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Synthesis of a series of multivalent homo-, and heteroglycosides and their anti-adhesion activities

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ABSTRACT

Adhesion of leukocytes to endothelium plays an important role in inflammatory diseases. We previously found that the tetravalent lactoside **Gu-4** was able to inhibit leukocyte-endothelial cell adhesion significantly and that CD11b was the target of **Gu-4** on the surface of leukocytes. In this report, we aimed to explore the relationship between structural characteristics of glycoclusters and anti-adhesion activity. Using selective glycosylation method and convergent strategy, we synthesized a new series of homoglycoclusters and heteroglycoclusters with diverse structures. And the bioactivities of these compounds were assessed by a static state cell-based adhesion assay. We found that when the linked saccharide fragments are the same, the anti-adhesion activities of compounds with flexible linkers were stronger than those with rigid scaffold such as the benzene ring, and the best flexible linker in the tested compounds was L-glutamic acid. When L-glutamic acid was employed as the linker, glycoclusters with four valences, but not other valences, exhibited the most significant anti-adhesion activity; however, no significant differences in anti-adhesion activity were found among the tetravalentglycosides that were made by linking glucose (**32**), mannose (**TMa-4**), cellobiose (**34**), or lactose (**Gu-4**). Thus, we conclude that a flexible linker with proper length, such as that of L-glutamic acid, and the linking of four saccharide fragments might be the preferable structural characteristics for the glycocluster compounds with potent anti-adhesion activity.

1. Introduction

Inflammation is an immune response to tissue injury and invasion of pathogens, in which recruitment of leukocytes from the blood to the tissue is the key process. However, excessive influx of leukocytes, under certain pathological conditions, may result in adverse reactions.[1–4] For example, the recruitment of leukocytes to the site of tissue injury can cause further tissue damages, leading to the deepening, broadening, and worsening of tissue injury and even irreversible tissue necrosis and complete loss of functions. Some related disorders include ischemia and reperfusion injury, shock, systemic septicemia, severe trauma and burns, acute lung injury, and several autoimmune disorders.[5–7] In all cases, leukocytes are recruited to the inflamed tissue by the sequentially adhesive interactions between the leukocytes and the endothelium mediated by cell-adhesion molecules (CAMs) on the surface of the interacting cells, and the different subsets of CAMs are responsible for the different steps in extravasation.[8–10]

The selectin family of CAMs serves to slow down the motion of leukocytes in the direction of flow and to promote the rolling of leukocytes along the vessel wall.[5,11–13] The initial contact is followed by the firm adhesion of leukocytes to the endothelial cells, which is mediated by the interaction of β1 and β2 integrins ($\alpha_L\beta_2$, CD11a/CD18, or $\alpha_M\beta_2$, CD11b/CD18) on leukocytes with their corresponding receptors on endothelial cells.[4] Strong adhesion is followed by the penetration of the leukocytes from the blood stream to the tissue.[14,15] Because of the important roles of CAMs in the multistep leukocyte recruitment cascade, they have been used as drug targets and their effective inhibitors are actively explored.[16–21]

CD11b/CD18 is a leukocyte integrin that is involved in various biological processes.[14–16,25] It plays an important role in the firm attachment of leukocytes to endothelium. It has been reported that CD11b forms complexes with glycosylphosphatidylinositol-linked membrane glycoproteins such as FcγRIIIB(CD16), the urokinase-plasminogen activator receptor (uPAR or CD87), or the LPS receptor CD14, thereby providing these surface bound molecules with transmembrane signaling capability.[26] The critical site on CD11b

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contributing to these functions is a lectin domain, which has been mapped to a region of CD11b located at the C-terminal of the I-domain binding sites for ICAM-1, fibrinogen, and iC3b. It has also been found that CD11b interacts specifically with certain sugars, including some polysaccharides containing glucose, *N*-acetyl-D-glucosamine, and mannose.[26a,27] Inhibiting the activity of CD11b/CD18 by using small oligosaccharides offered the possibility to design new drugs to treat several pathological events such as inflammation caused by bacterial infections.

In our previous research on the treatment of severe burn shock, a clinical syndrome associated with inadequate blood flow to vital organs and tissues,[1,6,22] we have demonstrated that multivalent lactosides can effectively inhibit the adhesion of leukocytes to endothelial cells.[23] In addition, our investigations on the anti-adhesion biological mechanism using labeled compounds **YAn-2** and **YGu-4** Fig. 1) have revealed that the target of multivalent lactosides is CD11b on the surface of leukocytes.[24] However, the contributions of structural components of the synthetic multivalent oligosaccharide, including the class, the valence of sugar and scaffold, to the anti-adhesion activity have not been systematically investigated. In this study, we have synthesized a series of glycoclusters consisting of galactose, glucose, and cellobiose, and a new series of multivalent lactosides with a variety of structures. In addition, mixed-type α-Man-β-Lac heteroglycoclusters have also been synthesized. The anti-adhesion activities of these newly synthesized compounds as well as previously prepared multivalent mannosides[28] were (Fig. 2) assessed.

2. Results and discussion

2.1. Synthesis of oligosaccharides

To study the structure–activity relationship and investigate the cluster effect[29–33] of multivalent lactosides, a series of analogues of **An-2** and **Gu-4** with diverse structures were synthesized using a convergent method.[34]

As depicted in Scheme 1, divalent lactoside **8** was obtained in a yield of 50% by treatment of octaacetyllactose **1** with 1,3-propandiol in the presence of $BF_3{\cdot}OEt_2$ at room temperature. Treatment of compound **8** with CH_3ONa/CH_3OH produced compound **14**, an analogue of **An-2**, in a yield of 96%.

Tetra-, hexa-, and octavalent lactosides have been synthesized to expand the structural variety of multivalent lactosides, to investigate the cluster effect of multivalent lactosides, and to explore the influence of linker flexibility on activity.[29b,35] For example, compound **6** reacted with linkers including butanedioic acid, pentanedioic acid, octanedioic acid, and 1,3,5-benzenetricarboxylic acid in dry THF in the presence of HOBt and DCC to form tetra- and hexavalent glycosides (**9**, **10**, **11**, and **12**) in 70–88% yields (Scheme 2). Treatment of compounds **9**, **10**, **11**, and **12** with

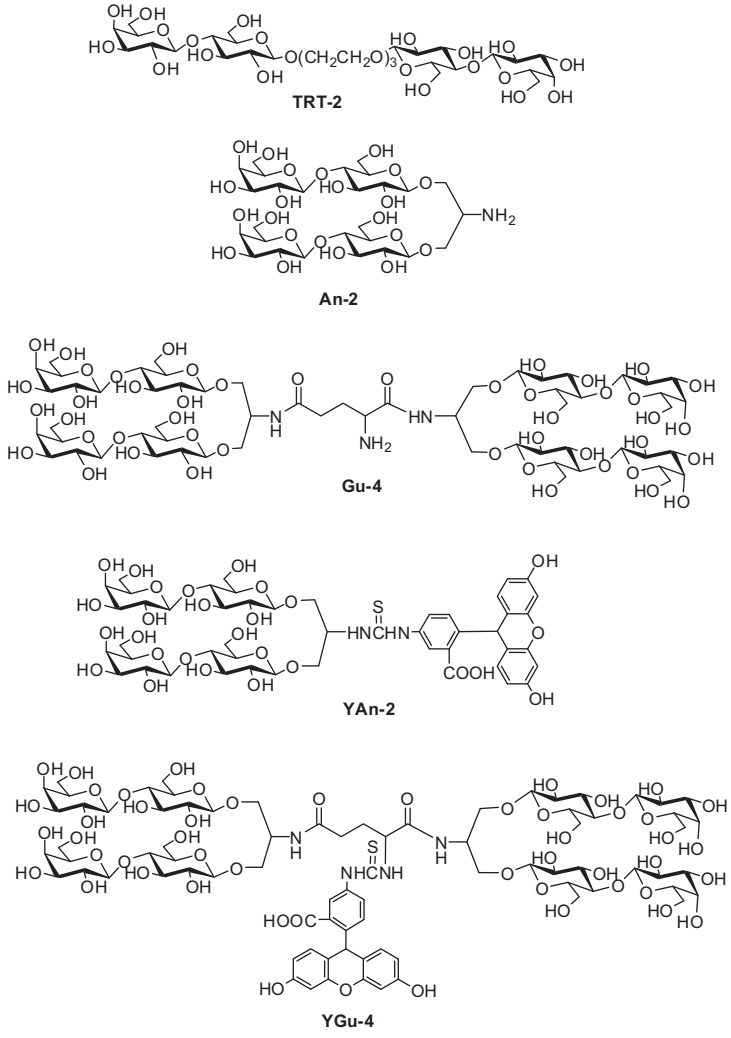


Figure 1. The structures of compounds previously prepared.

CH_3ONa/CH_3OH produced the final target compounds **15**, **16**, **17**, and **18**, respectively, in 94–97% yields (Scheme 2).

Tetravalent lactoside **7** reacted with octanedioic acid in dry THF in the presence of HOBt and DCC to form octavalent lactoside **13** in 50% yield (Scheme 3). Treatment of compound **13** with CH_3ONa/CH_3OH produced the target compound **19** in 94% yield (Scheme 3).

Considering our previous observations and the reported evidences of sugar specificity of CD11b, we intended to investigate which residue of lactose (galactose or glucose) plays a key role in adhesion inhibition. In this study, a series of glycoclusters consisting of galactose, glucose, and cellobiose, which have the same scaffold as **Gu-4**, have been synthesized.

Compounds **20**, **21**, and **22** were prepared by the reaction of pentaacetyl-β-D-glucopyranose **2**, pentaacetyl-β-D-galactopyranose **3**, and octaacetylcellobiose **4** with 2-carbobenzyloxyamino-1,3-propandiol in the presence of $BF_3{\cdot}OEt_2$ at room temperature, respectively (Scheme 4). Divalent glycoside units **23**, **24**, and **25** were obtained by treating compounds **20**, **21**, **or 22** with Pd/C under H_2 atmosphere at room temperature, respectively.

Tetravalent glycosides **26**, **27**, and **28** were prepared in 85–88% yield by the reaction of divalent glycoside units **23**, **24**, and **25** with *Cbz*-L-glutamic acid in dry THF in the presence of HOBt and DCC, respectively. The target compounds **32**, **33**, and **34** were obtained after two steps of deprotection as shown in Scheme 5.

The native polysaccharide ligands of CD11b are made of mixed sugar units. It was reported that an equimolar mixture of β-methylmannoside and β-methylglucoside was two to fourfold more efficient in blocking the lectin site of CD11b than the individual sugars at the same total hexose concentration.[27] Therefore, we synthesized some mixed-type α-Man-β-Lac heteroglycoclusters.[27,36] Donor **1** reacted with 2-carbobenzyloxyamino-1,3-propandiol (1:1) in the presence of $BF_3{\cdot}OEt_2$ at room temperature (Scheme 6) to yield compound **35** in 30% yield. Compound **35** reacted with donor **5** in the presence of TMSOTf to afford compound **36**, which was then treated with Pd/C under 0.4 MPa H_2 atmosphere at room temperature to produce the heteroglycoside **37** in 70% yield.

Synthesized glycoclusters **38**, **40**, **41**, or **42** were referred as tetravalent homoglycosides (Scheme 5). The target heteroglycocluster **43** (2 + 2), **44** (2 + 2), heteroglycocluster **45** (3 + 3), and heteroglycocluster **46** (4 + 4) were produced in 94–98% yields by treating the compounds **38**, **40**, **41**, or **42** with CH_3ONa/CH_3OH, respectively (Scheme 7).

2.2. Anti-adhesion assay

A static state anti-adhesion assay of leukocyte-endothelial cells was adopted.

Anti-adhesion activities of all synthesized compounds in this report (Table 1) as well as previously prepared multivalent mannosides[28] were determined and the results are shown as follows.

2.2.1. The effect of flexible linker on the anti-adhesion activity of tetravalent lactosides

As shown in Figure 3, compound **15**, which has a shorter spacer than that of **Gu-4**, and compound **17**, which has a longer spacer than **Gu-4**, showed lower anti-adhesion activity than **Gu-4** (p <0.05). Compound **16**, bearing a linker with the same length as that of **Gu-4** but having no amino group, also showed lower anti-adhesion activity than **Gu-4** (p <0.05). These results suggested that anti-adhesion activities of tetravalent lactosides might be influenced by the flexible linker, and the optimal flexible linker in our study was L-glutamic acid.

2.2.2. The cluster effect of homoglycosides

Tetravalent lactoside **Gu-4** bearing L-glutamic acid linker showed the highest anti-adhesion activity among the multivalent lactosides tested. The divalent, hexavalent, and octavalent derivatives of lactoside were not as effective as **Gu-4** in respect to their anti-adhesion activities (Fig. 4A). The similar pattern was observed for multivalent mannosides[28] (Fig. 4B). These observations suggested that the optimal valence of multivalent glycosides was four and the optimal flexible linker was L-glutamic acid in our study. In addition, these results suggest that the anti-adhesion activities of compounds with flexible linkers are stronger than those with rigid linkers such as the benzene ring.

2.2.3. The effects of different sugar residues on anti-adhesion activity

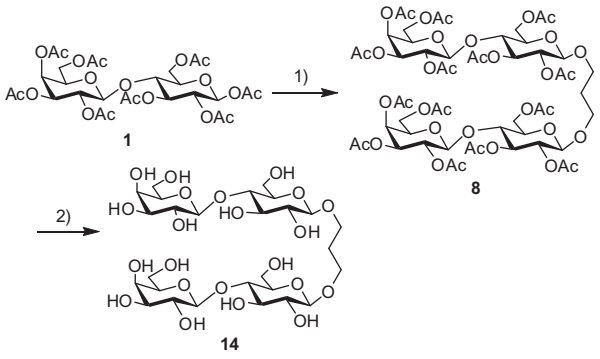
To understand the effect of different sugar residues on the anti-adhesion activity, a series of tetravalent glycosides consisting of galactose, glucose, mannose[28], and cellobiose with the same scaffold were synthesized, and their anti-adhesion activities were tested. As illustrated in Figure 5, all tetravalent glycosides, namely compound **32** (glucoside), **33** (galactoside), **TMa-4** (mannoside), and **34** (cellobioside), exhibited significant anti-adhesion activities compared with control (p <0.05), but no marked differences among these four glycosides were observed (p >0.05). In comparison with **Gu-4**, only **33** showed significantly lower anti-adhesion activity (p <0.05). These results suggested that tetravalent glycosides that contain mannose or glucose fragments possess significant anti-adhesion activity, which are consistent with previous reports. However, more compounds with various structural units need to be synthesized to study the activity–structure relationship concerning the composition of the structural unit and the anti-adhesion activity.

2.2.4. Anti-adhesion activities of mixed-type α-Man-β-Lac heteroglycoclusters

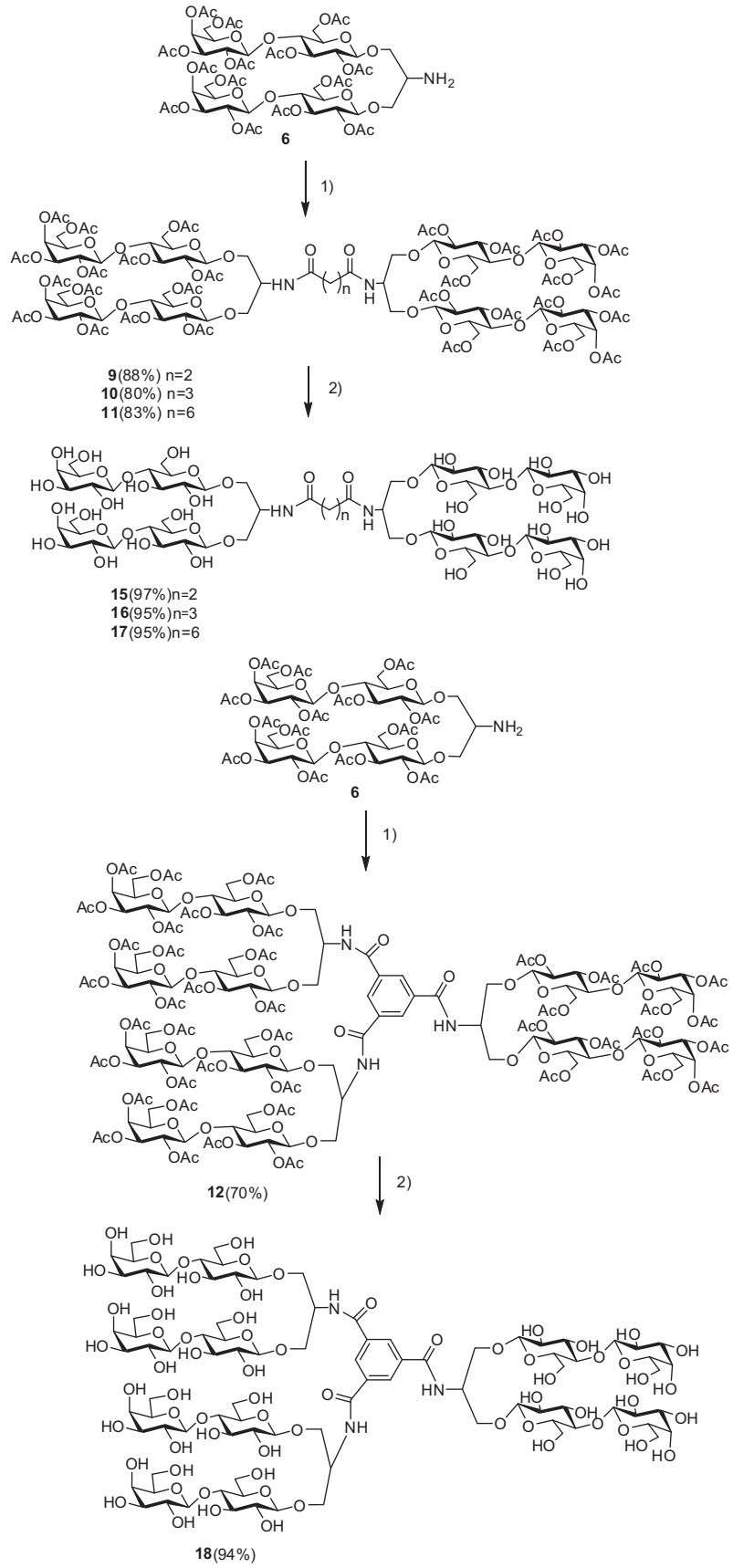
Some mixed-type α-Man-β-Lac heteroglycoclusters were synthesized to explore the effect of different types of saccharides. As shown in Figure 6, heteroglycocluster **46** and **43** exhibited the similar anti-adhesion activity as that of tetravalent homolacoside **Gu-4**. Heteroglycocluster **46** showed significantly higher anti-adhesion activity than its corresponding octavalent homolactoside **19** and octavalent homomannoside **TMa-8**. These results suggested that a heterocluster can afford better activity at higher valency. Further investigations are needed to find out the optimal combination of different types of saccharide and the suitable scaffold for increased anti-adhesion activity.

3. Conclusions

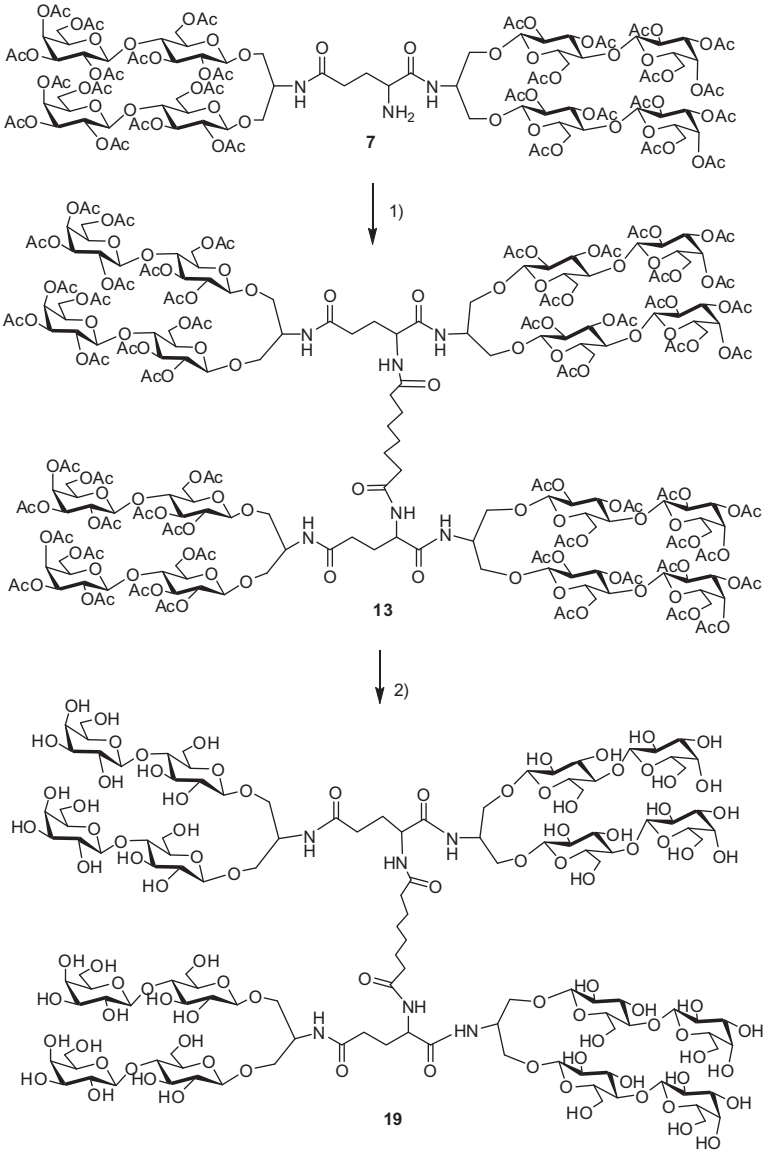
By employing a convergent strategy, we have successfully synthesized various multivalent homoglycosides and mixed-type α-



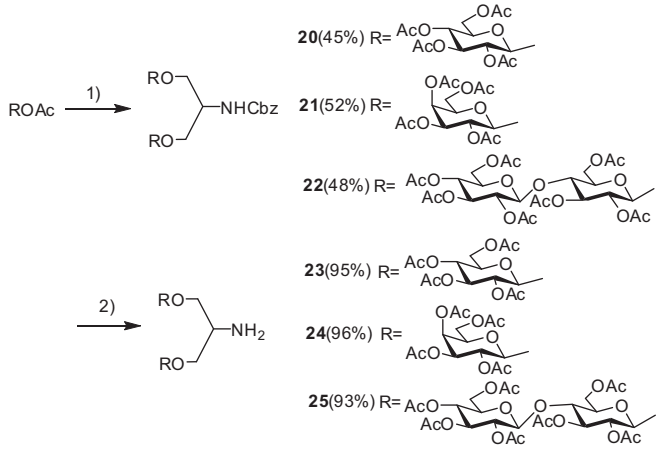
Scheme 1. Synthesis of divalent glycoside **14**. Reagents and conditions: (1) 1,3-propandiol, $BF_3{\cdot}OEt_2$,CH_2Cl_2, 50% yield; (2) CH_3ONa, CH_3OH, 96% yield.



Scheme 2. Synthesis of compounds **15**, **16**, **17**, and **18**. Reagents and conditions: (1) butanedioic acid(**9**), pentanedioic acid(**10**), octanedioic acid(**11**), 1,3,5-benzenetricarboxylic acid(**12**), respectively, HOBt, DCC, THF; yields: 70–88%; (2) CH_3ONa, CH_3OH, yields: 94–97%.

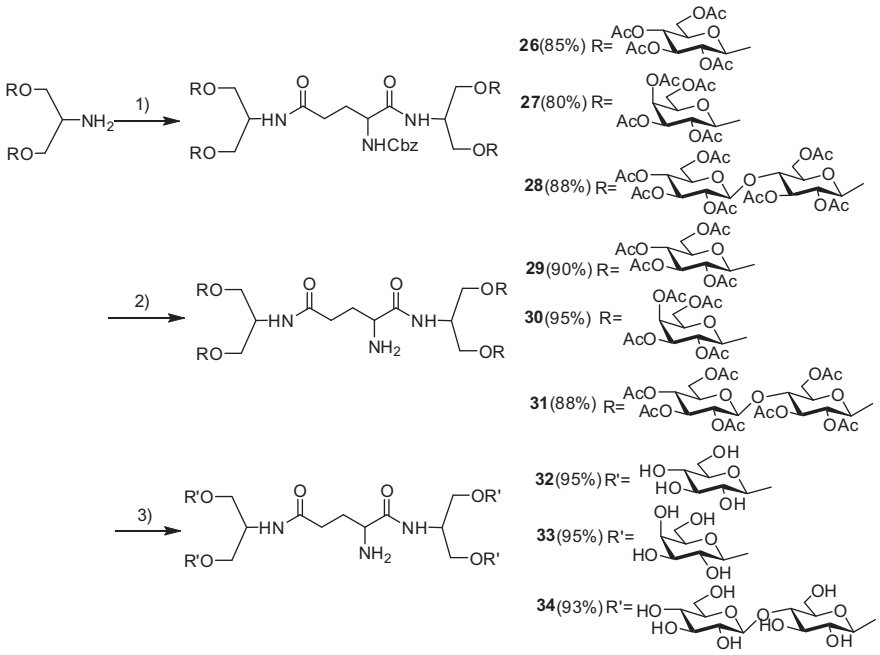


Scheme 3. Synthesis of compound **19**. Reagents and conditions: (1) octanedioic acid, HOBt, DCC, THF; 50% yield; (2) CH_3ONa, CH_3OH, 94% yield.

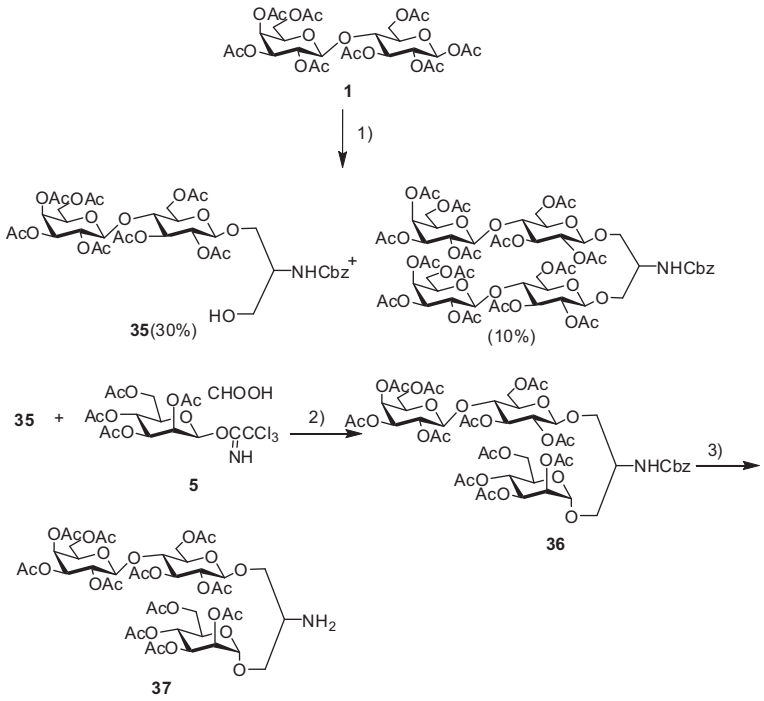


Scheme 4. Synthesis of divalent glycoside unite **23**, **24**, and **25**. Reagents and conditions: (1) 2-carbobenzyloxyamino-1,3-propandiol, $BF_3{\cdot}OEt_2$, CH_2Cl_2, yields: 45–52%; (2) Pd/C, H_2, CH_3OH, yields: 93–96%.

Man-β-Lac heteroglycoclusters that bear different saccharide fragments, different scaffolds, and different valences of saccharides. Through the assessments of inhibitory effects of these glycoclusters on leukocyte-endothelial cell adhesion, we found that the compounds with flexible linkers showed higher anti-adhesion activities than those with rigid scaffold, and the best flexible linker in the tested compounds was L-glutamic acid. When L-glutamic acid was used as the linker, the tetravalence glycosides that contained mannose or glucose fragments, such as compound **32** (glucoside), **TMa-4** (mannoside), **Gu-4** (lactoside), **34** (cellobioside), and **46** (mixed-type α-Man-β-Lac heteroglycocluster), exhibited the most potent anti-adhesion activities. Although further structural modifications are needed to improve the anti-adhesion activity and the understanding of the structure–activity relationship, our initial results provide experimental data on the design and synthesis of polysaccharides for the treatment of pathological conditions involving cell–cell adhesion.



Scheme 5. Synthesis of compounds **32**, **33**, and **34**. Reagents and conditions: (1) *Cbz*-L-glutamic acid, HOBt, DCC, THF, yields: 80–88%; (2) Pd/C, H_2, CH_3OH, yields: 88–95%; (3) CH_3ONa, CH_3OH, yields: 93–95%.



Scheme 6. Synthesis of heteroglycoside unit **37**. Reagents and conditions: (1) 2-carbobenzyloxyamino-1,3-propandiol, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 30% yield; (2) TMSOTf, CH_2Cl_2, 70% yield; (3) Pd/C, H_2, CH_3OH, 95% yield.

4. Experimental section

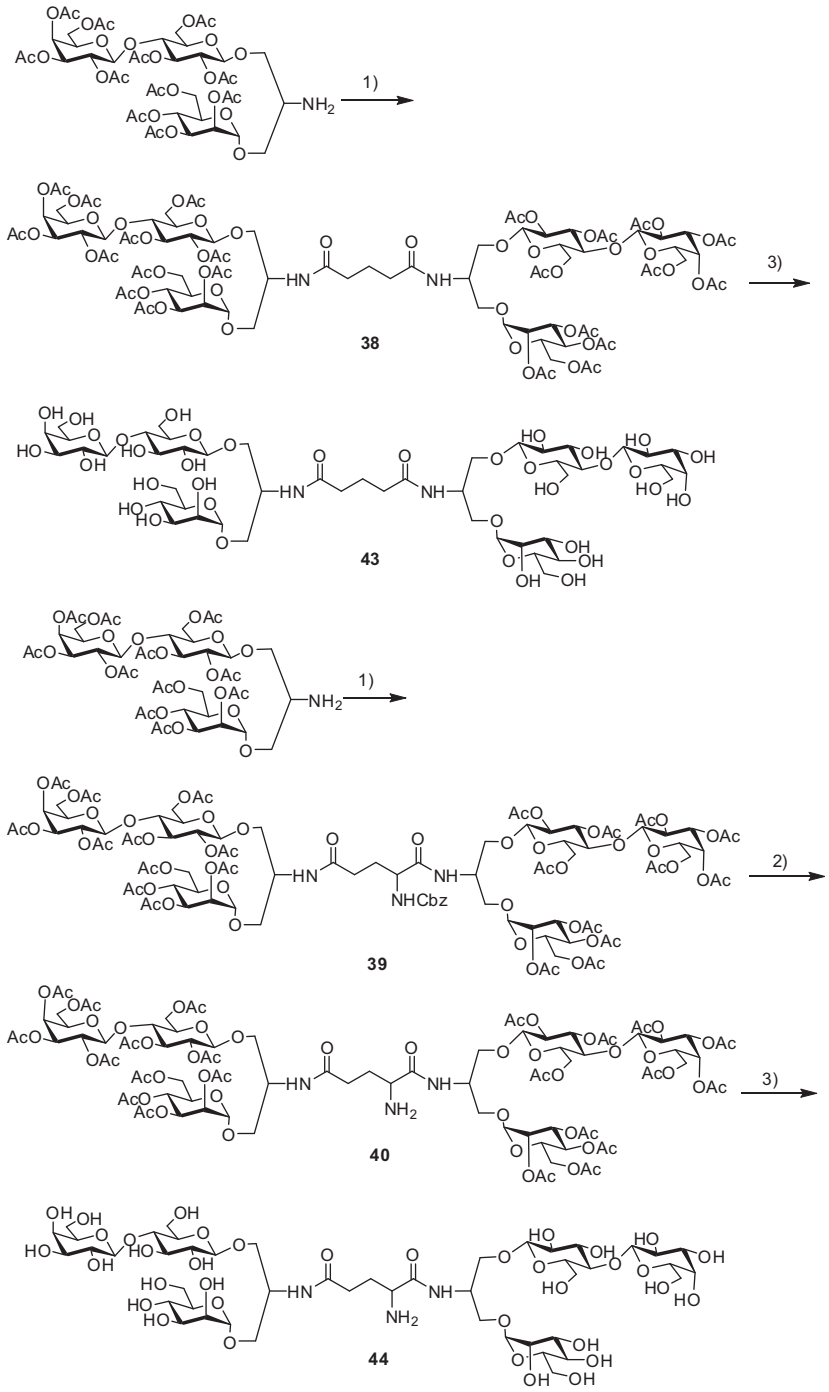
4.1. Synthesis

4.1.1. General methods

Optical rotations were recorded using an Optical Activity AA-10R polarimeter. NMR spectra were recorded on Bruker ARX-400, Varian JEOL-300, and Varian VRX500 spectrometers, with $CDCl_3$, CD_3OD, and D_2O as solvents. Elemental analyses were performed with a Perkin–Elmer 240C instrument. Mass spectra were recorded with an IBI-MDS Sciex Qstar mass spectrometer. Purity of the products was verified by TLC with Silica Gel GF_{254}. Column chromatography was performed with Silica Gel H_{60}.

4.1.2. 1,3-Di-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(14)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy]-propane (8)

To a solution of 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(14)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl acetate **1** (1.12 g,

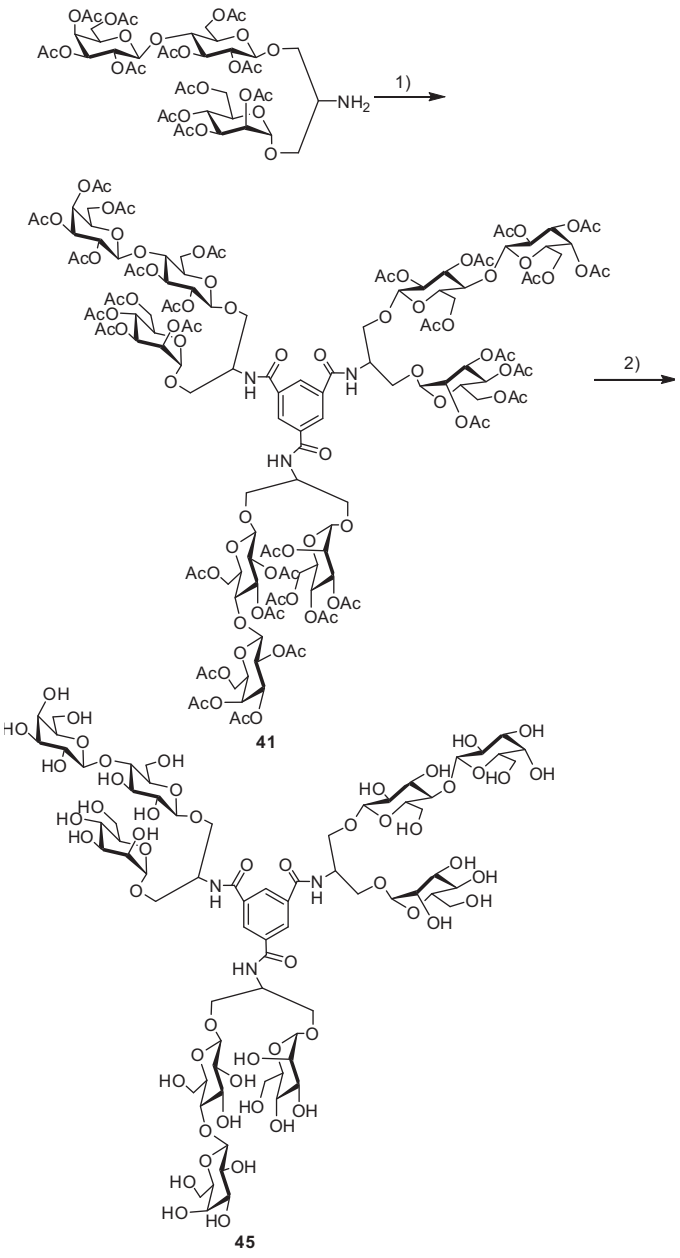


Scheme 7. Synthesis of heteroglycoside **43**, **44**, **45**, or **46**. Reagents and conditions: (1) pentanedioic acid(**38**), *Cbz*-L-glutamic acid(**39**), 1,3,5-benzenetricarboxylic acid(**41**), octanedioic acid(**42**), respectively, HOBt, DCC, THF; yields: 50–85%; (2) Pd/C, H_2, CH_3OH, 94% yield; (3) CH_3ONa, CH_3OH, yields: 93–95%.

1.65 mmol) and 1, 3-propandiol (0.05 g, 0.66 mmol) in dry CH_2Cl_2 (3 mL), $BF_3{\cdot}OEt_2$ (0.04 mL) was added and the mixture was stirred at room temperature for 24 h. The mixture was then diluted with CH_2Cl_2 (20 mL) and washed with aqueous sodium bicarbonate and water, dried (Na_2SO_4), and evaporated under diminished pressure. The crude product was purified by flash chromatography with 3:2 petroleum ether (60–90 °C)–acetone as eluent to afford 4 g of **8** as a colorless syrup in 50% yield; $[\alpha]_D^{25} = -7.0$ (*c* 1.15 in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ = 5.35 (d, 1H; H-4′), 5.18 (t, 1H; H-3), 5.11 (dd, 1H; H-2′), 4.95 (dd, 1H; H-3′), 4.87 (t, 1H; H-2), 4.48 (d, $J_{1,2}$ = 8.2 Hz, 1H; H-1), 4.41 (d, $J_{1',2'}$ = 8.0 Hz, 1H; H-1′), 4.46, 4.16–4.05 (m, 4H; H-6a,b, H-6a′,b′), 3.89–3.52 (m, 5H; H-4,5, H-5′, CH_2O), 2.15–1.97 (7s, 21H; CH_3CO), 1.82 (m, 1H; CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.3–169.0(7CO), 101.1 (C-1′), 100.7 (C-1), 76.3 (C-4), 72.7 (C-3), 72.6 (C-5), 71.7 (C-2), 71.0 (C-5′), 70.7 (C-3′), 69.1 (CH_2O), 66.6 (C-2′), 66.5 (C-4′), 62.0 (C-6′), 60.8 (C-6), 29.8 (CH_2), 20.8–20.5 ($7CH_3CO$); HRESI-TOF MS: *m*/*z* calcd for $C_{55}H_{76}O_{36}$: 1335.40140 $[M+Na]^+$; found: 1335.39940.

4.1.3. *N*-{2-[1,3-Di-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(14)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy)]-propanyl}-butane-1,4-diamide (9)

Succinic acid (0.02 g, 0.17 mmol) was dissolved in anhydrous THF (1 mL) that was cooled to 0 °C, then HOBt (0.08 g, 0.6 mmol)



Scheme 7 (*continued*)

and DCC (0.12 g, 0.6 mmol) were added. The mixture was stirred for 0.5 h at 0 °C. A solution (pH 8–9) of compound **6**[24b] (0.45 g, 0.34 mmol) in dry THF (2 mL) in the presence of NMM was added dropwise and the mixture was stirred at 0 °C for 2 h, followed by stirring at room temperature for 36 h. The mixture was concentrated and the residue was diluted with CH_2Cl_2 (10 mL). The solution was filtered and the filtrate was washed with aqueous sodium bicarbonate, aqueous citric acid, and water, dried (Na_2SO_4), and evaporated under diminished pressure. The crude product was purified by flash chromatography with 2:3 petroleum ether (60–90 °C)–acetone as eluent to afford **9** (0.24 g) as a colorless syrup in 88% yield; $[\alpha]_D^{25} = -19.8$ (*c* 1.62 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 6.14 (d, 1H; NHCO), 5.36 (d, 1H; H-4′), 5.20 (t, 1H; H-3), 5.13 (dd, 1H; H-2′), 4.93 (dd, 1H; H-3′), 4.87 (t, 1H; H-2), 4.49 (d, $J_{1,2}$ = 7.8 Hz, 2H; H-1), 4.45 (d, $J_{1',2'}$ = 7.8 Hz, 1H; H-1′), 4.53, 4.13–4.05 (m, 4H; H-6a,b, H-6a′,b′), 3.89–3.49 (m, 6H; H-4,5, H-5′, CH_2O, C*H*NH_2), 2.16–2.14 (s, 1H; $COCH_2$), 2.14–1.97 (7s, 21H; CH_3CO), 1.84 (t, 1H; CH_2); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 171.4, 170.4, 170.3, 170.1, 170.0, 169.7, 169.6, 169.0 (8CO), 101.0 (C-1′), 100.8 (C-1), 76.1 (C-4), 72.7 (C-3), 72.4 (C-5), 71.7 (C-2), 71.5 (C-5′), 70.9 (C-3′), 70.6 (CH_2O), 69.0 (C-2′), 66.6 (C-4′), 61.8 (C-6′), 60.(C-6), 48.3 (*C*HNHCO), 30.8 (*C*H_2CO), 20.9, 20.7, 20.6, 20.5 (7CH_3CO); Anal. Calcd for $C_{114}H_{156}N_2O_{74}$: C, 50.00; H, 5.74; N, 1.02. Found: C, 49.83; H, 5.95; N, 1.00.

4.1.4. *N*-{2-[1,3-Di-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(14)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy)]-propanyl}-pentane-1,5-diamide (10)

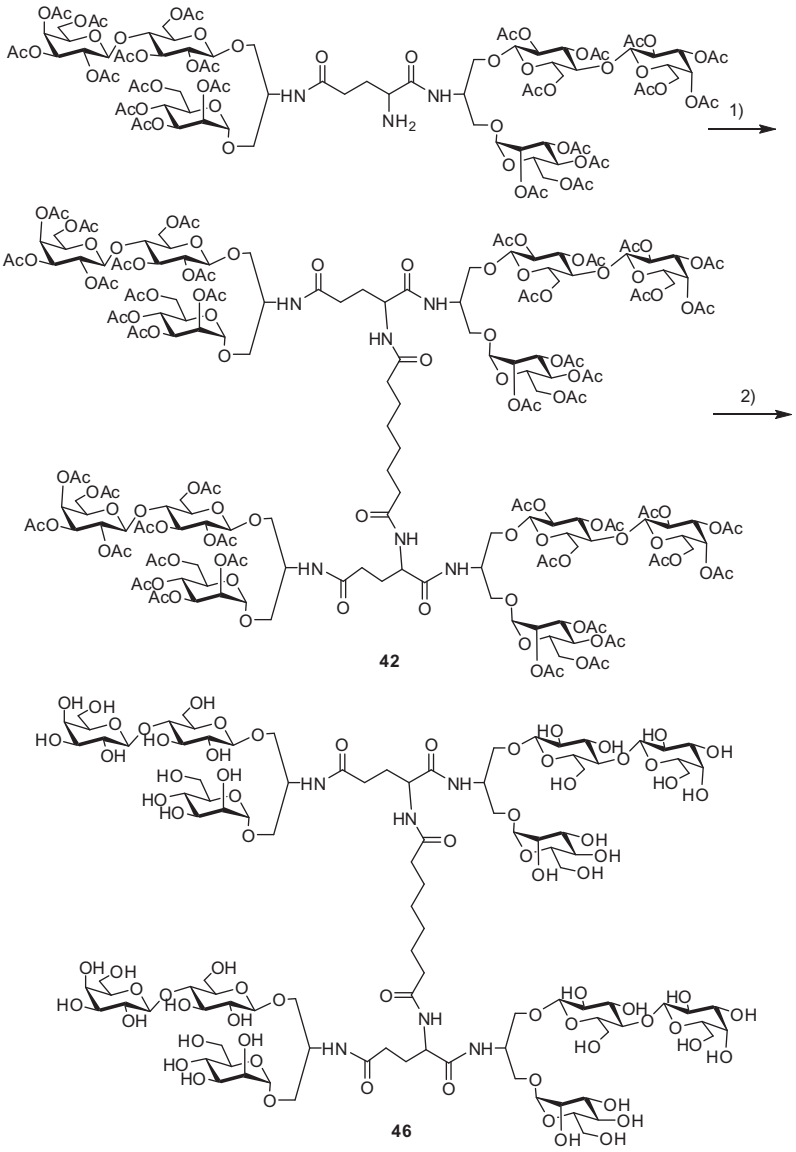
Compound **10** was prepared by the same procedure as described for the preparation of **9** using pentanedioic acid as linker and the crude product was purified by chromatography with 2:3 petroleum ether (60–90 °C)–acetone as eluent to afford **10** as a colorless syrup in 80% yield; $[\alpha]_D^{25} = -17.1$ (*c* 1.17 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.15 (d, 1H; NHCO), 5.33 (d, 1H; H-4′), 5.15 (dd, 1H; H-3), 5.08 (dd, 1H; H-2′), 4.94 (dd, 1H; H-3′), 4.83 (dd, 1H; H-2), 4.48 (d, $J_{1,2}$ = 8.0 Hz, 2H; H-1), 4.43 (d, $J_{1',2'}$ = 8.0 Hz, 1H; H-1′), 4.50, 4.15–4.03 (m, 4H; H-6a,b, H-6a′,b′), 3.88–3.45 (m, 6H; H-4,5, H-5′, CH_2O, C*H*NH_2), 2.16–1.94 (s, 1H; $COCH_2$, CH_3CO, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 172.1, 170.4, 170.3, 170.1, 170.0, 169.7, 169.6, 169.0 (8CO), 101.0, 100.7 (C-1, C-1′), 76.0 (C-4), 72.8 (C-3), 72.5 (C-5), 71.6 (C-2), 71.5 (C-5′), 70.9 (C-3′), 70.6 (CH_2O), 69.0 (C-2′), 66.6 (C-4′), 61.8 (C-6′), 60.7 (C-6), 48.2 (*C*HNHCO), 35.1 (*C*H_2CO), 21.5, 20.8, 20.7, 20.6, 20.5, 20.4 (7CH_3CO); HRESI-TOF MS: *m*/*z* calcd for $C_{115}H_{158}N_2O_{74}$: 2774.85931 $[M+Na]^+$; found: 2774.85496.

4.1.5. *N*-{2-[1,3-Di-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(14)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy)]-propanyl}-octane-1,8-diamide (11)

Compound **11** was prepared by the same procedure as described for the preparation of **9** using octanedioic acid as linker and the crude product was purified by chromatography with 2:3 petroleum ether (60–90 °C)–acetone as eluent to afford **11** as a colorless syrup in a yield of 83%.; $[\alpha]_D^{25} = -28.3$ (*c* 1.13 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 5.83 (d, 1H; NHCO), 5.35 (d, 1H; H-4′), 5.20 (t, 1H; H-3), 5.10 (dd, 1H; H-2′), 4.93 (dd, 1H; H-3′), 4.85 (t, 1H; H-2), 4.49 (d, $J_{1,2}$ = 7.8 Hz, 2H; H-1), 4.44 (d, $J_{1',2'}$ = 7.8 Hz, 1H; H-1′), 4.49, 4.14–4.04 (m, 4H; H-6a,b, H-6a′,b′), 3.91–3.48 (m, 6H; H-4,5, H-5′, CH_2O, C*H*NH_2), 2.18–2.13 (t, 1H; $COCH_2$), 2.14–1.97 (7s, 21H; CH_3CO), 1.59 (m, 1H; CH_2), 1.26 (m, 1H; CH_2); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 172.5, 170.3, 170.0, 169.7, 169.6, 169.5, 169.0 (8CO), 100.9 (C-1′), 100.7 (C-1), 76.1 (C-4), 72.7 (C-3), 72.4 (C-5), 71.6 (C-2), 71.4 (C-5′), 70.9 (C-3′), 70.6 (CH_2O), 69.0 (C-2′), 66.5 (C-4′), 61.8 (C-6′), 60.7 (C-6), 48.0 (*C*HNHCO), 36.2 (*C*H_2CO), 28.9 (CH_2), 25.3 (CH_2), 20.8, 20.7, 20.6, 20.4 (7CH_3CO); Anal. Calcd for $C_{118}H_{164}N_2O_{74}$: C, 50.72: H, 5.92; N, 1.00. Found: C, 50.62; H, 6.00; N, 0.98.

4.1.6. *N*-{2-[1,3-Di-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(14)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy)]-propanyl}-benzene-1,3,5-triamide (12)

Compound **12** was prepared by the same procedure as described for the preparation of **9** using benzene-1,3,5-tricarboxylic acid as linker and the crude product was purified by chromatography with 2:3 petroleum ether (60–90 °C)–acetone as eluent to afford **12** as a colorless syrup in 70% yield; $[\alpha]_D^{25} = -29.2$ (*c* 2.33 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.34 (3H; Ph), 7.08 (d, 1H; NHCO), 5.36 (d, 1H; H-4′), 5.22 (t, 1H; H-3), 5.12 (dd, 1H; H-2′), 4.98 (dd, 1H; H-3′), 4.88 (t, 1H; H-2), 4.56 (d, $J_{1,2}$ = 8.1 Hz, 2H; H-1), 4.52 (d, $J_{1',2'}$ = 8.1 Hz, 1H; H-1′), 4.52, 4.17–4.09 (m, 4H; H-6a,b, H-6a′,b′), 3.92–3.66 (m, 6H; H-4,5, H-5′, CH_2O, C*H*NH), 2.18–1.97 (7s, 21H; CH_3CO); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 170.3, 170.2, 170.0, 169.9, 169.6, 169.5, 168.9 (CO), 165.3, 134.7 (Ph), 100.9 (C-1′), 100.6 (C-1), 76.1 (C-4), 72.9 (C-3), 72.5 (C-5), 72.3



Scheme 7 (*continued*)

(C-2), 71.5 (C-5′), 70.9 (C-3′), 70.5 (CH_2O), 69.0 (C-2′), 66.6 (C-4′), 61.9 (C-6′), 60.7 (C-6), 49.2 (*C*HNHCO), 20.9, 20.7, 20.6, 20.5, 20.4 (7CH_3CO); MALDI-TOF MS: *m*/*z* calcd for $C_{174}H_{231}N_3O_{111}$: 4162.2 $[M+Na]^+$; found: 4162.8.

4.1.7. *N*-{2-*N*-{2-[1,3-Di-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(14)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy)] propanyl}pentane-1,5-diamidyl}-octane-1,8-diamide (13)

Compound **13** was prepared by the same procedure as described for the preparation of **9** using octanedioic acid as linker and compound **7**[24b] as the linking unit. The crude product was purified by chromatography with 1:2 petroleum ether (60–90 °C)–acetone as eluent to afford **13** as a colorless syrup in 50% yield; $[\alpha]_D^{25} = -13.5$ (*c* 0.89 in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ = 7.00 (d, 2H; NH), 6.71 (d, 2H; NH), 6.30 (d, 2H; NH), 5.35 (d, 8H; H-4′), 5.18 (t,8H; H-3), 5.09 (dd, 8H; H-2′), 4.96 (dd, 8H; H-3′), 4.84 (dd, 8H; H-2), 4.50 (d, $J_{1,2}$ = 8.0 Hz, 8H; H-1), 4.46 (d, $J_{1',2'}$ = 8.1 Hz, 8H; H-1′), 4.52, 4.14–4.05 (m, 32H; H-6a,b, H-6a′,b′), 3.90–3.51 (m, 46H; H-4,5, H-5′, CH_2O, COC*H*NH, *CH*NHCO), 2.32–1.97 (m, 23H; CH_3CO, $COCH_2$, CH_2-$CHNH_2$), 1.59, 1.24 (m, 8H; CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.5, 170.4, 170.3, 170.1, 170.0, 169.7, 169.6, 169.0 (CO), 101.0, 100.9 (C-1′), 100.8, 100.5 (C-1), 76.0 (C-4), 72.8, 72.7 (C-3), 72.6, 72.5 (C-5), 71.8, 71.6 (C-2), 70.9 (C-5′), 70.6 (C-3′), 69.5 (CH_2O), 69.1 (C-2′), 66.6 (C-4′), 61.9 (C-6′), 60.7 (C-6), 53.8, 52.1, 48.8, 48.5 (*C*HNHCO), 36.3, 32.3, 31.7, 29.6, 29.3, 29.0, 25.4 (CH_2CO, CH_2CHNH), 20.8, 20.7, 20.6, 20.5 (CH_2, CH_3); MALDI-TOF MS: *m*/*z* calcd for $C_{238}H_{328}N_6O_{150}$: 5694.8 $[M+Na]^+$; found: 5694.1.

4.1.8. 1,3-Di-[*O*-β-D-galactopyranosyl-(14)-β-D-glucopyranosyloxy]-propane (14)

A catalytic amount of sodium was added to a solution of compound **8** (0.1 g) in methanol (10 mL). The mixture was stirred at room temperature for 12 h, and the mixture was neutralized with H^+ cation exchange resin. The solution was filtered and concentrated and the residue was dissolved in 20 mL water and freeze-dried to give **14** as a white solid (0.054 g, 96%); $[\alpha]_D^{25} = -23.5$ (*c* 1.02 in H_2O); ^1H NMR (D_2O, 400 MHz): δ = 4.36, 4.31 (d, $J_{1,2}$ = $J_{1',2'}$ = 8.0 Hz, 2H; H-1, H-1′), 3.92–3.16 (m, 14H; H-2, 3,4,5,6a,b, H-2′,3′,4′,5′,6a′,b′, CH_2O), 1.83 (q, 1H; CH_2); ^{13}C NMR (D_2O, 100 MHz): δ = 102.9 (C-1′), 102.1 (C-1), 78.4 (C-4), 75.3, 74.8,

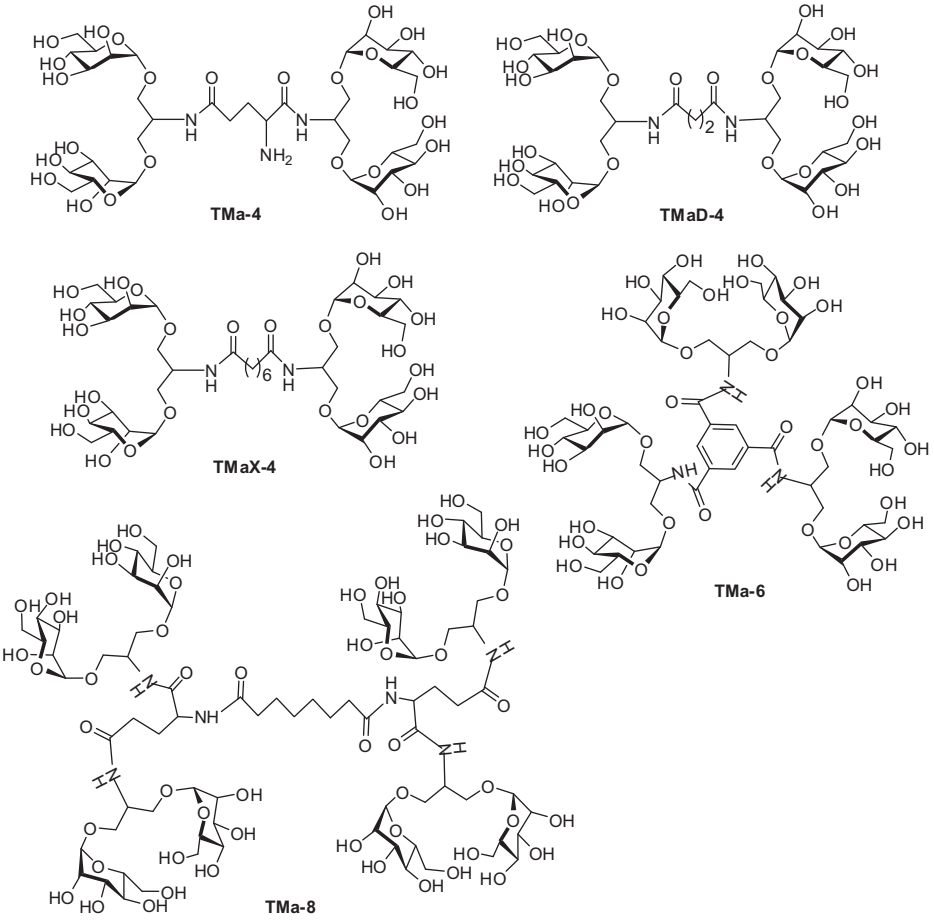


Figure 2. The structures of previously prepared multivalent mannosides.

Table 1
Anti-adhesion activities of all synthesized compounds[a]

Compound	Mean fluorescence intensity	±SD	*p* (vs control)
Control	1000	10	
An-2	1400	100	0.000
Gu-4	700	40	0.003
14	990	100	0.855
15	870	50	0.017
16	1100	70	0.080
17	870	90	0.016
18	1100	200	0.122
19	1000	50	0.605
32	770	70	0.002
33	840	90	0.018
34	720	100	0.000
43	820	70	0.017
44	1100	80	0.065
45	850	100	0.040
46	730	100	0.001
TMaD-4	860	70	0.018
TMaX-4	790	60	0.002
TMa-4	740	90	0.000
TMa-6	1000	100	0.645
TMa-8	940	60	0.285

[a] Compounds: 40 μM

74.4, 72.8, 72.5, 70.9, 68.5, 67.1 (C-2,3,5, C-2′,3′,4′,5′, CH_2O), 61.0, 60.1 (C-6, C-6′), 29.1 (CH_2); HRESI-TOF MS: *m*/*z* calcd for $C_{27}H_{48}O_{22}$: 747.25349 $[M+Na]^+$; found: 747.252965.

4.1.9. *N*-{2-[1,3-Di-(*O*-β-D-galactopyranosyl-(14)-β-D-glucopyranosyloxy)]-propanyl}-butane-1,4-diamide (15)

Compound **15** was prepared by the same procedure as described for the preparation of **14**. The yield was 97%; $[\alpha]_D^{25} = -46.5$ (*c* 1.29 in H_2O); ^1H NMR (D_2O, 300 MHz): δ = 6.10 (d, 1H; NHCO), 4.33 (d, $J_{1,2}$ = 7.8 Hz, 1H; H-1), 4.28 (d, $J_{1',2'}$ = 7.8 Hz, 1H; H-1′), 3.83–3.18 (m, 13H; H-2,3,4,5,6a,b, H-2′,3′,4′,5′,6a′,b′, CH_2O, *CH*NH_2), 2.40 (s, 1H; CH_2CO); ^{13}C NMR (D_2O, 75 MHz): δ = 175.4 (CO), 103.5 (C-1′), 103.1 (C-1), 78.9 (C-4), 75.9, 75.3, 74.8, 73.3, 73.1, 72.3, 71.5, 69.1 (C-2,3,5, C-2′,3′,4′,5′, CH_2O), 61.6, 60.6 (C-6, C-6′), 49.5 ($CHNH_2$), 31.5 ($COCH_2$); ESI-TOF MS: *m*/*z* calcd for $C_{58}H_{100}N_2O_{46}$: 1599.5 $[M+K]^+$; found: 1599.6.

4.1.10. *N*-{2-[1,3-Di-(*O*-β-D-galactopyranosyl-(14)-β-D-glucopyranosyloxy)]-propanyl}-pentane-1,5-diamide (16)

Compound **16** was prepared by the same procedure as described for the preparation of **14**. The yield was 95%; $[\alpha]_D^{25} = -26.7$ (*c* 1.05 in H_2O); ^1H NMR (D_2O, 400 MHz): δ = 4.36 (d, $J_{1,2} = J_{1',2'}$ = 8.0 Hz, 4H; H-1′, H-1), 3.85–3.18 (m, 13H; H-2,3,4,5,6a,b, H-2′,3′,4′,5′,6a′,b′, CH_2O, *CH*NH_2), 2.18 (m, 2H; CH_2CO), 1.75 (m, 1H; CH_2); ^{13}C NMR (D_2O, 100 MHz): δ = 176.0 (CO), 102.9, 102.5 (C-1, C-1′), 78.4 (C-4), 75.3, 74.8, 74.3, 74.2, 72.8, 72.5, 70.9, 68.7, 68.5 (C-2,3,5, C-2′,3′,4′,5′, CH_2O), 61.0, 60.1 (C-6, C-6′), 49.1 (CHNH), 34.9 ($COCH_2$), 21.7 ($COCH_2CH_2$); ESI-TOF MS: calcd for $C_{59}H_{102}N_2O_{46}$: 1597.6 $[M+Na]^+$; found: 1598.5.

4.1.11. *N*-{2-[1,3-Di-(*O*-β-D-galactopyranosyl-(14)-β-D-glucopyranosyloxy)]-propanyl}-octane-1,8-diamide (17)

Compound **17** was prepared by the same procedure as described for the preparation of **14**. The yield was 95%;

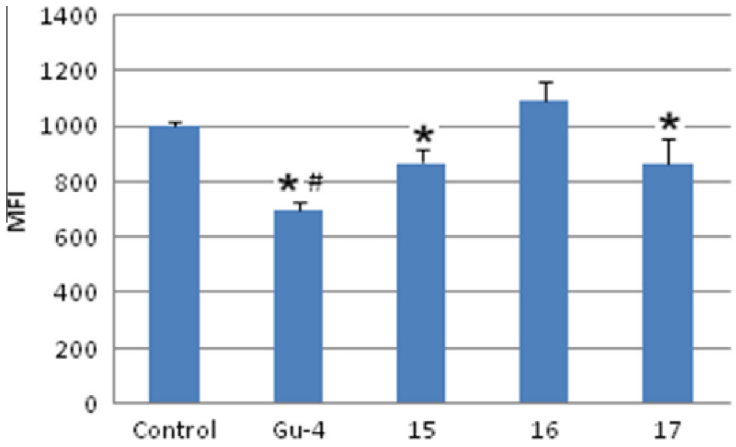


Figure 3. The effect of flexible linker on the anti-adhesion activity of tetravalent lactosides. $^{*}p$ <0.05 versus control. $^{\#}p$ <0.05 versus compound **15**, **16**, or **17**. MFI: mean fluorescence intensity.

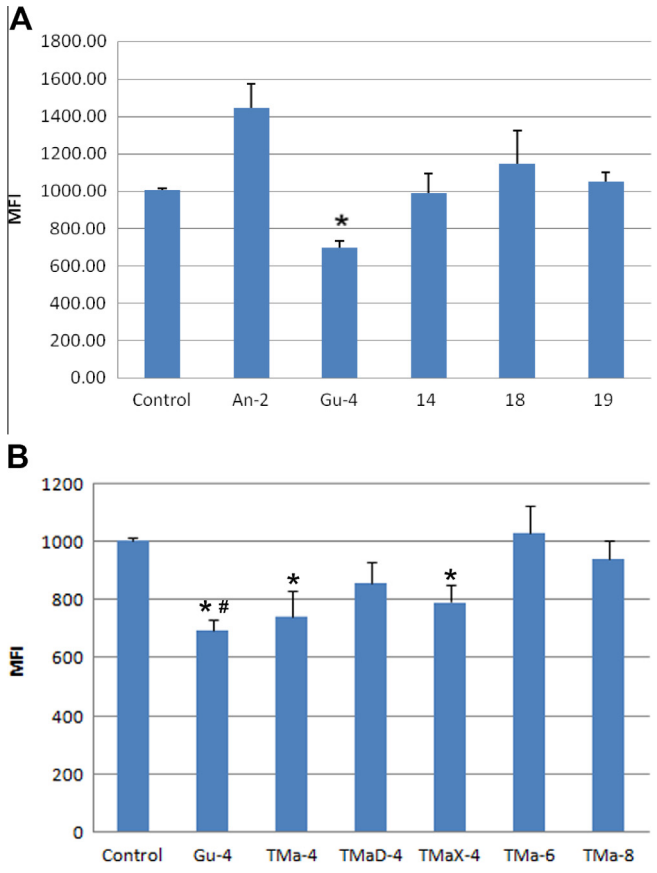


Figure 4. The cluster effect of homoglycosides. (A) The cluster effect of multivalent lactosides. $^{*}p$ <0.05 versus any other group. (B) The cluster effect of multivalent mannosides. $^{*}p$ <0.05 versus control. $^{\#}p$ <0.05 versus **TMaD-4**, **TMa-6**, or **TMa-8**. MFI: mean fluorescence intensity.

$[\alpha]_D^{25} = -24.4$ (*c* 1.31 in H_2O); ^{1}H NMR (D_2O, 300 MHz): δ = 5.79 (d, 1H; NHCO), 4.33 (d, $J_{1,2}$ = 7.5 Hz, 1H; H-1), 4.31 (d, $J_{1',2'}$ = 7.8 Hz, 1H; H-1′), 3.83–3.15 (m, 13H; H-2,3,4,5,6a,b, H-2′,3′,4′,5′,6a′,b′, CH_2O, $CHNH_2$), 2.10 (t, 1H; CH_2CO), 1.43(m, 1H, CH_2), 1.15(m, 1H; CH_2); ^{13}C NMR (D_2O, 75 MHz): δ = 177.8 (CO), 103.5 (C-1′), 103.1 (C-1), 78.9 (C-4), 75.9, 75.3, 74.8, 73.3, 73.1, 72.3, 71.5, 69.1 (C-2,3,5, C-2′,3′,4′,5′, CH_2O), 61.6, 60.6 (C-6, C-6′), 49.5 ($CHNH_2$), 36.3 ($COCH_2$), 28.4 (CH_2), 25.8 (CH_2); MALDI-TOF MS: *m*/*z* calcd for $C_{62}H_{108}N_2O_{46}$: 1639.6 $[M+Na]^+$; found: 1639.9.

4.1.12. *N*-{2-[1,3-Di-(*O*-β-D-galactopyranosyl-(14)-β-D-glucopyranosyloxy)]-propanyl}-benzene-1,3,5-triamide (18)

Compound **18** was prepared by the same procedure as described for the preparation of **14**. The yield was 94%; $[\alpha]_D^{25} = -33.6$ (*c* 1.31 in H_2O); ^{1}H NMR (D_2O, 300 MHz): δ = 8.18 (3H; Ph), 7.01 (d, 1H; CONH), 4.38 (d, $J_{1,2}$ = 7.5 Hz, 1H; H-1), 4.27 (d, $J_{1',2'}$ = 7.5 Hz, 1H; H-1′), 3.96–3.18 (m, 13H; H-2,3,4,5,6a,b, H-2′,3′,4′,5′,6a′,b′, CH_2O, $CHNH_2$); ^{13}C NMR (D_2O, 75 MHz): δ = 169.7 (CO), 135.4, 129.9 (Ph), 103.5 (C-1′), 103.1 (C-1), 78.9 (C-4), 75.9, 75.4, 74.8, 73.3, 73.1, 71.5, 69.2, 69.1 (C-2,3,5, C-2′,3′,4′,5′, CH_2O), 61.6, 60.6 (C-6, C-6′), 49.4 ($CHNH_2$); MALDI-TOF MS: *m*/*z* calcd for $C_{90}H_{147}N_3O_{69}$: 2396.8 $[M+Na]^+$; found: 2397.8.

4.1.13. *N*-{2-*N*-{2-[1,3-Di-(*O*-β-D-galactopyranosyl-(14)-β-D-glucopyranosyloxy)]propanyl}pentane-1,5-diamidyl}-octane-1,8-diamide (19)

Compound **19** was prepared by the same procedure as described for the preparation of **14**. The yield was 94%; $[\alpha]_D^{25} = -12.5$ (*c* 1.28 in H_2O); ^{1}H NMR (D_2O, 400 MHz): δ = 4.41 (d, $J_{1,2}$ = 7.7 Hz, 8H; H-1), 4.35 (d, $J_{1',2'}$ = 8.1 Hz, 8H; H-1′), 3.90–3.25 (m, 116H; H-2,3,4,5,6a,b, H-2′,3′,4′,5′,6a′,b′, CH_2O, $CHNH_2$), 2.32–2.19 (m, 8H; CH_2CO), 2.02–1.51 (m, 8H, CH_2), 1.24(m, 4H; CH_2); ^{13}C NMR (D_2O, 100 MHz): δ = 181.2, 177.1, 175.0, 173.5 (CO), 103.0 (C-1′), 102.7, 102.6 (C-1), 78.5 (C-4), 75.4, 74.8, 74.3, 72.9, 72.8, 72.6, 71.0, 68.8, 68.6 (C-2,3,5, C-2′,3′,4′,5′, CH_2O), 61.1, 60.2 (C-6, C-6′), 53.4, 49.4, 49.2, 49.0,35.4, 31.9 (CHNH, $COCH_2$),

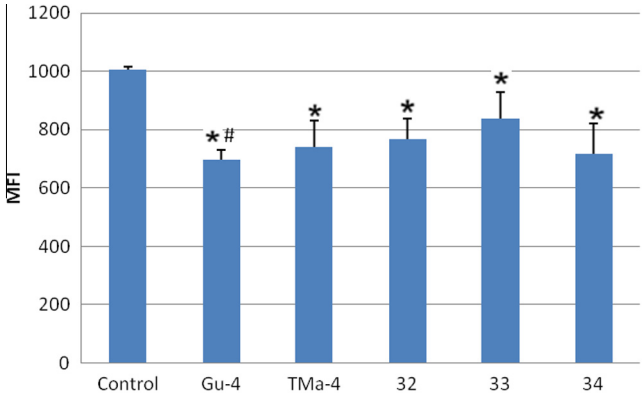


Figure 5. The effect of different sugar residues on the anti-adhesion activity. $^{*}p$ <0.05 versus control. $^{\#}p$ <0.05 versus compound **33**. MFI: mean fluorescence intensity.

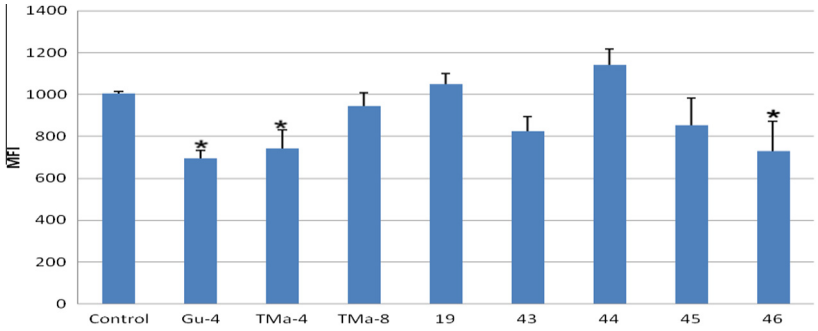


Figure 6. Anti-adhesion activities of mixed-type α-Man-β-Lac heteroglycoclusters. *p <0.05 versus control. MFI: mean fluorescence intensity.

28.0, 27.2, 25.2, 23.2 (CH_2); MALDI-TOF MS: m/z calcd for $C_{126}H_{216}N_6O_{94}$: 3341.2 $[M+Na]^+$; found: 3340.7.

4.1.14. 2-Carbobenzyloxyamino-1,3-di-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-propane (20)

To a solution of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl acetate **2** (2 g, 2.26 mmol) and 2-carbobenzyloxyamino-1,3-propandiol (0.2 g, 0.9 mmol) in dry CH_2Cl_2 (2 mL), $BF_3{\cdot}OEt_2$ (0.04 mL) was added and the mixture was stirred at room temperature for 24 h. The mixture was then diluted with CH_2Cl_2 (20 mL) and washed with aqueous sodium bicarbonate and water, dried (Na_2SO_4), and evaporated under diminished pressure. The crude product was purified by flash chromatography with 5:2 petroleum ether (60–90 °C)–acetone as eluent to afford 0.36 g of **20** as a colorless syrup in 45% yield; $[\alpha]_D^{25} = -5.7$ (c 0.70 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.34 (br, 5H, Ph), 5.29 (d, 2H, PhCH_2O), 5.19 (t, 2H, H-3), 4.97 (t, 2H; H-2), 4.49 (d, $J_{1,2}$ = 7.4 Hz, 2H; H-1), 4.26–3.56 (m, 10H; H-4,5, H-6a,b, CH_2O, CHNH), 2.08–2.00 (4s, 24H; CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.6, 170.1, 169.4, 169.3, 155.8 (CO), 136.3, 128.5, 128.2, 128.1 (Ph), 101.1 (C-1), 76.7 (C-4), 72.7 (C-3), 72.5 (C-5), 71.8 (C-2), 68.3 (CH_2O), 66.8 (PhCH_2O), 61.7 (C-6), 50.1 (CHNH), 20.7, 20.6, 20.5 (CH_3); HRESI-TOF MS: m/z calcd for $C_{39}H_{51}NO_{22}$: 908.28004 $[M+Na]^+$; found: 908.27912.

4.1.15. 2-Carbobenzyloxyamino-1,3-di-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)-propane (21)

Compound **21** was prepared by the same procedure as described for the preparation of **20** using 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl acetate **3** as donor and the crude product was purified by chromatography with 5:2 petroleum ether (60–90 °C)–acetone as eluent to afford **21** as a yellow syrup in 52% yield; $[\alpha]_D^{25} = -9.8$ (c 0.82 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.35–7.31 (m, 5H, Ph), 5.37 (d, 2H, H-4), 5.27 (d, 2H, PhCH_2O), 5.16 (dd, 2H; H-2), 5.00 (dd, 2H, H-3), 4.45 (d, $J_{1,2'}$ = 7.5 Hz, 2H; H-1), 4.18–3.57 (m, 9H; H-5, H-6a,b, CH_2O, CHNH), 2.15–1.97 (4s, 24H; CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.3, 170.2, 170.0, 169.4, 155.8 (CO), 136.3, 128.5, 128.2 (Ph), 101.7 (C-1), 70.8 (C-5′), 70.6 (C-3′), 68.9 (CH_2O), 68.8 (C-2′), 66.9 (C-4′), 66.8 (PhCH_2O), 61.2 (C-6′), 50.2 (CHNH), 20.6, 20.5 (CH_3); HRESI-TOF MS: m/z calcd for $C_{39}H_{51}NO_{22}$: 908.28004 $[M+Na]^+$; found: 908.27971.

4.1.16. 2-Carbobenzyloxyamino-1,3-di-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(14)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy]-propane (22)

Compound **22** was prepared by the same procedure as described for the preparation of **20** using 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(14)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl acetate **4** as donor and the crude product was purified by chromatography with 3:2 petroleum ether (60–90 °C)–acetone as eluent to afford **22** as a yellow syrup in 48% yield; $[\alpha]_D^{25} = -15.0$ (c 0.80 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.35–7.31 (m, 5H, Ph), 5.28 (d, 2H, PhCH_2O), 5.13 (t, 2H, H-3′), 5.06 (t, 2H; H-2′), 4.90 (t, 2H, H-3), 4.85 (t, 2H; H-2), 4.50 (d, $J_{1,2}$ = 7.9 Hz, 4H; H-1, H-1′), 4.46–4.01 (m, 8H; H-6a,b, H-6a′,b′), 3.80–3.49 (m, 13H; H-4,5, H-4′,5′, CH_2O, CHNH), 2.10–1.97 (7s, 42H; CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.4, 170.3, 170.2, 169.7, 169.6,169.3, 169.0, 155.7 (CO), 136.3, 128.5, 128.2, 128.1 (Ph), 101.0 (C-1′), 100.8 (C-1), 76.3,76.2 (C-4, C-4′), 72.9, 72.7 (C-3, C-3′), 72.3,72.1 (C-5, C-5′), 71.5, 70.9 (C- 2, C-2′), 67.8 66.8 (CH_2O, PhCH_2O), 61.7, 61.5 (C-6, C-6′), 50.1 (CHNH), 20.7, 20.6, 20.5, 20.4 (CH_3); HRESI-TOF MS: m/z calcd for $C_{63}H_{83}NO_{38}$: 1484.44908 $[M+Na]^+$; found: 1484.44526.

4.1.17. 2-Amino-1,3-di-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-propane (23)

Compound **20** (0.2 g, 0.23 mmol) was dissolved in MeOH (10 mL), Pd/C was added, and the mixture was stirred under 0.4 MPa H_2 atmosphere for 6 h at room temperature. The mixture was then filtered over Celite and the filtrate was concentrated under reduced pressure. The crude product was purified by chromatography with (2:3) petroleum ether (60–90 °C)–acetone as eluent. 0.16 g Colorless syrup was obtained in 95% yield; $[\alpha]_D^{25} = -11.8$ (c 0.68 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.22 (t, 2H; H-3), 5.08 (t, 2H; H-2), 4.61 (d, $J_{1,2}$ = 7.5 Hz, 2H; H-1), 4.65, 4.28–4.02 (m, 4H; H-6a,b), 3.87–3.67 (m, 9H; H-4,5, CH_2O, CHNH$_2$), 2.08–2.00 (4s, 24H; CH_3CO); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 170.6, 170.0, 169.3 (CO), 101.1 (C-1), 76.8 (C-4), 72.6 (C-3), 72.5 (C-5), 71.5 (C-2), 68.1 (CH_2O), 61.6 (C-6), 53.9 (CHNH$_2$), 20.7, 20.6, 20.5 (CH_3); HRESI-TOF MS: m/z calcd for $C_{31}H_{45}NO_{20}$: 752.26132 $[M+1]^+$; found: 752.26032.

4.1.18. 2-Amino-1,3-di-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)-propane (24)

Compound **24** was prepared by the same procedure as described for the preparation of **23**. The yield was 96%; $[\alpha]_D^{25} = -11.6$ (c 0.82 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.37 (d, 2H; H-4), 5.14 (dd, 2H; H-2), 5.00 (dd, 2H; H-3), 4.44 (d, $J_{1,2'}$ = 7.5 Hz, 2H; H-1), 4.47, 4.17–4.04 (m, 4H; H-6a,b), 3.92–3.43(m, 7H; H-5, CH_2O, CHNH$_2$), 2.14–1.97 (4s, 24H; CH_3CO); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 170.4, 170.2, 170.1, 169.4 (CO), 101.5 (C-1), 70.7 (C-5), 70.6 (C-3), 69.0 (CH_2O), 68.9 (C-2), 67.0 (C-4), 61.2 (C-6), 50.7 (CHNH$_2$), 20.8, 20.7, 20.6, 20.5 (CH_3); HRESI-TOF MS: m/z calcd for $C_{31}H_{45}NO_{20}$: 752.26132 $[M+1]^+$; found: 752.26018.

4.1.19. 2-Amino-1,3-di-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(14)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy]-propane (25)

Compound **25** was prepared by the same procedure as described for the preparation of **23**. The yield was 93%; $[\alpha]_D^{25} = -15.7$ (c 1.78 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$):

δ = 5.13 (dd, 2H, H-3′), 5.05 (t, 2H; H-2′), 4.90 (t, 2H, H-3), 4.86 (dd, 2H; H-2), 4.48 (d, $J_{1,2}$ = 7.8 Hz, 4H; H-1, H-1′), 4.53, 4.39–4.01 (m, 8H; H-6a,b, H-6a′,b′), 3.80–3.65 (m, 13H; H-4,5, H-4′,5′, CH_2O, $CHNH_2$), 2.14–1.97 (7s, 42H; CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.5, 170.4, 170.2, 169.8, 169.7,169.2, 169.0 (CO), 100.9, 100.6 (C-1, C-1′), 76.0 (C-4, C-4′), 73.0, 72.9 (C-3, C-3′), 72.2,71.9 (C-5, C-5′), 71.6, 71.4 (C-2, C-2′), 67.8 (CH_2O), 61.5 (C-6, C-6′), 50.9 ($CHNH_2$), 20.9, 20.6, 20.4 (CH_3); HRESI-TOF MS: *m*/*z* calcd for $C_{55}H_{77}NO_{36}$: 1328.43035[*M*+1]$^+$; found: 1328.42708.

4.1.20. *N*-{2-[1,3-Di-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyran osyloxy)-propanyl]}-2-carbobenzyloxyamino- pentane-1, 5-diamide (26)

N-Carbobenzyloxy-L-glutamic acid (0.03 g, 0.11 mmol) was dissolved in anhydrous THF (1 mL) that was cooled to 0 °C, then HOBt (0.05 g, 0.37 mmol) and DCC (0.08 g, 0.39 mmol) were added. The mixture was stirred for 0.5 h at 0 °C. A solution (pH 8–9) of compound **23** (0.17 g, 0.22 mmol) in dry THF (2 mL) in the presence of NMM was added dropwise and the mixture was stirred at 0 °C for 2 h, followed by stirring at room temperature for 36 h. The mixture was concentrated and the residue was then diluted with CH_2Cl_2 (10 mL). The solution was filtered and the filtrate was washed with aqueous sodium bicarbonate, aqueous citric acid and water, dried (Na_2SO_4), and evaporated under diminished pressure. The crude product was purified by flash chromatography with 1:1 petroleum ether (60–90 °C)–acetone as eluent to afford **26** (0.16 g) as a colorless syrup in 85% yield; $[\alpha]_D^{25} = -21.3$ (*c* 0.75 in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ = 7.37–7.32 (m, 5H; Ph), 6.29 (d, 1H; NH), 6.11 (d, 1H; NH), 5.24–5.07 (m, 6H, PhC*H*$_2$O, H-3), 5.05–4.94 (m, 4H; H-2), 4.51 (d, $J_{1,2}$ = 7.8 Hz, 4H; H-1), 4.58, 4.31–4.21 (m, 8H; H-6a,b), 4.17–3.59 (m, 19H; H-4,5, CH_2O, COC*H*NH, C*H*NHCO), 2.10–2.00 (m, 52H; CH_3CO, $COCH_2$, C*H*$_2$CHNH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 170.7, 170.6, 170.1, 169.4, 169.3, 169.2 (CO), 136.4, 128.5, 128.2, 128.0, 127.9 (Ph), 101.2, 100.9 (C-1), 76.7 (C-4), 72.7 (C-3), 72.5 (C-5), 71.9, 71.3 (C-2), 68.3, 68.2 (CH_2O), 66.8 (PhCH_2O), 61.8, 61.7 (C-6), 53.8 48.8 (*C*HNHCO), 31.7, 29.3 (*C*H_2CO, *C*H_2CHNH), 20.7, 20.6, 20.5 (*C*H_3); HRESI-TOF MS: *m*/*z* calcd for $C_{75}H_{101}N_3O_{44}$: 1748.58362 [*M*+1]$^+$; found: 1748.58307.

4.1.21. *N*-{2-[1,3-Di-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopy ranosyloxy)-propanyl]}-2-carbobenzyloxyamino-pentane-1, 5-diamide (27)

Compound **27** was prepared by the same procedure as described for the preparation of **26** using compound **24** as the linking unit and the crude product was purified by chromatography with 1:1 petroleum ether (60–90 °C)–acetone as eluent to afford **27** as a colorless syrup in 80% yield; $[\alpha]_D = -16.2$ (*c* 0.74 in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ = 7.36–7.34 (m, 5H; Ph), 6,30 (d, 1H; NH), 6.00 (d, 1H; NH), 5.38 (d, 4H; H-4), 5.18–5.11 (m, 6H; PhC*H*$_2$O, H-2), 5.05–5.00 (m, 4H; H-3), 4.47 (d, $J_{1,2'}$ = 7.8 Hz, 4H; H-1′), 4.50, 4.20–4.10 (m, 8H; H-6a,b), 3.97–3.59 (m, 15H; H-5, CH_2O, COC*H*NH$_2$, C*H*NHCO), 2.16–1.98 (m, 52H; CH_3CO, $COCH_2$, C*H*$_2$CHNH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.4, 170.3, 170.2, 170.1, 170.0, 169.7, 169.6 (CO), 136.3, 128.5, 128.1, 127.9 (Ph), 101.7, 101.6, 101.4 (C-1), 70.8, 70.7 (C-5), 70.6 (C-3), 69.1, 68.9 (C-2, CH_2O), 67.0, 66.9 (C-4, Ph*C*H$_2$O), 61.1 (C-6), 48.9, 48.5 (*C*HNHCO), 31.7, 29.2 (*C*H_2CO, *C*H_2CHNH), 20.8, 20.7, 20.6, 20.5 (*C*H_3); HRESI-TOF MS: *m*/*z* calcd for $C_{75}H_{101}N_3O_{44}$: 1748.58362 [*M*+1]$^+$; found: 1748.58289.

4.1.22. *N*-{2-[1,3-Di-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(14)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy)]-propanyl}-2-carbobenzyloxyamino-pentane-1,5-diamide (28)

Compound **28** was prepared by the same procedure as described for the preparation of **26** using compound **25** as the linking unit and the crude product was purified by chromatography with 1:1 petroleum ether (60–90 °C)–acetone as eluent to afford **28** as a colorless syrup in 88% yield; $[\alpha]_D = -6.6$ (*c* 1.22 in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ = 7.35–7.34 (m, 5H; Ph), 6,30 (d, 1H; NH), 6.00 (d, 1H; NH), 5.19–5.13 (m, 6H; PhC*H*$_2$O, H-3′), 5.07 (t, 4H; H-2′), 4.92 (t, 4H; H-3), 4.85 (t, 4H; H-2), 4.42 (d, $J_{1,2}$ = 8.0 Hz, 8H; H-1, H-1′), 4.53, 4.38–4.35 (m, 16H; H-6a,b, H-6a′,b′), 4.13–3.51 (m, 27H; H-4,5, H-4′,5′, CH_2O, COC*H*NH$_2$, C*H*NHCO), 2.18–1.98 (m, 88H; CH_3CO, $COCH_2$, C*H*$_2$CHNH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.4, 170.2, 169.7, 169.3, 169.0 (CO), 128.5, 128.1, 127.9 (Ph), 100.7 (C-1, C-1′), 76.4 (C-4, C-4′), 72.9, 72.8 (C-3, C-3′), 72.3 (C-5, C-5′), 71.9, 71.6 (C-2, C-2′), 67.7, 66.8 (CH_2O, Ph*C*H$_2$O), 61.5 (C-6, C-6′), 53.8 (*C*HNHCO), 31.7, 29.2 (*C*H_2CO, *C*H_2CHNH), 20.8, 20.6, 20.5 (*C*H_3); HRESI-TOF MS: *m*/*z* calcd for $C_{123}H_{165}N_3O_{76}$: 2901.92504 [*M*+1]$^+$; found: 2901.92480.

4.1.23. *N*-{2-[1,3-Di-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyran osyloxy)]-propanyl}-2-amino-pentane-1,5-diamide (29)

Compound **26** (0.1 g, 0.06 mmol) was dissolved in MeOH (10 mL), Pd/C was added, and the mixture was stirred under 0.4 MPa H_2 atmosphere for 6 h at room temperature. The mixture was then filtered over celite and the filtrate was concentrated under reduced pressure. The crude product was purified by chromatography with (1:2) petroleum ether (60–90 °C)–acetone as eluent. 0.08 g colorless syrup was obtained in 90% yield; $[\alpha]_D^{25} = -12.1$ (*c* 0.99 in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ = 5.07 (t, 8H; H-2, H-3), 4.46 (d, $J_{1,2}$ = 7.5 Hz, 4H; H-1), 4.22–3.68 (m, 27H; H-4,5,6a,b, CH_2O, COC*H*NH, C*H*NH$_2$), 2.10–1.92 (m, 52H; CH_3CO, $COCH_2$, C*H*$_2$CHNH$_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.8, 170.7, 170.1, 169.7, 169.4 (CO), 101.3, 100.8 (C-1), 76.7 (C-4), 72.6 (C-3), 72.0 (C-5), 71.3 (C-2), 68.3 (CH_2O), 61.9 (C-6), 53.8 (*C*HNHCO), 31.7, 29.3 (*C*H_2CO, *C*H_2CHNH$_2$), 20.8, 20.5 (*C*H_3); HRESI-TOF MS: *m*/*z* calcd for $C_{67}H_{95}N_3O_{42}$: 1614.54684 [*M*+1]$^+$; found: 1614.54614.

4.1.24. *N*-{2-[1,3-Di-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)]-propanyl}-2-amino-pentane-1,5-diamide (30)

Compound **30** was prepared by the same procedure as described for the preparation of **29.** The yield was 95%; $[\alpha]_D^{25} = -7.7$ (*c* 1.04 in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ = 5.38 (d, 4H; H-4), 5.14 (m, 4H; H-2), 5.01 (m, 4H; H-3), 4.47 (d, $J_{1,2}$ = 7.6 Hz, 4H; H-1), 4.50, 4.20–4.08 (m, 8H; H-6a,b), 4.00–3.55 (m, 15H; H-5, CH_2O, COC*H*NH, C*H*NH$_2$), 2.16–1.97 (m, 52H; CH_3CO, $COCH_2$, C*H*$_2$CHNH$_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.4, 170.3, 170.2, 170.1, 170.0, 169.6, 169.5, 169.4 (CO), 101.6, 101.5 (C-1), 70.7 (C-5), 70.5 (C-3), 68.9, 68.0 (C-2, CH_2O), 67.0 (C-4), 61.1 (C-6), 53.8, 48.4 (*C*HNHCO), 31.7, 29.2 (*C*H_2CO, *C*H_2CHNH$_2$), 20.7, 20.6, 20.5 (*C*H_3); HRESI-TOF MS: *m*/*z* calcd for $C_{67}H_{95}N_3O_{42}$: 1614.54684 [*M*+1]$^+$; found: 1614.54639.

4.1.25. *N*-{2-[1,3-Di-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(14)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy)]-propanyl}-2-amino-pentane-1,5-diamide (31)

Compound **31** was prepared by the same procedure as described for the preparation of **29.** The yield was 88%; $[\alpha]_D^{25} = -17.9$ (*c* 0.67 in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ = 5.16 (t, 4H; H-3′), 5.08 (t, 4H; H-2′), 4.92 (t, 4H; H-3), 4.83 (t, 4H; H-2), 4.54 (d, $J_{1,2} = J_{1',2'}$ = 7.7 Hz, 8H; H-1, H-1′), 4.52–4.01 (m, 16H; H-6a,b, H-6a′,b′), 3.79–3.51 (m, 27H; H-4,5, H-4′,5′, CH_2O, COC*H*NH, C*H*NH$_2$), 2.16–1.96 (m, 88H; CH_3CO, $COCH_2$, C*H*$_2$CHNH$_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.4, 170.1, 169.7, 169.2, 169.0 (CO), 100.6. (C-1, C-1′), 76.2 (C-4, C-4′), 72.9 (C-3, C-5), 71.9 (C-2, C-2′), 71.6, 71.5 (C-3′, C-5′), 67.8 (CH_2O), 61.8, 61.5 (C-6, C-6′), 53.8 (*C*HNHCO), 31.7, 29.2 (*C*H_2CO, *C*H_2CHNH$_2$), 20.9, 20.6, 20.5

(CH_3); HRESI-TOF MS: *m*/*z* calcd for $C_{115}H_{159}N_3O_{74}$: 2767.88826 $[M+1]^+$; found: 2767.88771.

4.1.26. *N*-{2-[1,3-Di-(*O*-β-D-glucopyranosyloxy)]-propanyl}-2-amino-pentane-1,5-diamide (32)

Compound **32** was prepared by the same procedure as described for the preparation of **14**. The yield was 95%; $[\alpha]_D^{25} = -25.9$ (*c* 1.7 in H_2O); ^1H NMR (400 MHz, D_2O): $\delta = 4.35$ (d, $J_{1,2} = 7.8$ Hz, 4H; H-1), 4.32–3.11 (m, 34H; H-2,3,4,5,6a,b, CH_2O, C*H*NH), 2.90–2.03 (m, 5H; CH_2CO, CH_2CHNH_2); ^{13}C NMR (100 MHz, D_2O): $\delta = 173.6$, 167.6 (CO), 102.8, 102.6 (C-1), 75.2, 72.7, 70.8, 68.7 (C-2,3,4,5, CH_2O), 61.1 (C-6), 48.9 (CHNH_2), 30.8 (CH_2CHNH_2), 23.3 (CH_2CO); HRESI-TOF MS: *m*/*z* calcd for $C_{35}H_{63}N_3O_{26}$: 942.37780 $[M+1]^+$; found: 942.37814.

4.1.27. *N*-{2-[1,3-Di-(*O*-β-D-galactopyranosyloxy)-propanyl]}-2-amino-pentane-1,5-diamide (33)

Compound **33** was prepared by the same procedure as described for the preparation of **14**. The yield was 95%; $[\alpha]_D^{25} = -53.7$ (*c* 0.67 in H_2O); ^1H NMR (400 MHz, D_2O): $\delta = 4.28$ (d, $J_{1,2} = 7.8$ Hz, 4H; H-1), 4.20–3.39 (m, 34H; H-2,3,4,5,6a,b, CH_2O, C*H*NH), 2.35–1.80 (m, 5H; CH_2CO, CH_2CHNH_2); ^{13}C NMR (100 MHz, D_2O): $\delta = 174.8$, 174.5 (CO), 103.2 (C-1), 76.0 (C-4), 75.7, 73.1, 69.7, 68.6 (C-2,3,5, CH_2O), 60.8 (C-6), 48.9 (CHNH_2), 30.8 (CH_2CHNH_2), 23.8 (CH_2CO); HRESI-TOF MS: *m*/*z* calcd for $C_{35}H_{63}N_3O_{26}$: 942.37780 $[M+1]^+$; found: 942.37835.

4.1.28. *N*-{2-[1,3-Di-(*O*-β-D-glucopyranosyl-(14)-β-D-glucopyranosyloxy)]-propanyl}-2-amino-pentane-1,5-diamide (34)

Compound **34** was prepared by the same procedure as described for the preparation of **14**. The yield was 93%; $[\alpha]_D^{25} = -90.6$ (*c* 1.06 in H_2O); ^1H NMR (400 MHz, D_2O): $\delta = 4.42$ (d, $J_{1,2} = J_{1',2'} = 7.8$ Hz, 8H; H-1, H-1′), 3.91–3.21 (m, 58H; H-2,3,4,5,6a,b, H-2′,3′,4′,5′,6a′,b′, CH_2O, C*H*NH), 2.94–1.96 (m, 4H; CH_2CO, CH_2CHNH_2); ^{13}C NMR (100 MHz, D_2O): $\delta = 174.2$ (CO), 102.6, 102.5, 102.4 (C-1, C-1′), 79.0, 78.8 (C-4, C-4′), 76.1, 75.6, 74.8, 74.3, 73.2, 72.9, 69.5, 68.8, 68.6 (C-2,3,5, C-2′,3′,5′, CH_2O), 60.7, 60.2, 60.1 (C-6, C-6′), 53.0, 49.3 (CHNH), 31.1, 23.3 (CH_2CO, CH_2CHNH_2); HRESI-TOF MS: *m*/*z* calcd for $C_{59}H_{103}N_3O_{46}$: 1590.58910 $[M+1]^+$; found: 1590.58890.

4.1.29. 2-Carbobenzyloxyamino-3-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(14)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy]-1-propanol (35)

To a solution of 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(14)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl acetate **1** (2 g, 2.95 mmol) and 2-carbobenzyloxyamino-1,3-propandiol (0.66 g, 2.95 mmol) in dry CH_2Cl_2 (10 mL), $BF_3{\cdot}OEt_2$ (0.04 mL) was added and the mixture was stirred at room temperature for 24 h. The mixture was then diluted with CH_2Cl_2 (20 mL) and washed with aqueous sodium bicarbonate and water, dried (Na_2SO_4), and evaporated under diminished pressure. The crude product was purified by flash chromatography with 3:2 petroleum ether (60–90 °C)–acetone as eluent to afford 0.75 g of **35** as a colorless syrup in 30% yield; $[\alpha]_D^{25} = +4.7$ (*c* 0.85 in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.34$ (m, 5H; Ph), 5.35 (d, 1H; H-4′), 5.26 (d, 2H, PhCH_2-O),5.17 (dd, 1H; H-3), 5.11 (dd, 1H; H-2′), 4.95 (dd, 1H; H-3′), 4.87 (t, 1H; H-2), 4.48 (d, $J_{1,2} = J_{1',2'} = 8.0$ Hz, 2H; H-1, H-1′), 4.55, 4.17–4.03 (m, 4H; H-6a,b, H-6a′,b′), 3.89–3.59 (m, 8H; H-4,5, H-5′, CH_2O, C*H*NH), 2.17–1.97 (7s, 21H; CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 170.3$, 170.1, 170.0, 169.9, 169.7, 169.6, 169.0(CO), 101.1 (C-1′), 100.6 (C-1), 76.1 (C-4), 72.8 (C-3), 72.5 (C-5), 72.4 (C-2), 71.4 (C-5′), 70.9(C-3′), 70.7, 69.1, 68.9 (CH_2O, PhCH_2O), 66.9 (C-2′), 66.6 (C-4′), 61.7 (C-6′), 60.8 (C-6), 51.8 (CHNH), 20.7, 20.6, 20.5 (CH_3); HRESI-TOF MS: *m*/*z* calcd for $C_{37}H_{49}NO_{21}$: 866.26948 $[M+Na]^+$; found: 866.26788.

4.1.30. 2-Carbobenzyloxyamino-1-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(14)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy]-3-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)-propane (36)

2,3,4,6-Tetra-*O*-acetyl-D-mannopyranosyl trichloroacetimidate **5** (1 g, 2.03 mmol) and compound **35** (1.14 g, 1.35 mmol) were dissolved in dry CH_2Cl_2 (3 mL) that was cooled to −20 °C, then TMSOTf (0.015 mL) was added and the mixture was stirred at room temperature for 24 h. The mixture was then diluted with CH_2Cl_2 (20 mL) and washed with aqueous sodium bicarbonate and water, dried (Na_2SO_4), and evaporated under diminished pressure. The crude product was purified by flash chromatography with 3:2 petroleum ether (60–90 °C)–acetone as eluent to afford 1.11 g of **36** as a colorless syrup in 70% yield; $[\alpha]_D^{25} = +8.3$ (*c* 0.98 in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.28$ (m, 5H; Ph), 5.27 (m, 1H; H-4′), 5.24–5.00 (m, 5H; H-3, H-2′, H-3″, PhCH_2O), 4.88 (dd, 1H; H-3′), 4.80–4.74 (m, 2H; H-2, H-1″), 4.41 (d, $J_{1,2} = J_{1',2'} = 7.6$ Hz, 2H; H-1, H-1′), 4.41, 4.25–4.00 (m, 7H; H-2″, H-6a,b, H-6a′,b′, H-6a″,b″), 3.88–3.37 (m, 9H; H-4,5, H-5′, H-4″,5″, CH_2O), 2.08–1.88 (8s, 33H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 170.5$, 170.3, 170.2, 170.0, 169.9, 169.8, 169.6, 169.5, 169.4, 169.3, 168.9 (CO), 136.2, 128.4, 128.2, 128.1, 128.0 (Ph), 101.0 (C-1′), 100.9 (C-1), 97.5 (C-1″), 76.1 (C-4), 76.0 (C-4″), 72.8 (C-3), 72.5 (C-5), 72.4 (C-2), 71.4 (C-3″), 70.9 (C-5′), 70.7 (C-3′), 69.6 (C-2″), 69.2, 69.0, 68.6 (CH_2O, PhCH_2O), 66.8 (C-2′), 66.6 (C-5″), 65.8 (C-4′), 62.2 (C-6′), 61.8 (C-6″), 60.8 (C-6), 49.8 (CHNH), 20.7, 20.6, 20.5 (CH_3); HRESI-TOF MS: *m*/*z* calcd for $C_{51}H_{67}NO_{30}$: 1196.36456 $[M+Na]^+$; found: 1196.36432.

4.1.31. 2-Amino-1-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(14)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy]-3-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)-propane (37)

Compound **37** was prepared by the same procedure as described for the preparation of **23**. The yield was 95%; $[\alpha]_D^{25} = +8.5$ (*c* 0.96 in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 5.36$ (d, 2H; H-4′), 5.30–5.00 (m, 5H; H-3, H-2′, H-3″, PhCH_2O), 4.93–4.61 (m, 3H; H-3′, H-2, H-1″), 4.56 (d, $J_{1,2} = J_{1',2'} = 7.3$ Hz, 2H; H-1, H-1′), 4.58, 4.26–4.00 (m, 7H; H-2″, H-6a,b, H-6a′,b′, H-6a″,b″), 3.97–3.39 (m, 10H; H-4,5, H-5′, H-4″,5″, CH_2O, C*H*NH), 2.23–1.98 (11s, 33H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 170.5$, 170.2, 170.1, 170.0, 169.5, 169.4, 169.0 (CO), 100.9 (C-1′), 100.8 (C-1), 97.5 (C-1″), 75.8 (C-4, C-4″), 73.0 (C-3), 72.6 (C-5), 71.3 (C-2), 70.9 (C-3″), 70.6 (C-5′), 70.2 (C-3′), 69.1 (C-2″, CH_2O), 66.6 (C-2′, C-5″), 65.5 (C-4′), 62.2 (C-6′, C-6″), 60.7 (C-6), 45.8 (CHNH_2), 20.7, 20.6, 20.4 (CH_3); HRESI-TOF MS: *m*/*z* calcd for $C_{43}H_{61}NO_{28}$: 1040.34584 $[M+Na]^+$; found: 1040.34420.

4.1.32. *N*-{2-[1-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(14)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy)-3-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)]-propanyl}-pentane-1,5-diamide (38)

Compound **38** was prepared by the same procedure as described for the preparation of **9** using pentanedioic acid as the linker and the crude product was purified by chromatography with 1:1 petroleum ether (60–90 °C)–acetone as eluent to afford **38** as a colorless syrup in 80% yield; $[\alpha]_D^{25} = +15.4$ (*c* 1.04 in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 6.13$ (d, 1H; NHCO), 6.04 (d, 1H; NHCO), 5.35 (d, 2H; H-4′), 5.29–5.07 (m, 6H; H-3, H-2′, H-3″), 4.98 (dd, 2H; H-3′), 4.87–4.81 (m, 4H; H-2, H-1″), 4.50 (d, $J_{1,2} = J_{1',2'} = 7.7$ Hz, 4H; H-1, H-1′), 4.55, 4.34–4.01 (m, 14H; H-2″, H-6a,b, H-6a′,b′, H-6a″,b″), 3.91–3.40 (m, 20H; H-4,5, H-5′, H-4″,5″, CH_2O, C*H*NH), 2.18–1.97 (m, 70H; CH_3, CH_2CO), 1.72 (m, 2H; CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 170.7$, 170.6, 170.4, 170.3, 170.1 170.0, 169.7, 169.6, 169.5, 169.0 (CO), 101.1 (C-1′), 100.9 (C-1), 98.0 (C-1″), 76.1 (C-4), 76.0 (C-4″), 72.9 (C-3), 72.5 (C-5), 71.6 (C-2), 71.5 (C-3″), 70.9 (C-5′), 70.6 (C-3′), 69.2 (C-2″), 69.0,

68.7 (CH_2O), 66.6, 65.9 (C-2′, C-5″), 65.8 (C-4′), 62.2 (C-6′), 61.6 (C-6″), 60.7 (C-6), 53.8 (*C*HNHCO), 31.7 (*C*H_2CO), 29.2 (CH_2), 20.8, 20.7, 20.6, 20.5 (CH_3); HRESI-TOF MS: *m*/*z* calcd for $C_{91}H_{126}N_2O_{58}$: 2197.68692 $[M+Na]^+$; found: 2197.68481.

4.1.33. *N*-{2-[1-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(14)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy)-3-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)]-propanyl}-2-carbobenzyloxyamino-pent- ane-1,5-diamide (39)

Compound **39** was prepared by the same procedure as described for the preparation of **9** using *N*-carbobenzyloxy-L-glutamic acid as the linker and the crude product was purified by chromatography with 1:1 petroleum ether (60–90 °C)–acetone as eluent to afford **39** as a colorless syrup in 85% yield; $[\alpha]_D^{25} = +16.0$ (*c* 1.00 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.32 (m, 5H; Ph), 6,10 (d, 1H; NH), 6.00 (d, 1H; NH), 5.32 (d, 2H; H-4′), 5.29–5.05 (m, 8H; H-3, H-2′, H-3″, PhCH_2O), 4.93 (dd, 2H; H-3′), 4.85–4.80 (m, 4H; H-2, H-1″), 4.47 (d, $J_{1,2} = J_{1',2'}$ = 7.8 Hz, 4H; H-1, H-1′), 4.48, 4.28–4.03 (m, 14H; H-2″, H-6a,b, H-6a′,b′, H-6a″,b″), 3.87–3.52 (m, 20H; H-4,5, H-5′, H-4″,5″, CH_2O, *C*HNH), 2.30–1.69 (m, 70H; CH_2, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.7, 170.6, 170.4, 170.3, 170.1 170.0, 169.7, 169.6, 169.5, 169.4, 169.0 (CO), 136.4, 128.5, 128.1, 127.9, 127.8 (Ph), 101.1 (C-1′), 100.6 (C-1), 97.4 (C-1″), 76.4 (C-4), 76.2 (C-4″), 72.8 (C-3), 72.6 (C-5), 71.5 (C-2), 71.0 (C-3″), 70.7 (C-5′), 70.1 (C-3′), 69.5, 69.1 (C-2″), 69.0, 68.6, 68.2, 67.8 (CH_2O, PhCH_2O), 66.8, 66.6 (C-2′), (C-5″), 65.7, 65.6 (C-4′), 62.4, 62.2 (C-6′), 61.9, 61.8 (C-6″), 60.7 (C-6), 53.8, 48.3 (*C*HNHCO), 31.7, 29.2 (*C*H_2CO, *C*H_2CHNH_2), 20.8, 20.7, 20.6, 20.5 (CH_3); HRESI-TOF MS: *m*/*z* calcd for $C_{99}H_{133}N_3O_{60}$: 2325.75601 $[M+1]^+$; found: 2325.75546.

4.1.34. *N*-{2-[1-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(14)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy)-3-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)]-propanyl}-2-amino-pentane-1,5-diamide (40)

Compound **40** was prepared by the same procedure as described for the preparation of **23**. The yield was 94%; $[\alpha]_D^{25} = +9.1$ (*c* 0.88 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6,21 (d, 4H; NH), 5.27 (d, 4H; H-4′), 5.23–4.98 (m, 12H; H-3, H-2′, H-3″), 4.89 (dd, 4H; H-3′), 4.80–4.75 (m, 8H; H-2, H-1″), 4.45 (d, $J_{1,2}$ = $J_{1',2'}$ = 7.6 Hz, 8H; H-1, H-1′), 4.51, 4.28–4.00 (m, 28H; H-2″, H-6a,b, H-6a′,b′, H-6a″,b″), 3.91–3.42 (m, 40H; H-4,5, H-5′, H-4″,5″, CH_2O, C*H*NH), 2.88–1.83 (m, 140H; CH_2CO, CH_3), 1.80–1.23 (m, 8H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.6, 170.4, 170.3, 170.1, 170.0, 169.8, 169.7, 169.6, 169.5, 169.0 (CO), 101.1 (C-1′), 100.9, 100.5 (C-1), 98.0, 97.8, 97.6, 97.4 (C-1″), 76.1, 76.0 (C-4, C-4″), 72.8 (C-3), 72.5 (C-5), 71.6 (C-2), 71.5 (C-3″), 70.9 (C-5′), 70.6 (C-3′), 69.4, 69.1,69.0 (C-2″), 68.7,68.6,68.2 (CH_2O), 66.6, (C-2′), 66.1 (C-5″), 65.7 (C-4′), 62.1 (C-6′), 61.7 (C-6″), 60.7 (C-6), 53.8, 52.1, 48.4, 41.9 (*C*HNHCO), 36.2, 31.7, 30.1, 29.6, 29.2, 28.9, 26.9 (*C*H_2CO, *C*H_2CHNH_2), 25.4, 24.9 (CH_2), 20.8, 20.7, 20.6, 20.4 (CH_3); HRESI-TOF MS: *m*/*z* calcd for $C_{91}H_{127}N_3O_{58}$: 2190.71587 $[M+1]^+$; found: 2190.71386.

4.1.35. *N*-{2-[1-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(14)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy)-3-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)]-propanyl}-benzene-1,3,5-triamide (41)

Compound **41** was prepared by the same procedure as described for the preparation of **9** using benzene-1,3,5-tricarboxylic acid as the linker and the crude product was purified by chromatography with 1:1 petroleum ether (60–90 °C)–acetone as eluent to afford **41** as a colorless syrup in 70% yield; $[\alpha]_D^{25} = +16.3$ (*c* 0.95 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.40 (3H; Ph), 7.44 (d, 2H; NHCO), 7.26 (d, 1H; NHCO), 5.35 (d, 3H; H-4′), 5.30–5.01 (m, 9H; H-3, H-2′, H-3″), 4.96 (dd, 3H; H-3), 4.91–4.86 (m, 6H; H-2, H-1″), 4.51 (d, $J_{1,2} = J_{1',2'}$ = 8.1 Hz, 6H; H-1, H-1′), 4.59, 4.46–4.06 (m, 21H; H-2″, H-6a,b, H-6a′,b′, H-6a″,b″), 4.00–3.57 (m, 30H; H-4,5, H-5′, H-4″,5″, CH_2O, C*H*NH), 2.15–1.95 (11s, 99H; CH_3-CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.7, 170.5, 170.4, 170.3, 170.2, 170.1 170.0, 169.9, 169.7, 169.6, 169.0, 165.9, 165.8 (CO), 134.9, 134.8, 129.0, 128.2 (Ph), 101.1, 101.0 (C-1′), 100.9, 100.8 (C-1), 97.7, 97.5 (C-1″), 76.1 (C-4, C-4″), 73.0 (C-3), 72.9 (C-5), 72.5 (C-2), 71.6 (C-3″), 70.9 (C-5′), 70.6 (C-3′), 69.2 (C-2″), 69.1, 68.9, 68.7 (CH_2O), 66.6, 65.8 (C-2′, C-5″), 65.7 (C-4′), 62.2 (C-6′), 61.8 (C-6″), 60.7 (C-6), 49.2, 48.9, 41.9 (*C*HNHCO), 20.8, 20.7, 20.6, 20.5 (CH_3); HRESI-TOF MS: *m*/*z* calcd for $C_{138}H_{183}N_3O_{87}$: 3276.00995 $[M+1]^+$; found: 3276.00940.

4.1.36. *N*-{2-*N*-{2-[1-(2,3,4,6-Tetra-*O*-acetyl-β-D-galacto pyranosyl-(14)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy)-3-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)] propanyl}pentane-1,5-diamidyl}-octane-1,8-diamide (42)

Compound **42** was prepared by the same procedure as described for the preparation of **9** using octanedioic acid as the linker and compound **41** as the linking unit. The crude product was purified by chromatography with 1:2 petroleum ether (60–90 °C)–acetone as eluent to afford **42** as a colorless syrup in 50% yield; $[\alpha]_D^{25} = +7.0$ (*c* 1.15 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.34 (d, 2H; H-4′), 5.30–5.06 (m, 6H; H-3, H-2′, H-3″), 4.95 (dd, 2H; H-3′), 4.82–4.80 (m, 4H; H-2, H-1″), 4.51 (d, $J_{1,2} = J_{1',2'}$ = 7.3 Hz, 4H; H-1, H-1′), 4.57, 4.32–4.05 (m, 14H; H-2″, H-6a,b, H-6a′,b′, H-6a″,b″), 3.88–3.50 (m, 19H; H-4,5, H-5′, H-4″,5″, CH_2O, CHNH), 2.63–1.71 (m, 70H; CH_2, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.6, 170.5, 170.2, 170.0, 169.6, 169.4, 169.0 (CO), 101.1 (C-1′), 100.7 (C-1), 97.5 (C-1″), 76.2 (C-4, C-4″), 72.9 (C-3), 72.6 (C-5), 71.6 (C-2), 71.0 (C-3″), 70.7 (C-5′), 70.3 (C-3′), 69.5, 69.1 (C-2″), 68.8 (CH_2O), 66.6 (C-2′), 65.7 (C-5″), 65.6 (C-4′), 62.4, 62.2 (C-6′), 61.8 (C-6″), 60.7 (C-6), 53.9 (*C*HNHCO), 31.7, 29.3 (*C*H_2CO, *C*H_2CHNH_2), 20.8, 20.7, 20.6, 20.5, 20.4 (CH_3); HRESI-TOF MS: *m*/*z* calcd for $C_{190}H_{264}N_6O_{118}$: 2260.75327 $[M+2]^{2+}$; found: 2260.75342.

4.1.37. *N*-{2-[1-(*O*-β-D-Galactopyranosyl-(14)-β-D-glucopyranosyloxy)-3-(*O*-α-D-mannopyranosyloxy)]-propanyl}-pentane-1,5-diamide (43)

Compound **43** was prepared by the same procedure as described for the preparation of **14**. The yield was 95%; $[\alpha]_D^{25} = +15.6$ (*c* 1.02 in H_2O); ^{1}H NMR (400 MHz, D_2O): δ = 5.09, 4.96 (d, 2H; NHCO), 4.78 (s, 2H; H-1″), 4.37 (d, $J_{1,2}$ = 7.9 Hz, 2H; H-1), 4.34 (d, $J_{1',2'}$ = 7.8 Hz, 2H; H-1′), 3.92–3.41 (m, 46H; H-2,3,4,5,6a,b, H-2′,3′,4′,5′,6a′,b′, H-2″,3″,4″,5″,6a″,b″, CH_2O, C*H*NH), 2.22 (t, 4H; CH_2CO), 2.07 (m, 2H; CH_2); ^{13}C NMR (100 MHz, D_2O): δ = 173.8, 173.4 (CO), 102.9, 102.6, 102.4 (C-1, C-1′), 100.1, 99.7 (C-1″), 78.5 (C-4), 77.0 (C-4″), 75.3 (C-3), 74.8 (C-5), 74.3, 74.2 (C-2), 73.1, 73.0 (C-3″), 72.9, 72.8 (C-5′), 72.5, 72.4 (C-3′), 70.9, 70.5 (C-2″), 69.9, 68.7, 68.5 (CH_2O), 67.0, 66.8 (C-2′), 66.6 (C-5″), 66.3 (C-4′), 61.0, 60.9 (C-6′), 60.7, 60.5 (C-6″), 60.1 (C-6), 48.9 (*C*HNHCO), 35.0 (*C*H_2CO), 22.0, 21.9, 20.3 (CH_2); HRESI-TOF MS: *m*/*z* calcd for $C_{47}H_{82}N_2O_{36}$: 1273.45450 $[M+Na]^+$; found: 1273.45558.

4.1.38. *N*-{2-[1-(*O*-β-D-Galactopyranosyl-(14)-β-D-glucopyran osyloxy)-3-(*O*-α-D-mannopyranosyloxy)]-propanyl}-2-amino-pentane-1,5-diamide (44)

Compound **44** was prepared by the same procedure as described for the preparation of **14**. The yield was 94%; $[\alpha]_D^{25} = +29.6$ (*c* 1.00 in H_2O); ^{1}H NMR (400 MHz, D_2O): δ = 5.39, 5.12 (d, 2H; NHCO), 4.64 (s, 2H; H-1″), 4.23 (d, $J_{1,2} = J_{1',2'}$ = 7.3 Hz, 4H; H-1, H-1′), 3.78–2.51 (m, 47H; H-2,3,4,5,6a,b, H-2′,3′,4′,5′,6a′,b′, H-2″,3″,4″,5″,6a″,b″, CH_2O, C*H*NH), 2.24–1.45 (m, 4H; CH_2); ^{13}C NMR (100 MHz, D_2O): δ = 173.5 (CO), 103.0, 102.6 (C-1, C-1′),

99.8 (C-1″), 78.6 (C-4), 76.4 (C-4″), 75.4 (C-3), 74.8 (C-5), 74.3 (C-2), 73.1 (C-3″), 72.8 (C-5′), 72.6, 72.4, (C-3′), 70.7, 70.6 (C-2″), 70.0, 68.7, 68.6 (CH_2O), 67.1 (C-2′), 66.9 (C-5″), 66.7 (C-4′), 61.1 (C-6′), 60.8 (C-6″), 60.2 (C-6), 48.9 (*C*HNHCO), 30.3 (*C*H_2CO), 20.5, 20.4 (CH_2); HRESI-TOF MS: *m*/*z* calcd for $C_{47}H_{83}N_3O_{36}$: 1266.48345 $[M+Na]^+$; found: 1266.48584.

4.1.39. *N*-{2-[1-(*O*-β-D-Galactopyranosyl-(14)-β-D-glucopyranosyloxy)-3-(*O*-α-D-mannopyranosyloxy)]propanyl}-benzene-1,3,5-triamide (45)

Compound **45** was prepared by the same procedure as described for the preparation of **14**. The yield was 95%; $[\alpha]_D^{25} = +21.8$ (*c* 1.10 in H_2O); ^{1}H NMR (400 MHz, D_2O): $\delta = 8.19$ (s, 3H; Ph) 4.78 (s, 3H; H-1″), 4.41 (d, $J_{1,2} = 8.0$ Hz, 3H; H-1), 4.30 (d, $J_{1',2'} = 7.7$ Hz, 3H; H-1′), 4.03–3.21 (m, 84H; H-2,3,4,5,6a,b, H-2′,3′,4′,5′,6a′,b′, H-2″,3″,4″,5″,6a″,b″, CH_2O, C*H*NH); ^{13}C NMR (100 MHz, D_2O): $\delta = 169.1$ (CO), 134.9, 129.3 (Ph), 102.9, 102.7, 102.4 (C-1, C-1′), 100.2, 99.7 (C-1″), 78.4 (C-4, C-4″), 75.3 (C-3), 74.8 (C-5), 74.3 (C-2), 72.9 (C-3″), 72.8 (C-5′), 72.5 (C-3′), 70.9, 70.5 (C-2″), 70.0, 68.9, 68.5 (CH_2O), 66.6 (C-2′, C-5″), 66.3 (C-4′), 61.0 (C-6′), 60.8 (C-6″), 60.1 (C-6), 50.3 (*C*HNHCO), 49.7 (*C*H_2CO); HRESI-TOF MS: *m*/*z* calcd for $C_{72}H_{117}N_3O_{54}$: 1910.63991 $[M+Na]^+$; found: 1910.63724.

4.1.40. *N*-{2-*N*-{2-[1-(*O*-β-D-Galactopyranosyl-(14)-β-D-glucopyrano-syloxy)-3-(*O*-α-D-mannopyranosyloxy)]-propanyl}pentane-1,5-diamidyl}-octane-1,8-diamide (46)

Compound **46** was prepared by the same procedure as described for the preparation of **14**. The yield was 93%; $[\alpha]_D^{25} = +22.6$ (*c* 1.06 in H_2O); ^{1}H NMR (400 MHz, D_2O): $\delta = 4.78$ (s, 4H; H-1″), 4.41 (d, $J_{1,2} = 7.8$ Hz, 4H; H-1), 4.37 (d, $J_{1',2'} = 7.8$ Hz, 4H; H-1′), 4.26–2.80 (m, 112H; H-2,3,4,5,6a,b, H-2′,3′,4′,5′,6a′,b′, H-2″,3″,4″,5″,6a″,b″, CH_2O, C*H*NH), 2.23–1.26 (m, 32H; CH_2CO, CH_2); ^{13}C NMR (100 MHz, D_2O): $\delta = 177.1$, 174.8, 173.6, 173.5 (CO), 102.9, 102.4 (C-1, C-1′), 100.2, 100.1, 99.6 (C-1″), 78.4 (C-4, C-4″), 75.3 (C-3), 74.8 (C-5), 74.2 (C-2), 72.8 (C-3″), 72.5 (C-5′, C-3′), 70.9 (C-2″), 70.5, 70.0, 68.5 (CH_2O), 66.7, 66.6 (C-2′), 66.2 (C-5″), 65.9 (C-4′), 61.0 (C-6′), 60.9 (C-6″), 60.1 (C-6), 49.4, 49.2, 48.8 (*C*HNHCO), 35.4, 31.9 (*C*H_2CO), 27.9, 25.1, 20.3 (CH_2); HRESI-TOF MS: *m*/*z* calcd for $C_{102}H_{176}N_6O_{74}$: 1358.00117 $[M+2Na]^{2+}$; found: 1358.00137.

4.2. Anti-adhesion assay

In brief, human umbilical vein endothelial cells (HUVECs) were cultured as previously described.[37] HUVECs were grown in 96-well microtiter plate (Greiner) until the formation of a monolayer, which were then used in adhesion assay. Neutrophils were collected from healthy volunteers and separated as previously described.[22] Neutrophils were dyed with Calcein-AM (Sigma C1359, 8 mM) for 40 min, followed by the addition of LPS (Sigma L2630) to a final concentration of 1 μg/mL, and the incubation was continued for another 15 min. After washed for three times, the neutrophils were divided into aliquots (1×10^5 cells per aliquot) and subjected to respective treatments with different synthesized polysaccharide sugars (40 μM), dissolved in 1×isotonic phosphate buffered saline (PBS), pH 6.0) for 10 min. The treated cells were then added to 96-well culture plate and co-incubated with HUVECs in HEPES CaMg buffer (0.05 M HEPES, 0.15 M NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4). After incubation for 20 min at 37 °C, the non-adherent cells were removed by washing with 1×PBS. Neutrophils adhered to HUVECs were quantitated by fluorescence measurement using a fluorescence plate reader (Bio-Tek SynergyII) at 485 nm/528 nm. The mean fluorescence intensity (MFI) of triplicate wells was used for statistical analysis (LPS-stimulated cells without polysaccharide treatment were used as control). The lower MFI indicated stronger interference of a compound on adhesion between neutrophils and HUVECs. Statistical analysis was performed with statistics package for social science (SPSS) version 13.0. One-way analysis of variance was performed followed by Student–Newman–Keuls multiple comparison tests. $p < 0.05$ was considered statistically significant.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carres.2013.06.011.

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