 1H, COC*H*$_2$CH$_2$NH), 2.39 (t, 1H, COC*H*$_2$CH$_2$NH), 2.21 (sl, 8H, *H*-β-G2), 1.90 (bs, 8H, *H*-δ-G2), 1.67 (bs, 6H, *H*-β-G0 and *H*-β-G1), 1.40−1.17 (m, 28H, *H*-δ-G0, G1 and G2, *H*-γ-G0, G1 and G2). ^{13}C NMR (75 MHz, D$_2$O): δ = 172.9, 172.6, 172.5, 168.8 (CO), 143.4, 143.1 (*C*=CH (α and ε)), 124.4, 123.8 (C=*C*H (α and ε)), 99.1, 99.0 (*C*-1), 72.5 (*C*-5), 70.1 (*C*-3), 69.6 (*C*-2), 66.3 (*C*-4), 63.2(*C*-α-G2), 62.8 (*C*-α-G2), 60.5 (*C*-6), 59.2 (OCH$_2$), 53.7 (*C*-α-G0), 53.3 (*C*-α-G1), 49.4 (*C*-ε-G2), 38.7(*C*-ε-G1), 38.2 (*C*-ε-G0), 35.2 (COCH$_2$

Scheme 3. Structures of Mannosylated L-Lysine Dendrons 5−7 (G(1)−G(3)) Having "Extended" α-Amino-L-lysine Scaffolds with 6-Aminocaproic Acid and Showing the Relaxed Accessibility of the Mannopyranoside Residues in the α-Branches



5
G(1) 4- Mer

6
G(2) 8-Mer

7
G(3) 16-Mer

CH_2NH), 34.0 ($COCH_2CH_2NH$), 30.2, 29.5, 29.2, 28.0, 27.2, 21.8, 21.7, 21.4 (*C-β*, *C-δ*, *C-γ* of G0, G1 and G2). ESI$^+$-HRMS: *m/z* calcd for the [M + 4H]$^{4+}$ ion of $C_{117}H_{188}N_{32}O_{56}$, 735.3284; found, 735.31218.

*G(3) 16-Mer (**4**).* Starting with Rink Amide resin (200 mg, 0.108 mmol), G(3) 16-Mer **4** was isolated according to protocol A as a white powder (95 mg, 0.016 mmol, 15% yield over 15 steps): R_f = 0.11, CH_3CN/H_2O 7:3. ^{1}H NMR (300 MHz, D_2O, 24 °C): δ = 8.16 (bs, 8H, C═C*H*(α)), 7.97(bs, 8H, C═C*H*(ε)), 5.39 (bs, 4H, *H*-α_α -G3), 5.28 (bs, 4H, *H*-α_ε- G3), 4.93 (s, 16H, *H*-1), 4.80−4.67 (m, 32H, OCH_2C═CH), 4.36 (bs, 16H, *H*-ε-G3), 4.15 (bs, 7H, *H*-α -G0, G1 and G2), 3.90−3.61 (m, 72H, *H*-2, *H*-3, *H*-4, *H*-5 and *H*-6), 3.45- 3.27 (m, 2H, $COCH_2CH_2NH$), 3.15, 2.90 (m, 14H, *H*-ε-G0, *H*-ε-G1, *H*-ε-G2), 2.44 (bs, 2H, $COCH_2CH_2NH$), 2.19 (bs, 16H, *H*-β-G3), 1.88−1.17 (m, 74H, *H*-β-G0, *H*-β-G1, *H*-β-G2, *H*-δ-G0 to G3, *H*-γ-G0 to G3). ^{13}C NMR (75 MHz, D_2O): δ = 173.1, 168.9 (CO), 143.2, 143.1 (*C*═CH (α and ε), 124.5, 123.0

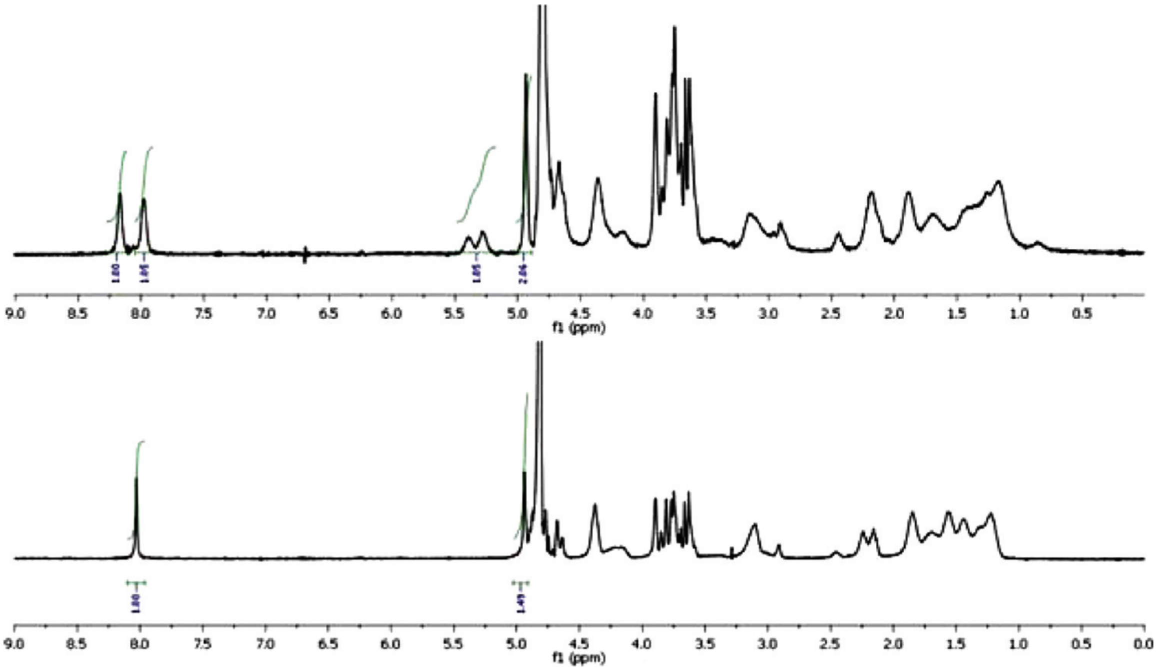


Figure 2. 300 MHz ^{1}H NMR (D_2O) of G(3) 16-mer mannosylated dendrons **4** (top) and **7** (bottom) showing the differences in chemical shifts of the triazole protons at δ 8.16, 7.97 and at δ 8.03 ppm, respectively.

(C═CH (α and ε)), 99.1, 98.9 (*C*-1), 72.5 (*C*-5), 70.1 (*C*-3), 69.6 (*C*-2), 66.3 (*C*-4), 63.2 (*C*-α-G3), 62.7 (*C*-α-G3), 60.4 (*C*-6), 59.2 (OCH_2), 53.4 (*C*-α-G2), 49.4 (*C*-ε-G3), 38.7(*C*-ε-G2), 35.2 ($COCH_2CH_2NH$), 34.0 ($COCH_2CH_2NH$), 30.2, 29.5, 29.2, 28.0, 27.2, 21.8, 21.7, 21.4 (*C*-β, *C*-δ, *C*-γ of G0, G1, G2 and G3). ESI$^+$-HRMS: *m*/*z* calcd for $[M + 4H]^{4+}$ ion of $C_{236}H_{378}N_{64}O_{112}$, 1479.14630; found, 1479.65508.

G(1) Tetramer (**5**). Starting with Rink Amide resin (200 mg, 0.108 mmol), G(1) tetramer **5** was isolated as a white powder (105 mg, 0.055 mmol, 51% yield over 11 steps): R_f = 0.15, CH_3CN/H_2O 7:3. ^{1}H NMR (300 MHz, D_2O, 24 °C): δ = 8.04 (s, 4H, C═C*H*), 4.95 (s, 4H, *H*-1), 4.83–4.66 (m, 8H, OCH_2C═CH), 4.40 (bs, 8H, CH_2CH_2N–N), 4.24–4.14 (m, 3H, *H*-α-G0 and *H*-α-G1), 3.91–3.63 (m, 24H, *H*-2, *H*-3, *H*-4, *H*-5 and *H*-6), 3.51–3.37 (m, 2H, $COCH_2CH_2NH$), 3.13 (bs, 6H, *H*-ε-G0 and H-ε-G1), 2.46 (t, 2H, $COCH_2CH_2NH$), 2.25, 2.17 (2*t*, 8H, $COCH_2CH_2CH_2$), 1.88–1.22 (m, 42H, *H*-β, *H*-γ, *H*-δ of G0 and G1, $COCH_2(CH_2)_3$). ^{13}C NMR (75 MHz, D_2O): δ = 177.4, 173.3, 177.1, 174.9, 174.8, 174.5 (CO), 144.2(C═CH), 125.8 (C═*C*H), 100.2 (*C*-1), 73.4 (*C*-5), 71.2 (*C*-3), 70.7 (*C*-2), 67.4 (*C*-4), 61.6 (*C*-6), 60.5 (OCH_2), 54.8, 54.5, 54.4 (*C*-α-G0 and *C*-α-G1), 50.9 (CH_2CH_2N–N), 39.6 (*C*-ε-G0 and G1), 36.5 ($COCH_2CH_2NH$), 36.3 ($COCH_2CH_2NH$), 35.9, 35.3, 31.3, 29.8, 28.6, 25.9, 25.7, 25.5, 25.4, 23.3, 23.0 (*C*-β, *C*- γ, *C*-δ of G0 and G1, $CO(CH_2)_4CH_2N$–N). ESI$^+$-HRMS: *m*/*z* calcd for doubly protonated ion of $C_{81}H_{136}N_{20}O_{32}$ $[M + 2H]^{2+}$, 951.4887; found, 951.4892.

G(2) Octamer (**6**). Starting from Rink Amide resin (200 mg, 0.108 mmol), according to protocol A, G(2) octamer **6** was isolated as a white powder (99 mg, 0.026 mmol, 24% yield over 13 steps): R_f = 0.15, CH_3CN/H_2O 7:3. ^{1}H NMR (300 MHz, D_2O, 23 °C): δ = 8.05 (s, 8H, C═C*H*), 4.96 (s, 8H, *H*-1), 4.86–4.67 (m, 16H, OCH_2C═CH), 4.41 (bs, 16H, CH_2CH_2N–N), 4.23–4.12 (m, 7H, *H*-α-G0 to G2), 3.91–3.63 (m, 48H, *H*-2, *H*-3, *H*-4, *H*-5, *H*-6), 3.51–3.37 (m, 2H, $COCH_2CH_2NH$), 3.13 (bs, 14H, *H*-ε- G0 to G2), 2.47 (t, 2H, $COCH_2CH_2NH$), 2.26, 2.19 (2*t*, 16H, $COCH_2CH_2CH_2$), 1.88–1.24 (m, 90H, *H*-β, *H*-γ, *H*-δ of G0 to G2, $COCH_2(CH_2)_3$); ^{13}C NMR (75 MHz, D_2O): δ = 176.1, 175.7, 173.5, 173.1, 172.9, (CO), 143.3 (C═CH), 124.6 (C═*C*H), 99.0 (*C*-1), 72.5 (*C*-5), 70.0 (*C*-3), 69.5 (*C*-2), 66.2 (*C*-4), 60.4 (*C*-6), 59.3 (OCH_2), 53.5, 53.2 (*C*-α-G0 to G2), 49.7 (CH_2CH_2N–N), 38.4 (*C*-ε-G0 to G2), 35.2 ($COCH_2CH_2NH$), 35.0 ($COCH_2CH_2NH$), 34.6, 34.0, 30.2, 28.6, 27.5, 24.7, 24.6, 24.3, 24.2, 22.1 (*C*-β, *C*-γ, *C*-δ of G0 to G1, $CO(CH_2)_4CH_2N$–N). ESI$^+$-HRMS: *m*/*z* calcd for the triple protonated ion of $C_{165}H_{276}N_{40}O_{64}$ $[M + 3H]^{3+}$, 1281.65968; found, 1281.65826.

G(3) 16-Mer (**7**). Starting with Rink Amide resin (200 mg, 0.108 mmol), G(3) 16-Mer **7** was isolated as a white powder (139 mg, 0.018 mmol, 17% yield over 17 steps): R_f = 0.15, CH_3CN/H_2O 7:3. ^{1}H NMR (300 MHz, D_2O, 23 °C): δ = 8.03 (s, 16H, C═C*H*), 4.94 (s, 16H, *H*-1), 4.87–4.64 (m, 32H, OCH_2C═CH), 4.37 (bs, 32H, CH_2CH_2N–N), 4.24–4.12 (m, 15H, *H*-α-G0 to G3), 3.89–3.63 (m, 96H, *H*-2, *H*-3, *H*-4, *H*-5, *H*-6), 3.49–3.36 (m, 2H, $COCH_2CH_2NH$), 3.09 (bs, 28H, *H*-ε-G0 to G3), 2.45 (bs, 2H, $COCH_2CH_2NH$), 2.15, 2.23 (2 × bs, 32H, $COCH_2CH_2CH_2$), 1.84–1.22 (m, 186H, *H*-β, *H*-γ and *H*-δ of G0 to G3, $COCH_2(CH_2)_3$). ^{13}C NMR (75 MHz, D_2O): δ = 176.0, 175.9, 175.7, 173.6, 173.4, (CO), 143.0 (C═CH), 124.5 (C═*C*H), 99.0 (*C*-1), 72.5 (*C*-5), 70.0 (*C*-3), 69.5 (*C*-2), 66.2 (*C*-4), 60.4 (*C*-6), 59.3 (OCH_2), 53.5, 53.2 (*C*-α-G0 to G2), 49.7 (CH_2CH_2N–N), 38.4 (*C*-ε-G0 to G2), 35.0 ($COCH_2CH_2NH$), 34.9 ($COCH_2CH_2NH$), 34.6, 34.0, 30.2, 28.6, 27.5, 24.7, 24.6, 24.3, 24.2, 22.1 (*C*-β, *C*-γ and *C*-δ of G0 to G1, $CO(CH_2)_4CH_2N$–N). ESI$^+$-HRMS: *m*/*z* calcd for $C_{333}H_{556}N_{80}O_{128}$ $[M + H]^+$, 7728.4369; found, 7728.51289.

■ RESULTS AND DISCUSSION

Synthesis of Mannosylated Glycodendrons. We[16] and others[17−19] have shown that glycodendrimers possess improved binding abilities against fimbriated *E. coli*. In particular, hyperbranched-L-lysine mannodendrimers having *p*-isothiocyanatophenyl linkers were shown to strongly inhibit the binding of suspension of ^{125}I-labeled, highly mannosylated neoglycoprotein to a type 1 fimbriated *E. coli* (K12) strain at subnanomolar concentrations.[29] In line with these first observations together with the improved binding capacities of 4-(mannopyranosyloxymethyl)-1*H*-1,2,3-triazole[16] analogues, we targeted the syntheses of two families of mannodendrons based on L-lysine scaffolds (Scheme 1). The difference between the two families was based on our intention to provide the second "extended" family with easier binding accessibilities of the mannosides at the α-branch of the L-lysine residues compared to that of the first "short" family. Using Rink amide resin and Fmoc chemistry, an initial Fmoc-β-alanine linker (**8**) was first introduced. Standard piperidine deprotection and ε-FmocHN-Lys(α-Fmoc)OH (**9**) coupling cycles with PyBOP-HOBt provided di- (G0) to hexadeca- (G3) valent amino-ending poly-L-lysine precursors. Diazo transfer reaction with triflyl azide (TfN$_3$, DCM, CuSO$_4$, K$_2$CO$_3$, MeOH, 18 h) directly on the solid phase afforded the required azido-ending dendrons.

For the synthesis of the first family of nonextended α-branches (Scheme 2), the resulting azidodendrons were mannosylated with prop-2-ynyl mannopyranoside **11**[16,27] under "click chemistry" (CuSO$_4$, vitamine C) to afford mannosylated dendrons (G(0)−G(3)). Trifluoroacetolysis (90% TFA, DCM) from the resin followed by standard Zemplén transesterification of the *O*-acetate protecting groups (NaOMe, MeOH, rt, 12 h) afforded the first series of mannodendrons **1**−**4** in 15−63% overall yields and in 9−15 consecutive steps (Scheme 2, Table 1). The diazo transfer reaction on the resin was followed by IR spectroscopy, which clearly showed the appearance of the typical azide stretching vibration at 2104 cm^{-1} (Figure 1) together with a negative Kaiser test (ninhydrin, disappearance of amines). After the copper-catalyzed 1,3-cycloaddition with sugar **11**, the complete disappearance of the azide stretching band in the IR spectra was observed (Figure 1) that was accompanied by the presence of a strong carbonyl ester vibration at 1748 cm^{-1}.

For the second "extended" family of mannosylated dendrons in which it was decided to incorporate an additional linker between the sugar and the α- and ε-amino (azido) functionalities of the L-lysine scaffolds, a 6-azidohexanoic acid derivative (**10**)[25] (Scheme 1) was used immediately (ByBOP, HOBt) after the Fmoc deprotection. Incorporation of the azide groups was again confirmed by IR spectroscopy (not shown) and a negative Kaiser test. The 1,3-cycloaddition with sugar **11** and deprotection as above afforded the fully mannosylated G(1) to G(3) mannosylated glycodendrons **5**−**7** (Scheme 3) in 17−51% overall yields and in 11−15 steps. The structural integrity of the final compounds was fully confirmed by NMR spectroscopy and ESI-high resolution mass spectrometry.

Interestingly and promisingly, the heterocyclic triazole protons between the two series of the "short" and "extended" mannosylated dendrons **1**−**4** and **5**−**7** appeared as two distinct signals at δ 8.16 and 7.97 and as a single signal at δ 8.03 ppm, respectively, in the ^{1}H NMR spectra (Figure 2). This initial observation clearly indicated that the chemical environments of the sugar moieties in the second extended series **5**−**7** were more closely related and similar in comparison to the "short" one in which the triazole moieties were nearest to the chiral α-amino lysine groups. Additionally, the protons of the α-amino groups of the shorter dendrons **1**−**4** appeared as distinct sets of signals at δ 5.28 and 5.39 ppm (δ 5.25 ppm for the dimer **1**), next to a triazole function, while they shifted upfield in the case of the extended series **5**−**7**.

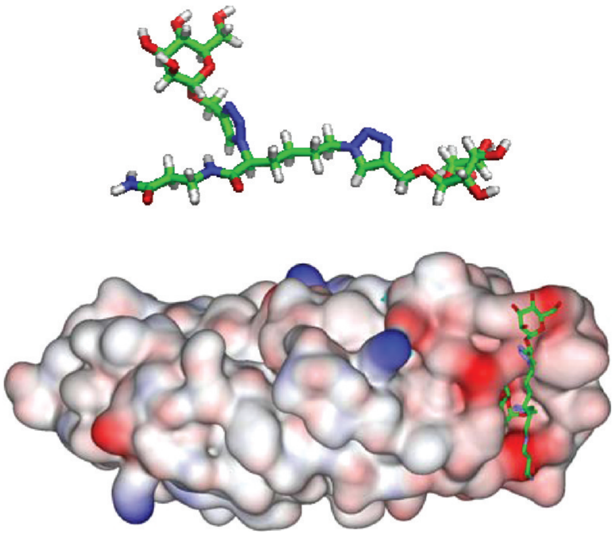


Figure 3. Computerized model of G(0) dimer **1** alone (top view) (extended conformation) and docked (bottom) into the X-ray crystal structure of *E. coli* FimH (pdb 1UWF)[14] into which butyl α-D-mannopyranoside was replaced by the α-branched mannoside residue of **1**.

Inhibition of Hemagglutination. To evaluate the relative binding potencies of our two families of short (**1**−**4**) and extended (**5**−**7**) mannosylated dendrimers bearing between two and 16 mannopyranoside residues, an inhibition of hemagglutination assay was used. Nonpathogenic but FimH-fimbriated *E. coli* K-12 strain BW25113 (FimH$^+$)[28] was treated with guinea pig erythrocytes, and the HAI titers were monitored in triplicate. The results are presented in Table 1, and the FimH$^-$ strain was used as negative control. The G(3)-16-mer (**4**) had the lowest titer with 2 μM and represented a 256-fold improvement when compared to the MeαMan monosaccharide (500 μM). When corrected for the saccharide valency, this result still indicated a 16-fold improvement. This result is, however, in slight contrast with the first three members of the family, wherein the HAI titers of G(0)−G(2) (**1**−**3**) were rather similar on a per saccharide-corrected basis, ranging from 2- to 4-fold better than the standard MeαMan monosaccharide. This observation is also in line with our previous data.[29]

In contrast, with the second extended series G(1)−G(3) (**5**−**7**), we observed a steady increase in potencies. The best candidate was also the G(3)-16-mer (**7**) which had an HAI titer of 1 μM, thus representing a 500-fold improvement over the monomer. Even when expressed on a per saccharide basis, 16-mer **7** was still 32-fold better than MeαMan. Somewhat surprisingly, both G(1) tetramers **2** and **5** had equipotent inhibitory potencies (62.5 μM; 2-fold better per saccharide). This indicated that the extended arm of the second series (**5**−**7**) experienced a positive effect at the G(2) generation only and that the effect was exacerbated at the G(3) level. Moreover, it also points to the fact that the improved potency could be strictly attributed to a multivalent or dendritic effect rather than on the use of a hydrophobic linker, at least at the higher generation. Given the appearance of single peaks in the HPLC profiles, it also agreed with the absence of self-aggregated species which could have been responsible for the improved binding.

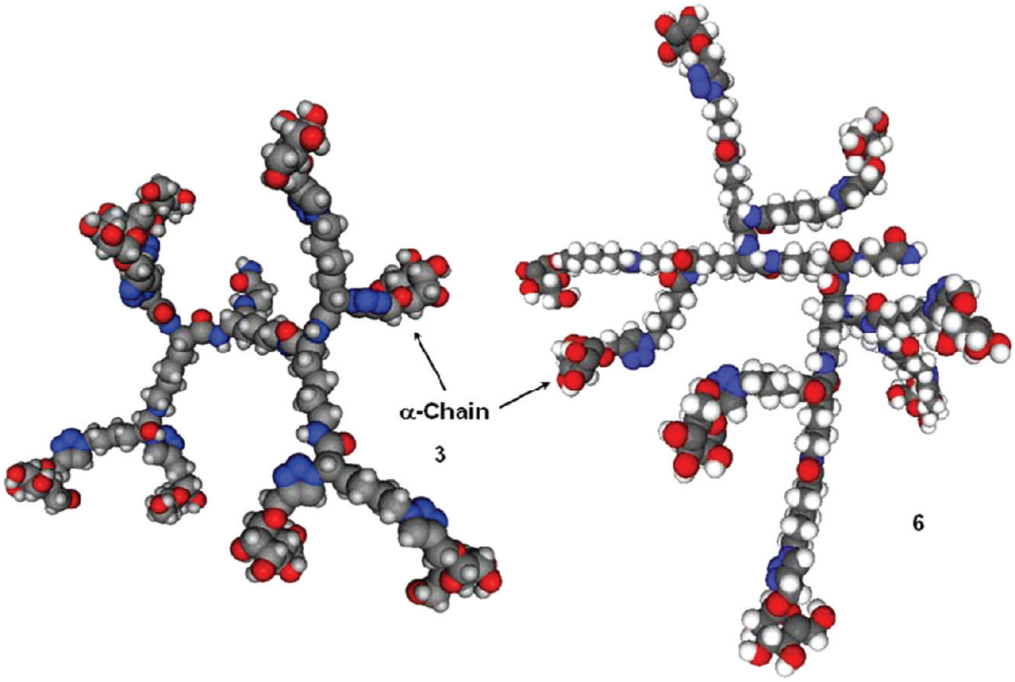


Figure 4. Extended conformations of G(2) mannodendrimers (8-mer) **3** (left) and **6** (right) clearly illustrating the improved accessibility of the mannopyranoside residues in the "extended" dendron family.

Although the lack of improved binding upon linker extension starts to be observable only at higher generations, it is important to realize that the entire piliated bacterium was being used in this assay by opposition to testing the single FimH protein which is much easier to inhibit given its monovalency. Figure 3 and Figure 4 also clearly illustrate the effect of lacking an extended linker (6-aminohexanoic acid) into the α- and ε-amino groups of the L-lysine moiety wherein the reduced sugar accessibility of the α-position is particularly evident. The better HAI titers obtained for the second "extended" mannodendrimer family may thus be easily explained on the basis of relative sugar accessibility.

■ CONCLUSIONS

The syntheses of two different families of mannosylated dendrons were efficiently accomplished using solid phase chemistry. Of particular interest was the observation that both the diazo transfer reaction and the 1,3-dipolar copper-catalyzed cycloaddition could be accomplished during the solid-phase steps. The 15 step syntheses of the G(3) dendrons were accomplished in an average of 16% overall yield. The second and extended family of mannodendrons (**5**−**7**), bearing an extra 6-aminohexanoic acid linker, showed better inhibitory potencies in inhibition of hemagglutination assays of guinea pig erythrocytes and fimbriated *E. coli*. The best candidate, **7**, with an HAI titer of 1 μM, showed a 32-fold better binding affinity when corrected on a per mannoside residue. This value compares favorably well with the ones recently published using fullerene[30] or pentaerythritol[31] scaffolds. However, a linear heptameric mannocluster built on sugar scaffolds and having a more hydrophobic heptyl aglycon showed better HAI titers than the one reported herein.[32]

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■ REFERENCES

(1) Gales, A. C.; Jones, R. N.; Gordon, K. A.; Sader, H. S.; Wilke, W. W.; Beach, M. L.; Pfaller, M. A.; Doern, G. V. Activity and spectrum of 22 antimicrobial agents tested against urinary tract infection pathogens in hospitalized patients in Latin America: report from the second year of the SENTRY antimicrobial surveillance program (1998). *J. Antimicrob. Chemother.* **2000**, *45*, 295−303.

(2) Jones, M. E.; Karlowsky, J. A.; Draghi, D. C.; Thornsberry, C.; Sahm, D. F.; Bradley, J. S. Rates of antimicrobial resistance among common bacterial pathogens causing respiratory, blood, urine, and skin and soft tissue infections in pediatric patients. *Eur. J. Clin. Microbiol. Infect. Dis.* **2004**, *23*, 445−455.

(3) von Baum, H.; Marre, R. Antimicrobial resistance of Escherichia coli and therapeutic implications. *Int. J. Med. Microbiol.* **2005**, *295*, 503−511.

(4) Roy, R. New trends in carbohydrate-based vaccines. *Drug Discovery Today: Technol.* **2004**, *1*, 327−336.

(5) Roy, R.; Shiao, T. C. Organic chemistry and immunochemical strategies in the design of potent carbohydrate-based vaccines. *Chimia* **2011**, *65*, 24−29.

(6) Sharon, N.; Ofek, I. Safe as mother's milk: Carbohydrates as future anti-adhesion drugs for bacterial diseases. *Glycoconjugate J.* **2000**, *17*, 659−664.

(7) Sharon, N. Carbohydrates as future anti-adhesion drugs for infectious diseases. *Biochim. Biophys. Acta* **2006**, *1760*, 527−537.

(8) Varki, A. Biological roles of oligosaccharides: all of the theories are correct. *Glycobiology* **1993**, *3*, 97−130.

(9) Lis, H.; Sharon, N. Lectins: Carbohydrate-specific proteins that mediate cellular recognition. *Chem. Rev.* **1998**, *98*, 637−674.

(10) Touaibia, M.; Roy, R. Glycodendrimers as anti-adhesion drugs against type 1 fimbriated E. coli uropathogenic infections. *Mini-Rev. Med. Chem.* **2007**, *7*, 1270−1283.

(11) Roy, R.; Touaibia, M. Application of multivalent mannosylated dendrimers in glycobiology. In *Comprehensive Glycoscience*; Kamerling, J. P., Ed.; Elsevier: Oxford, 2007; Vol. *3*, Chapter 3.36; pp 821−870.

(12) Knight, S. D.; Bouckaert, J. Structure, function, and assembly of type 1 fimbriae. *Top. Curr. Chem.* **2009**, *288*, 67−107.

(13) Hung, C.-S.; Bouckaert, J.; Hung, D.; Pinkner, J.; Widberg, C.; DeFusco, A.; Auguste, C. G.; Strouse, R.; Langermann, S.; Waksman, G.; Hultgren, S. J. Structure basis of tropism of Escherichia coli to the bladder during urinary tract infection. *Mol. Microbiol.* **2002**, *44*, 903−915.

(14) Bouckaert, J.; Berglund, J.; Schembri, M.; De Genst, E.; Cools, L.; Wuhrer, M.; Hung, C.-S.; Pinkner, J.; Slättegård, R.; Zavialov, A.; Choudhury, D.; Langermann, S.; Hultgren, S. J.; Wyns, L.; Klemm, P.; Oscarson, S.; Knight, S. D.; De Greve, H. Receptor binding studies disclose a novel class of high-affinity inhibitors of the Escherichia coli FimH adhesion. *Mol. Microbiol.* **2005**, *55*, 441−455.

(15) Firon, N.; Ashkenazi, S.; Mirelman, D.; Ofek, I.; Sharon, N. Aromatic alpha-glycosides of mannose are powerful inhibitors of the adherence of type 1 fimbriated Escherichia coli to yeast and intestinal epithelial cells. *Infect. Immun.* **1987**, *55*, 472−476.

(16) Touaibia, M.; Wellens, A.; Shiao, T. C.; Wang, Q.; Sirois, S.; Bouckaert, J.; Roy, R. Mannosylated G(0) dendrimers with nanomolar affinities to Escherichia coli FimH. *ChemMedChem* **2007**, *2*, 1190−1201.

(17) For a recent review see: Hartmann, M.; Lindhorst, T. K. The Bacterial Lectin FimH, a target for Drug discovery − carbohydrate inhibitors of type 1 fimbriae-mediated bacterial adhesion. *Eur. J. Org. Chem.* **2011**, 3583−3609.

(18) Han, Z.; Pinkner, J. S.; Ford, B.; Obermann, R.; Nolan, W.; Wildman, S. A.; Hobbs, D.; Ellenberger, T.; Cusumano, C. K.; Hultgren, S. J.; Janetka, J. W. Structure-based drug design and optimization of mannoside bacterial FimH antagonists. *J. Med. Chem.* **2010**, *53*, 4779−4792.

(19) Klein, T.; Abgottspon, D.; Wittwer, M.; Rabbani, S.; Herold, J.; Jiang, X.; Kleeb, S.; Lüthi, C.; Scharenberg, M.; Bezencon, J.; Gubler, E.; Pang, L.; Smiesko, M.; Cutting, B.; Schwardt, O.; Ernst, B. FimH antagonist for the oral treatment of urinary tract infections: from design and synthesis to in vitro and in vivo evaluation. *J. Med. Chem.* **2010**, *53*, 8627−8641.

(20) Chabre, Y. M.; Roy, R. Design and creativity in synthesis of multivalent neoglycoconjugates. *Adv. Carbohydr. Chem. Biochem.* **2010**, *63*, 165−393.

(21) Imberty, A.; Chabre, Y. M.; Roy, R. Glycomimetics and glycodendrimers as high affinity microbial anti-adhesins. *Chem.—Eur. J.* **2008**, *14*, 7490−7499.

(22) Roy, R. A decade of glycodendrimer chemistry. *Trends Glycosci. Glycotechnol.* **2003**, *15*, 291−310.

(23) Pieters, R. J. Interference with lectin binding and bacterial adhesion by multivalent carbohydrates and peptidic carbohydrate mimics. *Trends Glycosci. Glycotechnol.* **2004**, *16*, 243−254. Pieters, R. J. Intervention with bacterial adhesion by multivalent carbohydrates. *Med. Res. Rev.* **2007**, *27*, 796−816.

(24) Röckendorf, N.; Lindhorst, T. K. Glycodendrimers. *Top. Curr. Chem.* **2001**, *217*, 201−238.

(25) Kuil, J.; Branderhorst, H. M.; Pieters, R. J.; de Mol, N. J.; Liskamp, R. M. J. ITAM-derived phosphopeptide-containing as multivalent ligands for Syk tandem SH2 domain. *Org. Biomol. Chem.* **2009**, *7*, 4088−4094.

(26) Ruff, J. K. Sulfur oxyfluoride derivatives. II. *Inorg. Chem.* **1965**, *4*, 567−570. Note: Imadozyl azide gave similar results: Goddard-Borger, E. D.; Stick, R. V. An efficient, inexpensive, and shelf-stable diazotransfer reagent: imidazole-1-sulfonyl azide hydrochloride. *Org. Lett.* **2007**, *9*, 3797−3800.

(27) Chabre, Y. M.; Shiao, T. C.; Vidal, S.; Roy, R. Synthesis of prop-2-ynyl 2,3,4,6-tetra-*O*-acetyl-*α*-D-mannopyranoside. *Carbohydrate Chemistry: Proven Synthetic Methods*; Kovac, P., Ed.; Taylor & Francis: 2011; Vol. *1*, Chapter 31, pp 275−278.

(28) Baba, T.; Ara, T.; Hasegawa, M.; Takai, Y.; Okumura, Y.; Baba, M.; Datsenko, K. A.; Tomita, M.; Wanner, B. L.; Mori, H. Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the Keio collection. *Mol. Syst. Biol.* **2006**, *2*, 1−11.

(29) Nagahori, N.; Lee, R. T.; Nishimura, S.-I.; Pagé, D.; Roy, R.; Lee, Y. C. Inhibition of adhesion of type 1 fimbriated Escherichia coli to highly mannosylated ligands. *ChemBioChem* **2002**, *3*, 836−844.

(30) Durka, M.; Buffet, K.; Iehl, J.; Holler, M.; Nierengarten, J.-F.; Taganna, J.; Bouckaert, J.; Vincent, S. P. The functional valency of dodecamannosylated fullerenes with Escherichia coli FimH − towards novel bacterial antiadhesives. *Chem. Commun.* **2011**, *47*, 1321−1323.

(31) Gouin, S. G.; Wellens, A.; Bouckaert, J.; Kovensky, J. Synthetic multimeric heptyl mannosides as potent antiadhesives of uropathogenic Escherichia coli. *ChemMedChem* **2009**, *4*, 749−755.

(32) Almant, M.; Moreau, V.; Kovensky, J.; Bouckaert, J.; Gouin, S. G. Clustering of Escherichia coli type-1 fimbrial adhesions by using multimeric heptyl *α*-D-mannoside probes with a carbohydrate core. *Chem.—Eur. J.* **2011**, *17*, 10029−10038.