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Cu(II)-Self-assembling bipyridyl-glycoclusters and dendrimers bearing the Tn-antigen cancer marker: syntheses and lectin binding properties

René Roy* and Jin Mi Kim

Departement of Chemistry and Biochemistry, Université du Québec à Montréal, P.O. Box 8888, Succ. Centre-Ville, Montreal, Que. Canada H3C 3P8

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Abstract—Using the carbohydrate cancer marker, T_N-antigen (α-GalNAc-OR), covalently linked to a bipyridine core, square planar complexes were formed by self-assembly upon simple addition of Cu(II) sulfate. The required α-D-GalNAc-OR building block was constructed from 2-azidoethyl 2-acetamido-2-deoxy-α-D-glucopyranoside (GlcNAc) by epimerization at C-4 of a suitably protected derivative followed by conventional modifications to provide 2-aminoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranoside. The 2-aminoethyl aglycone was further elongated into a key monomer having an aminocaproic acid spacer together with their corresponding dimers using a double N-alkylation strategy of their *N*-bromoacetyl derivatives using mono-Boc-1,4-diaminobutane, respectively. The building blocks containing the bipyridyl dimers, having either a short or a long spacer arm, together with the tetramer built from the short spacer derivative were prepared in a convergent manner using 2,2′-bipyridine-4,4′-dicarboxylic acid chloride and the aminated sugar derivatives, respectively. Copper(II)-nucleated GalNAc derivatives containing four and eight residues were obtained from an aqueous solution of the bipyridyl derivatives. The relative inhibitory potencies of these glycodendrimers were evaluated against monomeric allyl α-D-GalNAc using a solid-phase competition assay with asialoglycophorin and horseradish peroxidase-labeled lectin *Vicia villosa*. The di- and tetra-valent bipyridyl clusters showed up to 87-fold increased inhibitory properties (IC_{50} 7.14, 1.82, 4.09 μM, respectively) when compared to the monomer (IC_{50} 158.3 μM) while the Cu(II)-complexes showed up to a 259-fold increase potencies (IC_{50} 0.61 μM) with the octamer showing the highest affinity. However, when expressed on a per-saccharide basis, the tetramer Cu(II) nucleated derivative, possessing the longest inter-sugar distances showed the highest affinity (IC_{50} 0.63 μM).

1. Introduction

Recently, there have been numerous studies toward the construction of various neoglycoconjugates[1] bearing multiple copies of surface carbohydrate groups to demonstrate the 'cluster effect', which is characterized by enhanced carbohydrate–protein interactions on cell surfaces.[2] This cluster effect is expected when the multivalent glycosides interact with more than one receptor binding site simultaneously and cooperatively, resulting in better cellular recognition and cross-linking. Therefore, the preparation of multivalent carbohydrate ligands that would bind to the carbohydrate recognition sites on proteins would contribute to a better understanding of this effect together with the development of better therapeutic inhibitors.

The conventional method for the preparation of carbohydrate clusters and carbohydrate dendrimers includes convergent[3] or divergent[4] approaches. However, these procedures require lengthy and reiterative stepwise synthesis. This issue can be overcome by employing newly developed synthetic methods such as metal associated self-assembly,[5,6] where hyper-branched dendrimers were prepared by nucleating readily accessible building blocks (dendrons) around the metal ions like ruthenium (II),[7–10] iron (II),[6,11] or copper (II).[12] In this self-assembling method, pre-made dendrons are non-covalently assembled around a coordinating metal and its dendritic structure is governed by the coordination of the selected metal and the degree of branching in the dendron.

It has been previously demonstrated that Fe(II)-induces the trimerization 2-acetamido-2-deoxy-D-galactopyranoside ligands (GalNAc),[13,14] where the GalNAc residue was directly coupled to 5-(bromomethyl)-5′-methylbipyridine. This cluster exhibited increased binding affinity toward the phytohemagglutinin *Vicia villosa* B_4 (VVA). Notwithstanding the enhancement of binding affinity observed in carbohydrate dendrimers, an extension of this self-assembly concept to the synthesis of glycodendrimers is unprecedented.

Herein, the syntheses and the relative binding properties of square planar Cu(II)-assisted self-assembling of glycoclusters

Keywords: glycodendrimer; lectin; self-assembly; *Vicia villosa*; GalNAc.

* Corresponding author. Tel.: +1-514-987-3000x2546; fax: +1-514-987-4054; e-mail: roy.rene@uqam.ca

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and glycodendrimers are presented (Fig. 1). The carbohydrate moiety in these glycoclusters and glycodendrimers includes the tumor-associated Tn-antigen (GalNAcα-*O*-Ser).[15,16] The inhibitory capacities of the synthetic GalNAc-containing ligands were evaluated by an Enzyme-Linked Lectin Assay (ELLA),[17] in which the synthetic ligands inhibited the binding of VVA lectin to asialoglycophorin, a glycoprotein found on human erythrocyte membrane.

2. Results and discussion

2.1. Synthesis of GalNAc derivatives from GlcNAc

Since the required *N*-acetyl-D-galactosamine (GalNAc) is a relatively costly starting material, we prepared the α-D-GalNAc glycoside using a modification of a published procedure using the readily available C-4 epimer *N*-acetyl-D-glucosamine (GlcNAc, **1a**).[18] The key building block for the synthesis of the carbohydrate ligand bearing the GalNAc residues was 2-aminoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranoside **5** (a mimic of α-D-GalNAc homoserine) prepared according to Scheme 1.

2-Azidoethyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (**2b**) was prepared in three steps from *N*-acetylglucosamine **1a** which was glycosylated with 2-chloroethanol in the presence of acetic acid to give **1b** (reflux, 4 h, 75%). Substitution of the chloride by an azide group could be completed at this stage with the unprotected sugar moiety. However, monitoring the reaction mixture was not facile due to the fact that the resulting azide derivative had the same R_f value as that of the starting material. Even though the ^{1}H NMR spectrum showed different signal patterns for the 2-chloroethyl glycoside **1b** in comparison to those of the 2-azidoethyl GlcNAc derivative **1c**, quantitative evaluation of the transformation was still difficult due to overlapping multiplets between δ 3.0–3.5 ppm. As an alternative method, **1b** was first regioselectively di-*O*-benzoylated at both C-3 and C-6 positions (2.3 equiv. BzCl, pyridine, −60°C, 77%) and the resulting chloride **2a** was converted into the azide **2b** by a S_N2 substitution (10 equiv. NaN_3, 1 equiv. NaI, CH_3CN,

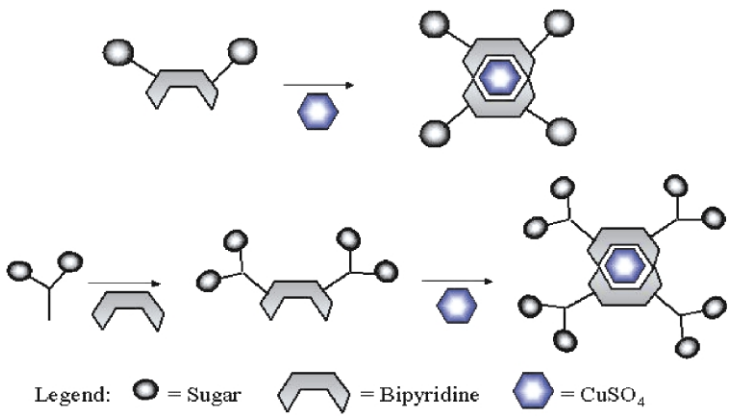
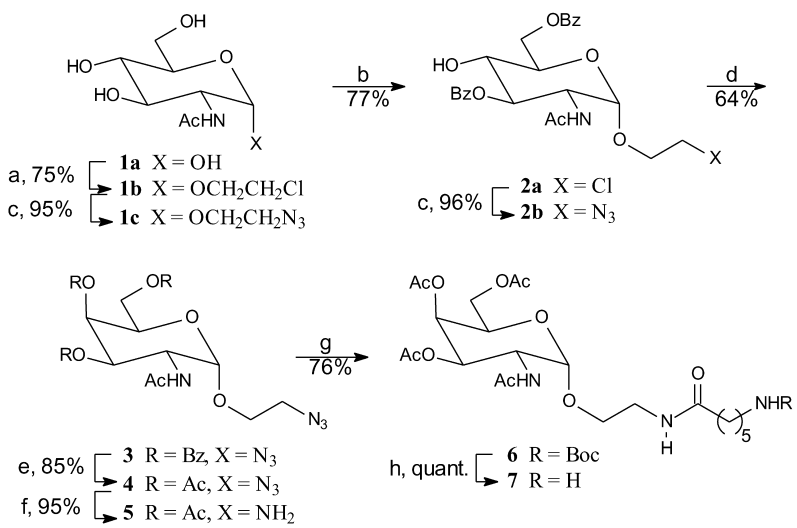
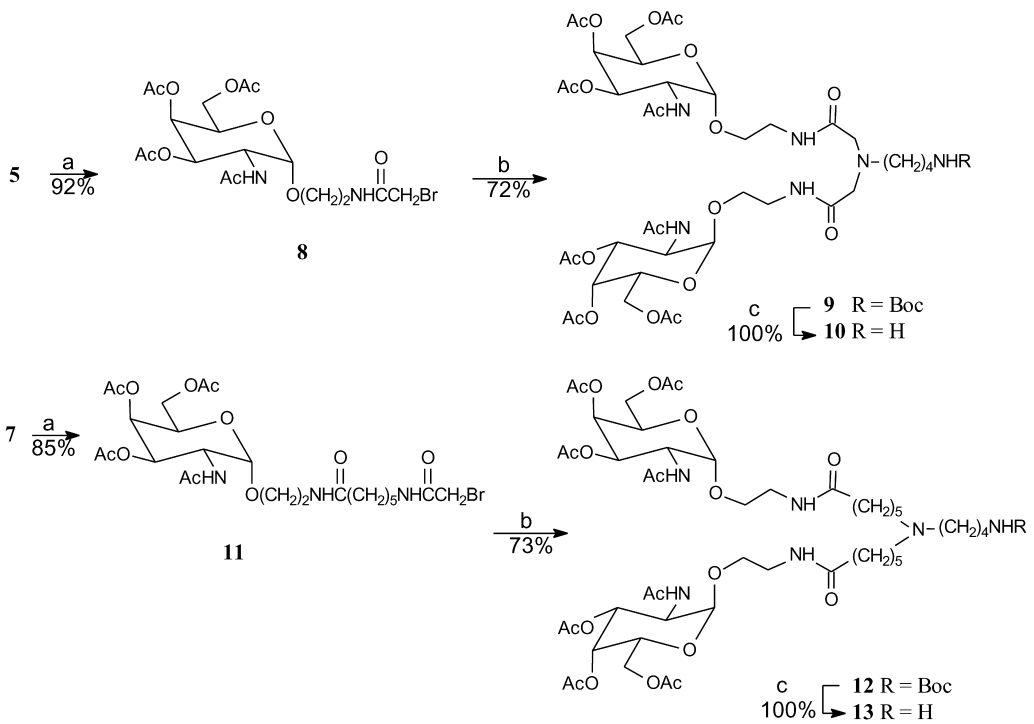


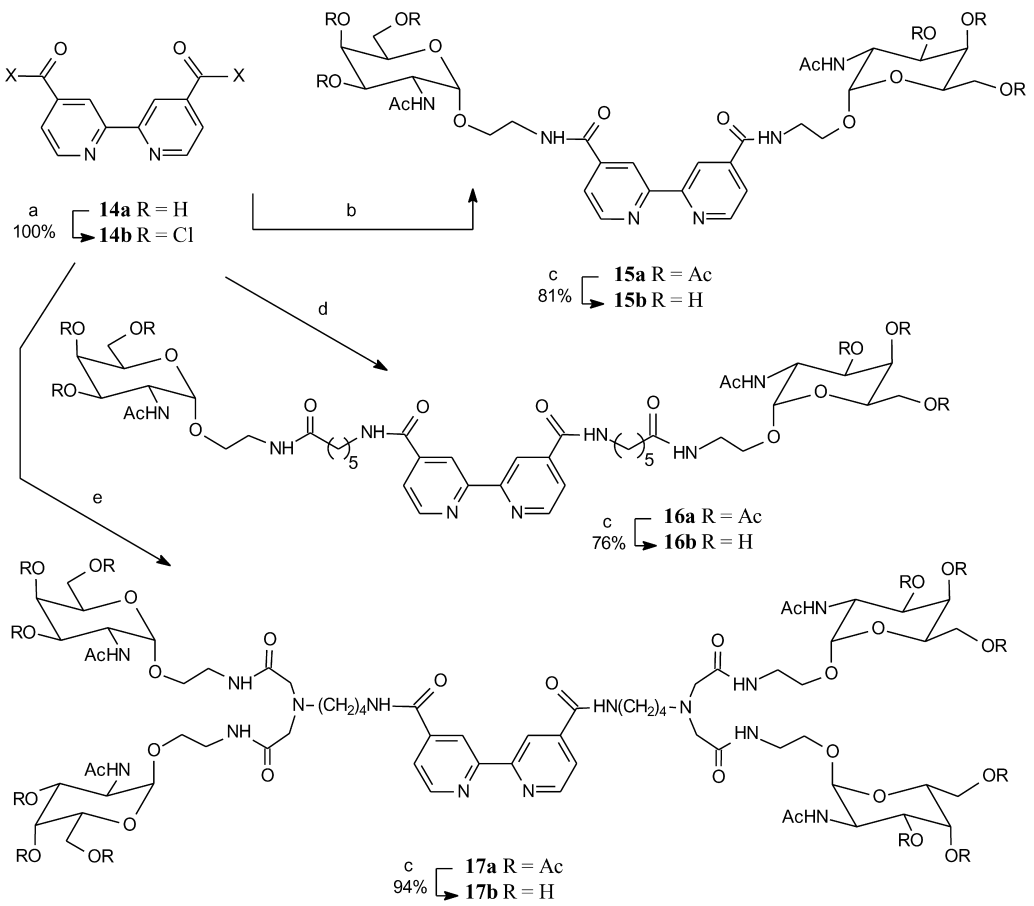
Figure 1. General self-assembling strategy.



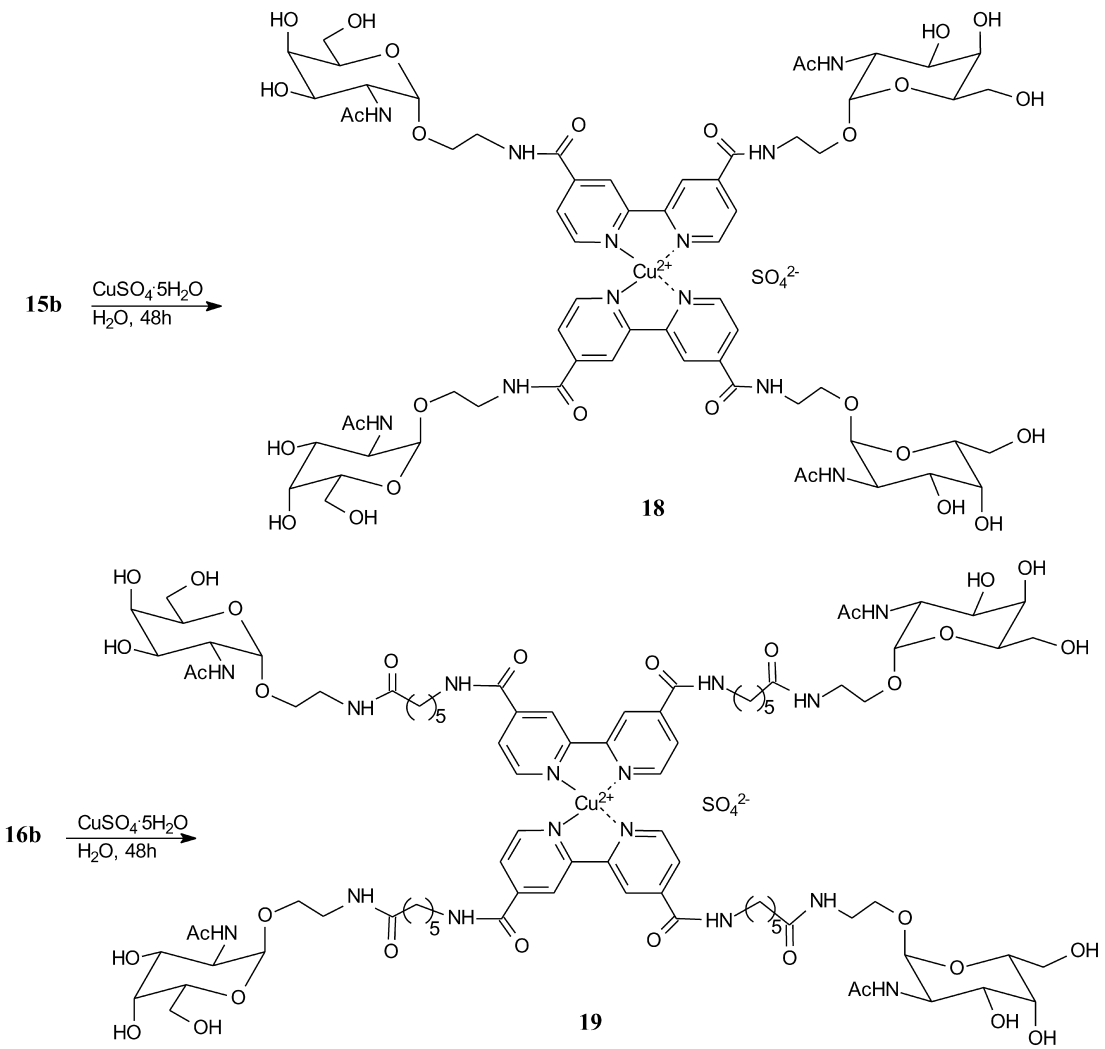
Scheme 1. *Reagents and conditions*: (a) $ClCH_2CH_2OH$, HOAc (1.2 equiv.), △, 4 h, 75%; (b) BzCl (2.3 equiv.), pyridine, CH_2Cl_2, −60°C, 3 h, 77%; (c) NaN_3 (10 equiv.), NaI, CH_3CN, △, 48 h, 96%; (d) (i) triflic anhydride (1.5 equiv.), pyridine, CH_2Cl_2, −15°C, 2 h, (ii) BzONa (5 equiv.), DMF, room temperature, 20 h, 64%; (e) (i) NaOMe, MeOH, 3 h, (ii) Ac_2O, pyridine, 85%; (f) H_2, Pd/C, HOAc (1 equiv.), MeOH, 24 h, 95%; (g) *N*-Boc-caproic acid (1.2 equiv.), DIPEA (2.5 equiv.), TBTU (1.2 equiv.), CH_2Cl_2, 0°C, 30 min, 76%; (h) 20% TFA, CH_2Cl_2, 2 h, quant.



Scheme 2. *Reagents and conditions*: (a) $BrCH_2COCl$ (1.2 equiv.), DIPEA (2.5 equiv.), CH_2Cl_2, 0°C, 30 min; (b) $BocN(CH_2)_4NH_2$ (0.4 equiv.), DIPEA (1.2 equiv), CH_3CN, 60°C, 48 h, 72% (**9**), 73% (**12**); (c) 20% TFA, CH_2Cl_2, 2 h, quant.



Scheme 3. *Reagents and conditions*: (a) $SOCl_2$, reflux, 2 h., quant.; (b) **5** (2.2 equiv.), Et_3N, CH_2Cl_2, 0–23°C, 3 h; (c) NaOMe, MeOH, 3 h, room temperature, **15b** (81%), **16b** (76%). **17b** (94%); (d) **7** (2.2 equiv.), Et_3N, CH_2Cl_2, 0–23°C, 3 h; (e) **10** (2.2 equiv.), Et_3N, CH_2Cl_2, 0–23°C, 3 h.



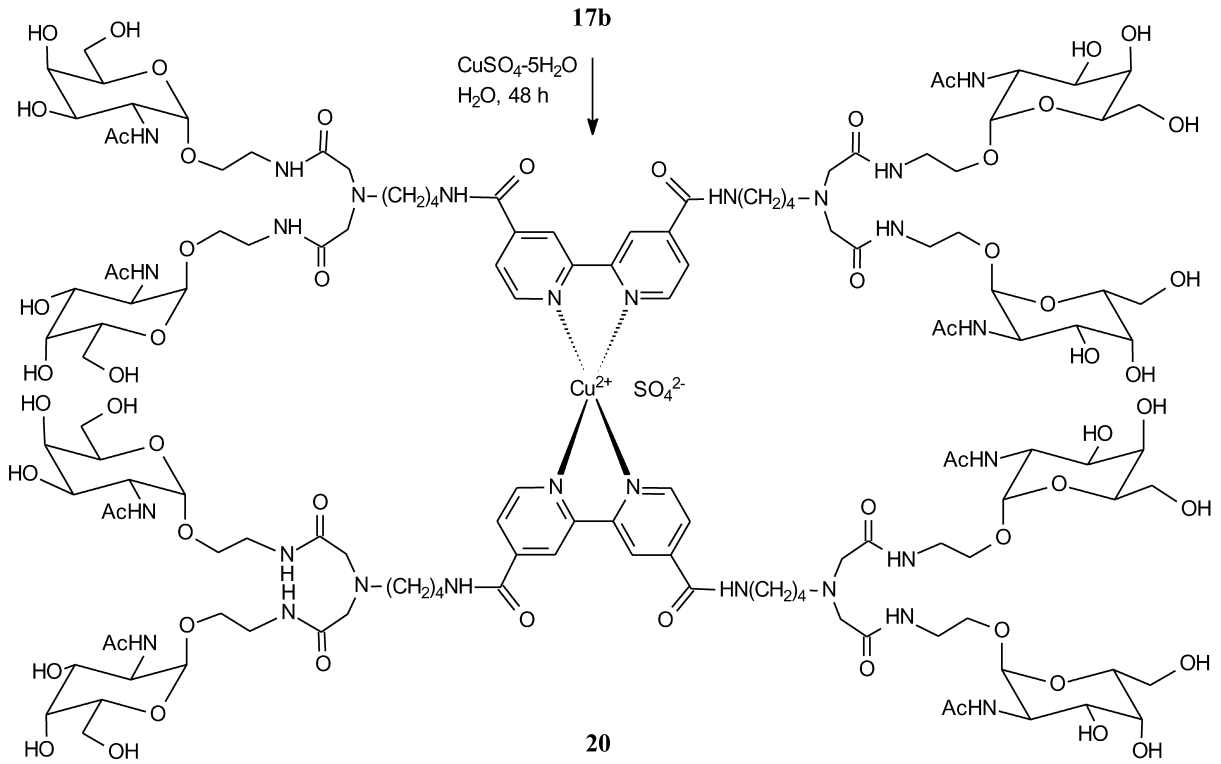
Scheme 4.

reflux, 48 h, 96%). Due to the relatively slow reaction rate, a large excess of NaN_3 and auxiliary NaI were used. The reaction was monitored by ^1H NMR and the well-resolved aglycon-protons of the product clearly indicated complete transformation. Epimerization at C-4 of **2b** to provide the corresponding GalNAc derivative **3** was also accomplished by a substitution reaction. To this end, the hydroxyl group at C-4 was transformed into a triflate derivative which was then displaced by sodium benzoate (DMF, room temperature, 20 h, 64% for 2 steps) to afford **3**. For the purpose of monitoring the coupling reactions to the bipyridyl residue, whose resonances appeared at δ 8.8, 8.3 and 7.6 ppm in the ^1H NMR spectra, the benzoyl groups were exchanged for acetyl protecting groups prior to further synthesis (i) NaOMe, MeOH; (ii) Ac_2O, pyridine) to give **4** in 85% yield. The azide functionality was further transformed into an amine by hydrogenation in the presence of an equimolar amount of acetic acid (H_2–Pd/C, AcOH, MeOH) to afford the key building block **5**. In order to synthesize ligands with longer spacer arms, peracetylated 2-aminoethyl GalNAc derivative **5** was first elongated with *N*-Boc-6-aminocaproic acid[19] (TBTU, DIPEA, CH_2Cl_2, 1 h, 76%) to afford **6** and then coupled to the bipyridine core after removing the *N*-Boc protecting group (20% TFA, CH_2Cl_2, 2 h).

2.2. Syntheses of the dendritic building blocks

In order to provide access to self-assembling structures of increasing carbohydrate valencies, dimeric GalNAc building blocks were prepared having both short and long spacer-armed acyl bromides. Compounds **8** and **11** were derived from the corresponding GalNAc amines **5** and **7**, respectively, by treatment with bromoacetyl chloride (1.2 equiv.) in the presence of DIPEA (2.5 equiv.) in 85–92% yield (CH_2Cl_2, 0°C, 30 min) using our previously established N,N-dialkylation strategy (Scheme 2). A minimum of two equivalents of bromoacetylated GalNAc derivatives **8** or **11** were heated with mono-*N*-Boc-1,4-diaminobutane (1 equiv., DIPEA (6 equiv.), 60°C, CH_3CN, 48 h). The progress of the reaction was monitored by TLC. The mono-substituted products were initially formed, followed by the slow appearance of the disubstituted ones having higher R_f values than their corresponding monomers. These dialkylated products were purified by silica gel column chromatography (72–73%) and the *N*-Boc group was next removed by trifluoroacetolysis (20% TFA, CH_2Cl_2, 2 h) to afford dimeric amines **10** and **13**, respectively.

The building blocks (dendrons) were synthesized by coupling the corresponding glycosylated amines **5**, **7**, **10**,



Scheme 5.

and **13** to a 2,2′-bipyridine core. In general, the coupling was accomplished by adding 2,2′-bipyridine-4,4′-dicarboxylic acid chloride **14b** in CH_2Cl_2 to a solution containing GalNAc glycosylated amines **5**, **7**, **10**, and **13** in Et_3N at 0°C, where the 2,2′-bipyridine-4,4′-dicarboxylic acid chloride was obtained by treating the commercial diacid **14a** with thionyl chloride ($SOCl_2$, 2 h, reflux) (Scheme 3). As the coupling reaction proceeded, the color of the reaction turned pinkish brown. However, the solution was decolorized upon treatment with 1 M NaOMe in MeOH during de-*O*-acetylation process (Scheme 3). The de-*O*-acetylated bipyridyl dimers **15b** and **16b** were precipitated out from the solution under Zemplén conditions (1 M NaOMe, MeOH, 23°C, 3 h; 81% for **15b**, 76% for **16b**). The bipyridyl dendron with higher valency (**17b**) was prepared using the same strategy by coupling **10** to **14** (94%) followed by de-*O*-acetylation. These di- and tetra-valent de-*O*-acetylated GalNAc ligands were soluble in MeOH, thus allowing their purification by size exclusion column chromatography on Sephadex LH20 in MeOH (**15b**: 81%; **16b**: 76%; **17b**: 94%).

2.3. Copper(II) sulfate self-assembling of dendrons

The pre-made dendrons, **15b**–**17b**, were nucleated around copper(II) ($CuSO_4$–$5H_2O$) to provide symmetric glyco-clusters and glycodendrimers in an efficient manner (Schemes 4 and 5). Glycodendrimers with square planar geometry were prepared using 2 M equiv. of dendrons and one equimolar amount of $CuSO_4{\cdot}5H_2O$ (H_2O, 48 h, 23°C). After 48 h of stirring, the bluish solution was lyophilized to afford light bluish-purple colored powder. The UV–vis spectra of the bipyridyl ligands without the metal ion showed UV absorptions corresponding to the aromatic bipyridyl moiety only (λ_{max} (ε, $M^{-1}\,cm^{-1}$)=236–240 (1.3–2.5×10^4), 293–296 (5.8–15×10^3)). The absorption spectra of these complexes in water showed similar patterns for all the prepared Cu(II)-(bipy-GalNAc)$_2$ complexes (λ_{max} (ε, $M^{-1}\,cm^{-1}$)=312–314 (1.2–1.4×10^4)) (Table 1).

The Cu(II) complexes having α-GalNAc-bipyridyl ligands appeared to have symmetrical geometries with their stoichiometries based on the elution peaks shown in the size exclusion chromatography (LH20 column in water). In addition, the 500 MHz ^{1}H NMR spectra of the clustered Cu(II) complexes in D_2O displayed significantly broadened signals for the aromatic bipyridyl cores near the para-magnetic Cu(II) ions, while those of the spacer and sugar residues, albeit broader than their monomer counterpart, clearly showed the expected unique signals, thus confirming

Table 1. UV–Vis (water) data of bipyridyl α-GalNAc building blocks and their self-assembled ligands

Ligands	M.W.	Conc. (×10^{-6} M)	λ (nm) (ε, $M^{-1}\,cm^{-1}$)
15b	736.32	27.2	236 (25331), 294 (15206)
16b	962.49	20.8	240 (12849), 293 (5775)
17b	1600.77	12.5	240 (9768), 293 (5604)
18	1632.19	12.3	312 (13306)
19	2085.70	9.59	256 (10950), 314 (3650)
20	3360.46	5.95	314 (12433)

Table 2. FAB-MS of bipyridyl GalNAc building blocks

Ligands	Formula	M.W. (calculated)[a]	M.W.+1 (found)[a]
15b	$C_{32}H_{44}N_6O_{14}$	736.30 (737.2994)	737.38 (737.2830)
16b	$C_{44}H_{66}N_8O_{16}$	962.47 (963.4675)	963.47 (963.4680)
17b	$C_{68}H_{108}N_{14}O_{30}$	1600.74 (1601.7434)	1601.78 (1601.6491)

[a] The values in parentheses are from high resolution mass spectrometry.

Table 3. MALDI-TOF Mass spectrometry of self-assembled GalNAc ligands

Ligands	Formula	M.W. (calculated)	M.W. (found)
18	$C_{64}H_{88}N_{12}O_{28}Cu$	1535.51	1536.06
19	$C_{88}H_{132}N_{16}O_{32}Cu$	1987.85	1989.39
20	$C_{136}H_{216}N_{28}O_{60}Cu$	3264.40	3267.76

Matrices used for these experiments were THAP (2,4,6-trihydroxyacetophenone) and 2,5-DHB (2,5-dihydrobenzoic acid).

the symmetry in their structural arrangements. All the synthetic bipyridyl dimers (**15b** and **16b**) and the tetramer (**17b**) were fully characterized by FAB-MS (Table 2) while matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) was used to characterize the bidentate metal coordinated Cu(II) complexes, using 2,5-dihydroxybenzoic acid (DHB) or 2,4,6-trihydroxy acetophenone (THAP) as the matrix (Table 3).

2.4. Lectin binding properties of the self-assembled GalNAc clusters

The binding studies of the synthetic bipyridyl ligands **15b**–**17b** and Cu(II)-(bipy-GalNAc)$_n$ complexes **18**–**20** demonstrated increased inhibitory potencies when compared to their analogous building blocks. The solid phase competitive inhibition assay, ELLA, was performed using asialoglycophorin as coating material, a glycoprotein present on type MN human blood group rich in exposed α-GalNAc residues. The peroxidase-labeled plant lectin *V. villosa* (VVA-HRP) was used as a carbohydrate binding protein.[20] All the synthetic GalNAc ligands inhibited the binding of asialoglycophorin to VVA-HRP with greater efficacy than the monomeric allyl α-D-GalNAc which was used as a standard inhibitor (IC_{50} 158.3 μM). As shown from the relative IC_{50} values (Fig. 2), the complexes clearly exhibited enhanced inhibitory behaviors following the expected 'cluster effect'. The dimeric bipyridyl GalNAc **15b** and **16b** were 22 (IC_{50} 7.14 μM) and 87 (IC_{50} 1.82 μM) times more potent than allyl α-D-GalNAc monomer.[21] Interestingly, the tetrameric bipyridyl derivative **17b** (IC_{50} 4.09 μM) showed less potency than **15b** and **16b** (38-fold) which could be attributed to a shorter intra-sugar distances between each of the branches. However, the Cu(II)-(bipy-GalNAc)$_n$ tetramers **18** (IC_{50} 2.60 μM, 61-fold), **19** (IC_{50} 0.63 μM, 251-fold), and octamer **20** (IC_{50} 0.61 μM, 259-fold) displayed much stronger inhibitory properties than their precursors. These observations clearly demonstrated that the structural arrangement and the flexibility of the molecules played important roles in their relative binding affinities. The ligands with longer aglycon spacer showed better inhibition than those having shorter ones. Considering the relative potency of the synthetic glycodendrimers on the basis of their carbohydrate contents, the best binding affinity to VVA was shown with derivative **19** (63-fold on a per-saccharide basis) having the longest inter-sugar distances. It is worth mentioning that one of the low energy conformers of these copper(II)-nucleated species may adopt a 'ladder-like' structure (see Figure 3 for **20**). Work is in progress to construct an analogous octamer derived from building block dimer **13** which should possess all the required features expected for high binding affinity.

3. Conclusions

The conventional method for the synthesis of glycodendrimers requires lengthy and reiterative procedures. This issue was surmounted by a novel self-assembling methodology. In this strategy, the pre-made building blocks (dendrons) were assembled around a metal ion, copper (II), to generate copper(II)-coordinated tetramers and octamers having square planar geometries. These metallated GalNAc-bearing glycodendrimers were characterized using spectrometric analyses, ^{1}H- and ^{13}C NMR, MALDI-TOF mass spectrometry, and UV–VIS spectroscopy.

The potential of these self-assembled GalNAc-bearing dendrimers to cross-link and precipitate lectin VVA was confirmed by the formation of colored precipitates between VVA and copper(II) coordinated glycodendrimers. When tested in ELLA using asialoglycophorin as coating antigen and horseradish peroxidase-labeled VVA for detection, these metal associated glycodendrimers exhibited markedly increased inhibitory potential. The best candidates for efficient binding were found to be those built around a longer aglycon spacer followed by nucleation to Cu(II) with relative inhibitory properties up to 259-fold (**20**) better than the monomer. Even when expressed on a per-saccharide basis, an increase of up to 63-fold was observed for tetramer **19**. These findings confirm that the aglycone spacer and the increase in the valency of sugar residues in neoglycoconjugates

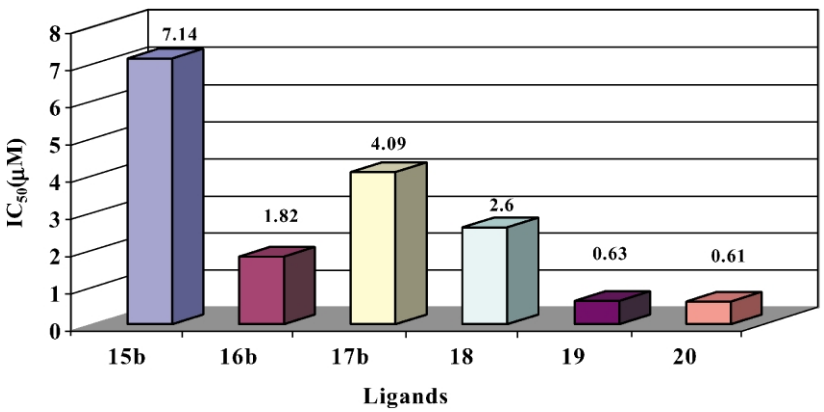


Figure 2. Relative IC_{50} values of GalNAc ligands with allyl α-D-GalNAc monomer (IC_{50} 158.3 μM).

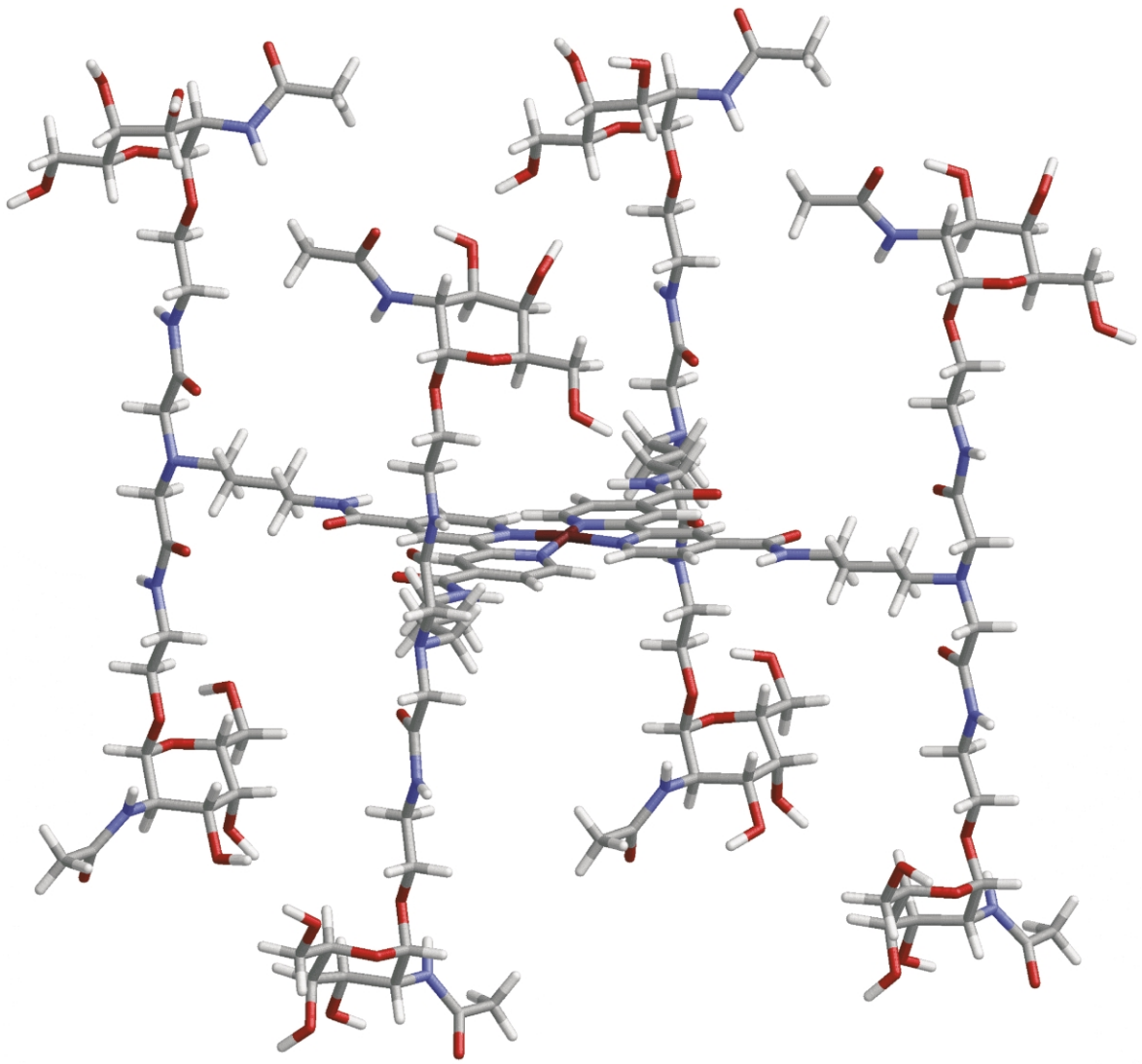


Figure 3. Computer-generated low energy conformer of compound **20** illustrating a 'ladder-type' geometry.

are responsible for an increase in binding affinity of carbohydrate–protein interactions. Clustering of the above bipyridyl clusters around iron(II) will also be reported shortly.

4. Experimental

4.1. General methods

^{1}H and ^{13}C NMR were recorded on a Brüker AMX500, Varian XL300, or Gemini 200 spectrometer at 500, 300, and 200 MHz for proton and at 125.7, 75.4, and 50.3 MHz for carbons, respectively. Proton chemical shifts (δ) are given relative to internal chloroform (7.24 ppm) for $CDCl_3$ solutions, and to HOD (4.76 ppm) for D_2O solutions. Carbon chemical shifts are given relative to $CDCl_3$ (77.0 ppm). Assignments were based on shift correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), and 1- and 2-dimentional distortionless enhancement by polarization transfer (DEPT) experiments. Mass spectra were obtained using a VG 7070-E spectrometer (EI and CI) or Kratos IIH instrument (FAB-glycerol). Xenon was used as the neutral carrier atom in FAB-MS experiments. MALDI-TOF was employed for the characterization of self-assembling compounds using 2,5-dihydroxybenzoic acid (DHB) or 2,4,6-trihydroxy acetophenone (THAP) as the matrix. Optical rotations ($[\alpha]_D$) were measured on a Perkin–Elmer 241 polarimeter and were run at room temperature. UV–VIS spectra were recorded on a Gilford Response I instrument using quartz cuvettes. Elemental analyses were performed by the analytical services of the Department of Chemistry of the University of Ottawa. Optical densities (O.D.) for enzyme linked lectin assays (ELLA) were measured on a Dynatech MR600 Microplate Reader. Thin layer chromatography (TLC) was performed using silica gel 60-F254 glass plates. Reagents used for developing plates include ceric sulfate (1% w/v) and ammonium molybdate (2.5% w/v) in 10% (v/v) aqueous sulfuric acid, ninhydrin (0.4% w/v) in aqueous pyridine (4% v/v), or UV light. TLC plates were heated to $\approx$150°C when necessary. Purifications were performed by gravity or flash column chromatography on silica gel 60 (230–400 Mesh, E. Merck No. 9385). Purifications were also performed via preparative size

exclusion chromatography using Sephadex LH 20 column. Columns were connected to a Pharmacia Peristaltic Pump P3 and eluted with distilled H_2O or methanol. Waters Differential Refractometer R401 or R403 apparatus was used for detection and recorded on a Linear 1200 or 2000 chart recorder. Fractions were collected using LKB 2112 Redirac or Pharmacia Model 5051 fraction collectors. Lyophilization was carried out on a VIRTIS-24 freeze dryer. All chemicals and solvents used in experiments were of reagent grade. Further purifications were performed, when necessary, following published procedures. Asialoglycophorin and peroxidase labeled *V. villosa* B_4 (VVA) lectin were purchased from Sigma. The computer generated low energy conformations were calculated using the CaChe software (Fujitsu Inc., OR).

4.1.1. 2-Chloroethyl 2-acetamido-2-deoxy-α-D-glucopyranoside (1b). To a solution of *N*-acetyl-D-glucosamine (**1a**) (10.0 g, 45.2 mmol) in 2-chloroethanol (150 mL) was added dropwise acetyl chloride (4.26 g, 54.2 mmol) at 0°C. The reaction mixture was heated at 70°C for 4 h and stirred at room temperature for another 4 h. The solution was concentrated and the brownish oily residue was dissolved in ethanol. The solution was decolorized using charcoal and filtered through a celite pad. The concentrated residue was purified by silica gel chromatography to yield **1b** (9.64 g, 75%) as a syrupy residue; $[\alpha]_D$=+147.9 (*c* 1.0, MeOH); ^{1}H NMR (D_2O) δ 1.98 (s, 3H, NAc), 3.40–3.50 (m, 1H), 3.65–3.95 (m, 9H), 4.89 (d, 1H, $J_{1,2}$=3.5 Hz, H-1); ^{13}C NMR (D_2O) δ 23.3 (CH_3), 45.0 (CH_2), 55.1 (C-2), 62.0 (C-6), 69.7 (CH_2), 71.4 (C-4), 72.3 (C-3), 73.5 (C-5), 98.6 (C-1), 176.0 C=O); CI-MS (*m*/*z*) calcd for $C_{10}H_{18}NO_6Cl$: 283.1; found: 283.9 (M^++1, 72.8%), 285.9 (32.6%).

4.1.2. 2-Azidoethyl 2-acetamido-2-deoxy-α-D-glucopyranoside (1c). 2-Chloroethyl 2-acetamido-2-deoxy-α-D-glucopyranoside (0.10 g, 0.353 mmol) was dissolved in CH_3CN (3 mL) by heating the solution. NaN_3 (0.23 g, 3.53 mmol) and NaI (0.053 g, 0.353 mmol) were then added. The reaction mixture was heated at 60°C for 7 h. The reaction mixture was concentrated and the residue purified using short silica gel column eluting with 95:5 $CHCl_3$/MeOH which yielded **1c** (0.097 g, 95%) as an oil; $[\alpha]_D$=+103.9 (*c* 1.0, MeOH); ^{1}H NMR (D_2O) δ 1.00 (s, 3H, NAc), 3.35–3.50 (m, 3H), 3.55–3.95 (m, 7H), 4.89 (d, 1H, $J_{1,2}$=3.5 Hz, H-1); CI-MS (*m*/*z*) calcd for $C_{10}H_{18}N_4O_6$: 290.1; found: 291.0 (M^++1, 12.6%).

4.1.3. 2-Chloroethyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (2a). 2-Chloroethyl 2-acetamido-2-deoxy-α-D-glucopyranoside **1b** (6.89 g, 24.3 mmol) was dissolved in a mixture of pyridine (66 mL) and CH_2Cl_2 (33 mL) at −60°C to which benzoyl chloride (7.86 g, 55.9 mmol) in CH_2Cl_2 (10 mL) was added through a dropping funnel over a 1 h period at −60°C. The reaction was monitored by thin layer chromatography (TLC) and the reaction was quenched by adding MeOH as the tribenzoylated product started to appear on TLC. The reaction mixture was diluted with $CHCl_3$ (50 mL) and washed with 5% aqueous HCl (2×100 mL), saturated $NaHCO_3$ (2×100 mL) and then water (1×100 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated to afford an oily residue. Purification of the crude product by silica gel chromatography eluting with 98:2 CH_2Cl_2/MeOH yielded 9.20 g (77%) of **2a** a white foam; ^{1}H NMR ($CDCl_3$) δ 1.82 (s, 3H, NAc), 3.62–4.18 (m, 7H, H-5, H-4, $2CH_2$, OH), 4.45 (ddd, 1H, $J_{2,3}$=10.3 Hz, $J_{2,NH}$=9.3 Hz, H-2), 4.56 (dd, 1H, $J_{6,6'}$=12.6 Hz, $J_{5,6'}$=2.6 Hz, H-6′), 4.73 (dd, 1H, $J_{5,6}$=3.9 Hz, H-6), 4.90 (d, 1H, $J_{1,2}$=3.6 Hz, H-1), 5.39 (dd, 1H, $J_{3,4}$=10.3 Hz, H-3), 6.00 (d, 1H, NH), 7.30–7.68 (m, 6H, Ar_{meta}, Ar_{para}), 7.98, 8.03 (2dd, *J*=8.6, 1.2 Hz, Ar_{ortho}); CI-MS (*m*/*z*) calcd for $C_{24}H_{26}NO_8Cl$: 491.1; found: 492.1 (M^++1, 79.1%), 493.9 (45.8%), 494.9 (12.2%).

4.1.4. 2-Azidoethyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (2b). 2-Chloroethyl 2-acetamido-3,4-di-*O*-benzoyl-2-deoxy-α-D-glucopyranoside **2a** (6.86 g, 14.0 mmol) was dissolved in CH_3CN (100 mL) and NaN_3 (9.10 g, 0.14 mmol) and NaI (2.10 g, 14.0 mmol) were added to the solution. The reaction mixture was heated at 60°C for 48 h and then concentrated. The residue was treated with CH_2Cl_2 (100 mL) and the solution was washed with water (2×30 mL), saturated $NaHCO_3$ (2×30 mL), then brine (1×20 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated. Silica gel chromatography eluting with 9:1 EtOAc/Hexanes provided **2b** (6.68 g, 96%) as a white foam; $[\alpha]_D$=+77.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.84 (s, 3H, NAc), 3.27 (d, 1H, $J_{4,OH}$=4.6 Hz, OH), 3.35 (ddd, 1H, $J_{a,b}$=13.4 Hz, $J_{a,d}$=5.5 Hz, $J_{a,c}$=2.9 Hz, $CH_aH_bN_3$), 3.54 (ddd, 1H, $J_{b,a}$=13.4, $J_{b,c}$=7.9 Hz, $J_{b,d}$=3.0 Hz, CH_aH_bN3), 3.67 (ddd, 1H, $J_{c,d}$=10.7 Hz, $J_{c,b}$=7.9 Hz, $J_{c,a}$=2.9 Hz, OCH_cH_d), 3.95 (ddd, 1H, $J_{d,c}$=10.7 Hz, $J_{d,a}$=5.5 Hz, $J_{d,b}$=3.0 Hz, OCH_c-H_d), 3.85 (ddd, 1H, $J_{4,5}$=9.5 Hz, H-4), 4.02 (ddd, 1H, H-5), 4.49 (ddd, 1H, $J_{2,3}$=10.2 Hz, $J_{2,NH}$=9.9 Hz, H-2), 4.55 (dd, 1H, $J_{5,6}$=2.2 Hz, $J_{6,6'}$=12.2 Hz, H-6), 4.76 (dd, 1H, $J_{5,6'}$=4.2 Hz, H-6′), 4.93 (d, 1H, $J_{1,2}$=3.6 Hz, H-1), 5.35 (dd, 1H, $J_{3,4}$=9.3 Hz, H-3), 5.88 (d, 1H, NH), 7.41 7.45 (2dd, 4H, $J_{m,o}$=7.3 Hz, $J_{m,p}$=7.4 Hz, Ar_{meta}), 7.54, 7.58 (2dd, 2H, Ar_{para}), 8.01, 8.05 (2d, 4H, Ar_{ortho}); ^{13}C NMR ($CDCl_3$) δ 23.0 (CH_3), 50.4 (CH_2N_3), 51.4 (C-2), 63.3 (C-6), 67.3 (OCH_2), 68.9 (C-4), 70.7 (C-3), 74.4 (C-5), 97.8 (C-1), 128.5 (Ar_{meta}), 129.1, 129.5 (Ar_{ipso}'s), 129.7, 129.9 (Ar_{ortho}'s), 133.3, 133.5 (Ar_{para}'s), 166.9, 167.8, 170.3 (C=O's); CI-MS (*m*/*z*) calcd for $C_{24}H_{26}N_4O_8$: 498.2; found: 499.0 (M^++1, 64.8%), 411.9 (glycon, 100%). Anal. calcd for: C, 57.83; H, 5.26; Found: C, 57.73; H, 5.42.

4.1.5. 2-Azidoethyl 2-acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-α-D-galactopyranoside (3). To a solution of trifluoromethanesulfonic anhydride (2.0 mL, 12.1 mmol) in CH_2Cl_2 (30 mL) cooled at −15°C was added dropwise through a dropping funnel a solution of pyridine (1.95 mL, 24.1 mmol) in CH_2Cl_2 (10 mL). A solid appeared during the initial phase of the reaction, but dissolved after the complete addition of pyridine. A solution of 2-azidoethyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (**2b**) (4.0 g, 8.03 mmol) in CH_2Cl_2 (10 mL) was added to the reaction mixture and the solution was stirred at −15°C for 1 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with cold 5% aqueous HCl (1×30 mL), saturated $NaHCO_3$ (2×30 mL) and then water (1×30 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated to afford the intermediate triflate as a white foam. This product was used for the next reaction without further purification.

The above triflate was dissolved in DMF (20 mL) and sodium benzoate (5.78 g, 0.040 mmol) was added. The reaction mixture remained heterogeneous and was stirred at room temperature for 20 h. The reaction mixture was diluted with $CHCl_3$ (30 mL) and washed thoroughly and successively with brine (2×30 mL) and water (3×30 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated. Purification of the crude product by silica gel chromatography eluting with 7:3 EtOAc/Hexanes yielded **3** (3.07 g, 64%) as a white foam; triflate ^{1}H NMR ($CDCl_3$) δ 1.83 (s, 3H, NAc), 3.35–3.46 (m, 1H, CH_aN_3), 3.52–3.77 (m, 2H, $OCH_cCH_bN_3$), 3.90–4.02 (m, 1H, OCH_d), 4.30–4.47 (m, 2H, H-6′, H-5), 4.58 (ddd, 1H, $J_{2,3}$=10.8 Hz, $J_{2,NH}$=9.6 Hz, H-2), 4.81 (dd, 1H, $J_{6,5'}$=11.9 Hz, $J_{5,6}$=1.4 Hz, H-6), 4.96 (d, 1H, $J_{1,2}$=3.5 Hz, H-1), 5.33 (dd, 1H, $J_{3,4}$=9.7 Hz, H-3), 5.75 (dd, 1H, $J_{4,5}$=10.7 Hz, H-4), 5.92 (d, 1H, NH), 7.40–7.66 (m, 6H, Ar_{meta}, Ar_{para}), 8.04, 8.09 (2dd, $J_{o,m}$=6.6 Hz, $J_{o,p}$=1.2 Hz, Ar_{ortho}); FAB-MS (pos. *m/z*) calcd for $C_{25}H_{25}N_4O_{10}SF_3$: 630.12; found: 631.19 (M^++1, 25.2%); **3**: $[\alpha]_D$=+103.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.84 (s, 3H, NAc), 3.33 (ddd, 1H, $J_{a,b}$=13.4 Hz, $J_{a,d}$=5.5 Hz, $J_{a,c}$=2.9 Hz, CH_aH_b-N_3), 3.50 (ddd, 1H, $J_{b,a}$=13.4 Hz, $J_{b,c}$=7.9 Hz, $J_{b,d}$=3.0 Hz, $CH_aH_bN_3$), 3.69 (ddd, 1H, $J_{c,d}$=10.7 Hz, $J_{c,b}$=7.9 Hz, $J_{c,a}$=2.9 Hz, OCH_cH_d), 3.95 (ddd, 1H, $J_{d,c}$=10.7 Hz, $J_{d,a}$=5.5 Hz, $J_{d,b}$=3.0 Hz, OCH_cH_d), 4.37 (dd, 1H, $J_{6,6'}$=9.8 Hz, $J_{5,6'}$=4.3 Hz, H-6′), 4.51–4.57 (m, 2H, H-5, H-6), 4.94 (ddd, 1H, $J_{2,3}$=11.3 Hz, $J_{2,NH}$=9.5 Hz, H-2), 5.12 (d, 1H, $J_{1,2}$=3.5 Hz, H-1), 5.57 (dd, 1H, $J_{3,4}$=3.3 Hz, H-3), 5.92 (d, 1H, H-4), 6.01 (d, 1H, NH), 7.25, 7.36 (2t, 4H, Ar_{meta}), 7.43 (t, 3H, Ar_{para}, Ar_{meta}), 7.49, 7.56 (2t, 2H, $J_{m,p}$=7.4 Hz, Ar_{para}), 7.80, 7.96, 8.06 (3d, 6H, $J_{o,m}$=7.3 Hz, Ar_{ortho}); ^{13}C NMR ($CDCl_3$) δ 23.15 (CH_3), 48.21 (C-2), 50.43 (CH_2), 62.53 (C-6), 67.50 (CH_2), 67.60 (C-3), 68.25 (C-4), 69.03 (C-5), 98.20 (C-1), 128.37, 128.42, 128.62 (Ar_{meta}'s), 128.84, 129.07, 129.37 (Ar_{ipso}'s), 129.63, 129.83, 129.94 (Ar_{ortho}'s), 133.24, 133.38, 133.55 (Ar_{para}'s), 165.71, 166.00, 166.39, 170.43 (C═O's); FAB-MS (pos. *m/z*) calcd for $C_{31}H_{30}N_4O_9$: 602.20; found: 603.19 (M^++1, 2.0%). Anal. calcd for: C, 61.79; H, 5.02; Found: C, 61.77; H, 4.95.

4.1.6. 2-Azidoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranoside (4). 2-Azidoethyl 2-acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-α-D-galactopyranoside (**3**) (8.5 g, 17.1 mmol) was dissolved in MeOH (100 mL) and 1 M sodium methoxide was added dropwise until the pH of the solution reached ~9.0. The solution was stirred at room temperature for 3 h. When the reaction was complete, the solution was treated with Amberlite IR (H) resin for 15 min. to neutralize the base. The resin was filtered off and the filtrate was concentrated to dryness to provide the de-*O*-acetylated compound.

The dried residue was dissolved in pyridine (20 mL) and acetic anhydride (15 mL) was added. The solution was stirred at room temperature for 16 h and then concentrated under reduced pressure. The residue was dissolved in $CHCl_3$ (30 mL) and washed with 5% aqueous HCl (2×20 mL), saturated $NaHCO_3$ (2×20 mL), water (1×20 mL), then dried over anhydrous Na_2SO_4. Purification of the crude product by silica gel chromatography eluting with 4:1 EtOAc/Hexanes yielded **4** (6.05 g, 85%) as a white foam;$[\alpha]_D$=+50.2 (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.94, 1.98, 2.03, 2.14 (4 s, 12H, OAc, NAc), 3.33 (ddd, 1H, $J_{d,c}$=13.5 Hz, $J_{d,b}$=2.9 Hz, $J_{d,a}$=5.5 Hz, H-d), 3.52 (ddd, 1H, $J_{c,d}$=13.5 Hz, $J_{c,a}$=3.0 Hz, $J_{c,b}$=8.0 Hz, H-c), 3.65 (ddd, 1H, $J_{b,a}$=10.8 Hz, $J_{b,c}$=8.0 Hz, $J_{b,d}$=2.9 Hz, H-b), 3.89 (ddd, 1H, $J_{a,b}$=10.8 Hz, $J_{a,c}$=3.0 Hz, $J_{a,d}$=5.5 Hz, H-a), 4.05–4.12 (m, 2H, H-6′s), 4.16 (dt, 1H, $J_{5,6}$=6.9 Hz, $J_{4,5}$=1.0 Hz, H-5), 4.60 (ddd, 1H, $J_{2,3}$=11.4 Hz, $J_{2,NH}$=9.6 Hz, H-2), 4.93 (d, 1H, $J_{1,2}$=3.6 Hz, H-1), 5.16 (dd, 1H, $J_{2,3}$=11.4 Hz, $J_{3,4}$=3.3 Hz, H-3), 5.38 (dd, 1H, $J_{3,4}$=3.3 Hz, $H_{4,5}$ 1.1 Hz, H-4), 5.64 (d, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 20.6 (OAc), 23.1 (NAc), 47.4 (C-2), 50.3 (CH_2), 61.8 (C-6), 66.9 (CH_2), 67.2 (C-3), 674 (C-4), 68.0 (C-5), 97.9 (C-1), 170.1, 170.2, 170.3, 170.8 (C═O's); CI-MS (*m/z*) calcd for $C_{16}H_{24}N_4O_9$: 416.15; found: 417.0 (M^++1, 91.0%). Anal. calcd C, 46.14; H, 5.81; N, 13.46; found: 46.21; H, 5.85; N, 13.40.

4.1.7. 2-Aminoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranoside hydrochloride (5). To a solution of 2-azidoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranoside (**4**) (1.0 g, 2.40 mmol) in MeOH (100 mL) was added 10% Pd/C (0.20 g) and acetic acid (0.14 g, 2.40 mmol). The heterogeneous solution was bubbled with H_2 gas for 24 h. The reaction mixture was filtered through a celite pad and the filtrate was gently stirred with Amberlite IRA (Cl^-) resin (2.0 g) for 24 h. The resin was filtered off and the filtrate was concentrated to provide **5** (0.97 g, 95%) as a white foam; ^{1}H NMR ($CDCl_3$) δ 1.94, 1.99, 2.01, 2.11 (4s, 12H, OAc, NAc), 3.27 (bs, 2H, CH_2N), 3.64–3.73 (m, 3H, OC*H*H, NH_2), 3.90–4.08 (m, 3H, OCH*H*, H-6′s), 4.28 (dd, 1H, $J_{5,6}$=6.0 Hz, H-5), 4.53 (ddd, 1H, $J_{2,NH}$=9.4 Hz, $J_{2,3}$=11.3 Hz, H-2), 4.91 (d, 1H, $J_{1,2}$=3.3 Hz, H-1), 5.27 (dd, 1H, $J_{3,4}$=3.1 Hz, H-3), 5.35 (d, 1H, H-4), 7.67 (d, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 20.6 (OAc), 22.8 (NAc), 39.3 (CH_2), 47.0 (C-2), 62.0 (C-6), 63.5 (CH_2), 66.9 (C-3), 67.1 (C-4), 68.2 (C-5), 98.1 (C-1), 170.4, 170.4, 171.0, 171.2 (C═O's); FAB-MS (pos. *m/z*) calcd for $C_{16}H_{26}N_2O_9$: 390.16; found: 391.23 (M^++1, 98.4%).

4.1.8. *N*-Boc-6-aminocaproic acid. ε-Aminocaproic acid (1.2 g, 9.15 mmol) and NaOH (0.73 g, 18.3 mmol) were dissolved in water (5 mL). A solution of di-*t*-butyl dicarbonate (2.0 g, 9.15 mmol) in CH_2Cl_2 (15 mL) was added to the aqueous solution at 0°C and stirred at room temperature for 48 h. The progress of the reaction was monitored by ninhydrin test. When the reaction was complete, the solution was acidified by adding conc. HCl dropwise. The organic layer was separated from the aqueous layer, which was then extracted with $CHCl_3$ (2×20 mL). The combined organic phase was dried over anhydrous Na_2SO_4 and then concentrated. Purification of the crude product by silica gel chromatography eluting with 18:1:1 $CHCl_3$/CH_3CN/CH_3OH yielded 1.94 g (92%) of a colorless oil which physical data corresponded to those published;[19] ^{1}H NMR ($CDCl_3$) δ 1.32 (quintet, 2H, *J*=6.7 Hz, H-e), 1.39 (s, 9H, *t*-Bu), 1.45 (quintet, 2H, *J*=7.4 Hz, H-f), 1.59 (quintet, 2H, *J*=7.6 Hz, H-d), 2.29 (t, 2H, *J*=7.4 Hz, H-c), 3.05 (t, 2H, *J*=6.8 Hz, H-g), 4.59 (bs, 1H, NH), 10.3 (bs, 1H, CO_2H); ^{13}C NMR ($CDCl_3$) δ 24.3 (C-d), 26.1 (C-e), 28.3 (*t*-Bu), 29.6 (C-f), 33.9 (C-c), 40.2 (C-g), 79.1 (CMe_3), 156.0, 178.9 (C═O's); CI-MS (*m/z*) calcd for $C_{22}H_{21}NO_4$: 231.1; found: 232.0 (M^++1, 2.3 %).

4.1.9. *N*-Boc-6-aminocaproic acid derivative (6). 2-Aminoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranoside hydrochloride (**5**) (1.06 g, 2.48 mmol) and *t*-butyl *N*-carbamylhexanoic acid (0.86 g, 3.72 mmol) were dissolved in CH_2Cl_2 (20 mL) and the solution was cooled to 0°C. TBTU (1.19 g, 3.72 mmol) and DIPEA (1.20 mL, 6.89 mmol) were added to the solution and the reaction mixture was stirred at 0°C for 1 h. The reaction solution was washed with 5% aqueous HCl (2×10 mL), saturated $NaHCO_3$ (2×10 mL) and water (1×10 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated. Purification of the crude product by silica gel chromatography eluting with 18:1:1 $CHCl_3$/CH_3CN/MeOH afforded 1.14 g (76%) of a white foam; $[\alpha]_D$=+68.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.25–1.69 (m, 6H, H-d, H-e, H-f), 1.38 (s, 9H, *t*-Bu), 1.94, 1.97, 2.00, 2.11 (4s, 12H, OAc, NAc), 2.14–2.27 (m, 2H, H-c), 3.05 (t, 2H, *J*=6.6 Hz, H-g), 3.25–3.40 (m, 1H, H-b), 3.50–3.69 (m, 3H, H-a, H-b), 4.02–4.05 (m, 2H, H-6′s), 4.14–4.20 (m, 1H, H-5), 4.53 (ddd, 1H, $J_{2,3}$=11.4 Hz, $J_{2,NH}$=9.5 Hz, H-2), 4.71–4.75 (m, 1H, N*H*Boc), 4.83 (d, 1H, $J_{1,2}$=3.0 Hz, H-1), 5.06 (dd, 1H, $J_{2,3}$=11.4 Hz, $J_{3,4}$=3.1 Hz, H-3), 5.31 (d, 1H, H-4), 6.40–6.55 (br, 2H, NHAc, NHCO); ^{13}C NMR ($CDCl_3$) δ 20.7 (OAc), 23.0 (NAc), 25.2 (C-d), 26.2 (C-e), 28.3 (*t*-Bu), 29.6 (C-f), 36.3 (C-c), 39.3 (C-g), 41.3 (C-b), 47.4 (C-2), 62.0 (C-6), 66.7 (C-3), 67.2 (C-4), 68.4 (C-5), 68.4 (C-a), 79.2 (*C*Me$_3$), 98.6 (C-1), 156.1, 170.3, 170.5, 170.7, 170.8, 173.7 (C=O's); FAB-MS (pos. *m*/*z*) calcd for $C_{27}H_{45}N_3O_{12}$: 603.30; found: 604.34.

4.1.10. 2-Bromoacetamidoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranoside (8). A solution of 2-aminoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranoside hydrochloride (**5**) (0.40 g, 0.89 mmol) in CH_2Cl_2 (10 mL) was treated with DIPEA (0.39 mL, 2.22 mmol) at 0°C. Bromoacetyl chloride (88 mL, 1.07 mmol) in CH_2Cl_2 (5 mL) was then added dropwise through a dropping funnel at 0°C. After 20 min, the reaction solution was washed with 5% aqueous HCl (1×10 mL), saturated $NaHCO_3$ (1×10 mL) and water (1×10 mL). The dried (Na_2SO_4) organic phase was concentrated and silica gel chromatography of the crude product eluting with 19:1 $CHCl_3$/MeOH yielded 0.42 g (92%) of a white foam; $[\alpha]_D$=+75.1 (*c* 1.0, $CHCl_3$), ^{1}H NMR ($CDCl_3$) δ 1.96, 2.02, 2.13 (3s, 12H, OAc, NAc), 3.45–3.64 (m, 3H, OC*H*H, CH_2N), 3.70–3.78 (m, 1H, OCH*H*), 3.89 (s, 2H, CH_2Br), 4.05–4.18 (m, 3H, H-5, H-6′s), 4.56 (ddd, 1H, $J_{2,NH}$=9.6 Hz, $J_{2,3}$=11.3 Hz, H-2), 4.87 (d, 1H, $J_{1,2}$= 3.5 Hz, H-1), 5.12 (dd, 1H, $J_{3,4}$=3.3 Hz, H-3), 5.33 (d, 1H, H-4), 6.00 (d, 1H, NHAc), 6.93 (b, 1H, NHCO); ^{13}C NMR ($CDCl_3$) δ 20.7 (OAc), 23.3 (NAc), 29.2 (CH_2), 39.8 (CH_2), 47.6 (C-2), 62.0 (C-6), 66.9 (C-3), 67.2 (C-4), 67.3 (CH_2), 68.3 (C-3), 98.2 (C-1), 166.0, 170.3, 170.6, 171.0 (C=O's); FAB-MS (pos. *m*/*z*) calcd for $C_{18}H_{27}N_2O_{10}Br$: 510.08; found: 511.06 (M^++1, 14.6%), 513.05 (M^++3, 13.6%).

4.1.11. Dimer (9). A solution containing 2-bromoacetamidoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranoside (**8**) (0.42 g, 0.819 mmol), mono-*N*-Boc-1,4-diaminobutane (77 mg, 0.410 mmol) and DIPEA (0.2 mL, 1.23 mmol) in CH_3CN (5 mL) was heated at 60°C for 48 h. The reaction solution was concentrated and dissolved in CH_2Cl_2 (10 mL). The organic solution was washed with saturated $NaHCO_3$ (1×5 mL) and water (1×5 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated. Purification by silica gel chromatography of the crude product eluting with 18:1:1 $CHCl_3$/MeCN/MeOH afforded 0.31 g (72%) of a white foam; $[\alpha]_D$=+91.4 (*c* 0.22, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: (protons assignments are numbered from a–e from the anomeric oxygen throughout) 1.40 (s, 9H, *t*-Bu), 1.48 (bs, 4H, H-d, H-e), 1.95, 1.96, 2.01, 2.12 (4s, 24H, OAc, NAc), 2.67 (b, 2H, H-c), 3.10–3.15 (m, 2H, H-f), 3.18 (bs, 4H, $COCH_2$N), 3.37–3.45 (m, 2H, H-b′), 3.52–3.61 (m, 4H, H-a′, H-b), 3.70–3.76 (m, 2H, H-a), 4.02–4.11 (m, 4H, H-6′s), 4.17 (dd, 2H, $J_{5,6}$=6.3 Hz, H-5), 4.57 (ddd, 2H, $J_{2,3}$=11.4 Hz, $J_{2,NH}$=9.5 Hz, H-2), 4.82–4.86 (m, 1H, NHBoc), 4.87 (d, 2H, $J_{1,2}$=3.4 Hz, H-1), 5.10 (dd, 2H, $J_{3,4}$=2.9 Hz, H-3), 5.33 (d, 2H, H-4), 6.76 (b, 2H, NHAc), 7.63 (b, 2H, NHCO); ^{13}C NMR ($CDCl_3$) δ 20.7 (OAc), 22.9 (NAc), 23.0 (C-d), 27.3 (C-e), 28.4 (*t*-Bu), 39.0 (C-b), 39.6 (C-f), 47.4 (C-2), 55.0 (C-c), 58.6 ($COCH_2$N), 62.0 (C-6), 66.7 (C-3), 67.2 (C-4), 67.4 (C-a), 68.4 (C-5), 79.4 (CMe$_3$), 98.4 (C-1), 156.4, 170.4, 170.6, 170.8, 171.4 (C=O's); FAB-MS (pos. *m*/*z*) calcd for $C_{45}H_{72}N_6O_{22}$: 1048.47; found: 1049.50 (M^++1, 18.1%). Anal. calcd C, 51.50; H, 6.92; N, 8.01; found: C, 51.18; H, 6.86; N, 7.88.

4.1.12. Bromoacetyl derivative (11). Compound **7** (0.43 g, 0.72 mmol) was treated with 20% TFA in CH_2Cl_2 (5 mL) at room temperature for 2 h and the solvent was evaporated. The residue was then co-evaporated with toluene twice to remove residual TFA. The de-protected amine salt was dissolved in CH_2Cl_2 (20 mL) and DIPEA (0.31 mL, 1.8 mmol) was added at 0°C. A solution of bromoacetyl chloride (70 mL, 0.87 mmol) in CH_2Cl_2 (5 mL) was added dropwise and the reaction solution was stirred at 0°C for 20 min. The solution was washed with 5% aqueous HCl (1×10 mL), saturated $NaHCO_3$ (1×10 mL) and water (1×10 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated. Silica gel chromatography of the crude product eluting with 18:1:1 $CHCl_3$/CH_3CN/MeOH yielded **11** (0.38 g, 85%) of an off-white foam; $[\alpha]_D$=+74.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.34 (quintet, 2H, *J*=7.5 Hz, H-e), 1.51–1.62 (m, 2H, H-f), 1.63–1.70 (m, 2H, H-d), 1.95, 1.98, 2.01, 2.13 (4s, 12H, OAc, NAc), 2.15–2.28 (m, 2H, H-c), 3.22–3.38 (m, 3H, H-b, H-g), 3.53–3.62 (m, 2H, H-a, H-b′), 3.68–3.72 (m, 1H, H-a′), 3.84 (s, 2H, CH_2Br), 4.04–4.09 (m, 2H, H-6′s), 4.15 (dd, 1H, $J_{5,6}$=6.5 Hz, H-5), 4.54 (ddd, 1H, $J_{2,3}$=11.4 Hz, $J_{2,NH}$= 9.5 Hz, H-2), 4.86 (d, 1H, $J_{1,2}$=3.5 Hz, H-1), 5.07 (dd, 1H, $J_{2,3}$=11.4 Hz, $J_{3,4}$=3.3 Hz, H-3), 5.33 (d, 1H, H-4), 6.29 (m, 1H, NHCO), 6.46 (d, 1H, AcNH), 6.75 (m, 1H, NHCO); ^{13}C NMR ($CDCl_3$) δ 20 6 (OAc), 23.0 (NAc), 24.8 (C-d), 25.9 (C-e), 28.8 (C-f), 29.0 (CH_2Br), 36.1 (C-c), 39.1 (C-g), 39.3 (C-b), 47.4 (C-2), 61.9 (C-6), 66.6 (C-3), 67.1 (C-4), 68.3 (C-5), 68.3 (C-a), 98.4 (C-1), 166.3, 170.3, 170.5, 170.8, 173.3 (C=O's); FAB-MS (pos. *m*/*z*) calcd for $C_{24}H_{38}N_3O_{11}Br$: 623.17; found: 624.26 (M^++1, 9.9%), 626.26 (M^++3, 10.1%). Anal. calcd C, 46.22; H, 6.15; N, 6.74; found: 46.43; H, 6.03; N, 6.65.

4.1.13. Dimer (12). A mixture of bromide **11** (0.44 g, 0.71 mmol), mono-*N*-Boc-1,4-diaminobutane (66 mg, 0.35 mmol) and DIPEA (0.18 mL, 1.06 mmol) in CH_3CN

(3 mL) was heated at 60°C for 48 h. The reaction solution was concentrated and the residue was dissolved in $CHCl_3$ (10 mL). The solution was washed with saturated with $NaHCO_3$ (1×5 mL), water (1×5 mL) and dried over anhydrous Na_2SO_4. Silica gel chromatography of the concentrated residue eluting with 18:1:1 $CHCl_3$/CH_3CN/MeOH yielded **12** (0.33 g, 73%) of a white foam; $[\alpha]_D$=+59.1 (*c* 1.0, $CHCl_3$), ^{1}H NMR ($CDCl_3$) δ 1.24–1.32 (quintet, 4H, *J*=7.4 Hz, $2CH_2$), 1.39 (s, 9H, *t*-Bu), 1.47–1.53 (m, 8H, $4CH_2$), 1.93, 1.96, 2.00, 2.1 (4s, 24H, 6OAc, 2NAc), 2.13–2.17 (t, 4H, *J*=7.4 Hz, $2CH_2CONH$), 3.02–3.33 (m, 14H, $5CH_2$, 4C*H*H), 3.45–3.52 (m, 2H, 2CH*H*), 3.55–3.66 (m, 2H, CH_2), 3.68–3.75 (m, 2H, 2C*H*H), 4.02–4.10 (m, 4H, 2H-6′s), 4.12–4.16 (m, 2H, 2H-5), 4.54 (ddd, 2H, $J_{2,3}$=11.4 Hz, $J_{2,NH}$=9.5 Hz, 2H-2), 4.84 (d, 2H, $J_{1,2}$=3.6 Hz, 2H-1), 4.93–4.99 (m, 1H, NHBoc), 5.10 (dd, 2H, $J_{3,4}$=3.3 Hz, 2H-3), 5.32 (d, 2H, 2H-4), 6.90–7.05 (b, 4H, 4NH), 7.50–7.65 (b, 2H, 2NH); ^{13}C NMR ($CDCl_3$) δ 20.7 (OAc), 23.0 (NAc), 25.0 (CH_2), 26.1 (CH_2), 27.0 (CH_2), 28.4 (*t*-Bu), 29.0 (CH_2), 36.2 (CH_2), 38.6 (CH_2), 39.0 (CH_2), 39.7 (CH_2), 42.0 (CH_2), 47.4 (C-2), 58.8 (CH_2), 62.0 (C-6), 66.7 (C-3), 67.3 (C-4), 68.1 (CH_2), 68.5 (C-5), 79.4 (*C*Me_3), 98.4 (C-1), 156.6, 170.4, 170.5, 170.6, 170.7, 173.5 (C=O's); FAB-MS (pos. *m*/*z*) calcd for $C_{57}H_{94}N_8O_{24}$: 1274.64; found: 1275.58 (M^++1, 10.2%).

4.1.14. Coupling of 5 with bipyridine (15b). A solution of 2,2′-bipyridine-4,4′-dicarboxylic acid **14a** (54.2 mg, 0.222 mmol) in $SOCl_2$ (3 mL) was refluxed for 2 h and the solution was concentrated to provide a yellowish solid. To a solution of 2-aminoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranoside hydrochloride (**5**) (0.19 g, 0.444 mmol) in CH_2Cl_2 (10 mL) were added Et_3N (0.15 mL, 1.11 mmol) at 0°C and previously prepared solution of 4,4′-bis(chlorocarbonyl)-2,2′-bipyridine **14a** in CH_2Cl_2 through a dropping funnel. The solution was stirred at 0°C for 3 h. During the reaction, the solution turned into a pink color. After the reaction was completed, the solution was concentrated and the residue **15a** was dissolved in MeOH (10 mL). The methanolic solution was treated with 1 M NaOMe until pH 9 and stirred at room temperature for 3 h. The de-*O*-acetylated product was precipitated out from the solution and the filtration of the product through a fritted glass funnel provided **15b** (0.13 g, 81%) as a white solid; *O*-acetylated compound **15a**: ^{1}H NMR ($CDCl_3$) δ 1.93, 2.10 (2s, 24H, OAc, NAc), 3.55–3.72 (m, 4H, H-b, H-b′), 3.75–3.86 (m, 4H, H-a, H-a′), 4.02–4.10 (m, 4H, H-5, H-6′), 4.21 (t, 2H, $J_{5,6}$=6.3 Hz, H-6), 4.54 (ddd, 2H, $J_{2,NH}$=9.5 Hz, $J_{2,3}$=11.2 Hz, H-2), 4.89 (d, 2H, $J_{1,2}$=3.5 Hz, H-1), 5.14 (dd, 2H, $J_{3,4}$=2.0 Hz, H-3), 5.33 (d, 2H, H-4), 6.45 (d, 2H, NHAc), 7.62 (b, 2H, NHCO), 7.76 (d, 2H, H-d), 8.66 (s, 2H, H-g), 8.71 (d, 2H, H-e); ^{13}C NMR ($CDCl_3$) δ 21.3 (OAc), 23.5 (NAc), 40.6 (C-b), 48.1 (C-2), 62.8 (C-6), 67.5 (C-3), 67.9 (C-a), 68.7 (C-4), 68.9 (C-5), 99.8 (C-1), 118.6 (C-g), 123.1 (C-d), 143.3 (C-f), 150.6 (C-e), 156.3 (C-c), 166.5, 170.9, 171.1, 171.2, 171.4 (C=O's); FAB-MS (pos. *m*/*z*) calcd for $C_{44}H_{56}N_6O_{20}$: 988.35; found: 989.37 (M^++1, 54.8%); **15b**: $[\alpha]_D$=+38.8 (*c* 0.5, DMSO); FAB-HRMS (pos. *m*/*z*) calcd for $C_{32}H_{45}N_6O_{14}$: 737.2994; found: 737.2830 (M^++1, 38.4%). Anal. calcd for $C_{32}H_{44}N_6O_{14}$ C, 66.64; H, 7.69; N, 14.57; found: C, 66.84; H, 7.78; N, 14.45.

4.1.15. Coupling of 7 with bipyridine (16b). 4,4′-Bis(chlorocarbonyl)-2,2′-bipyridine **14b** was prepared as above from **14a** (51.8 mg, 0.212 mmol) in $SOCl_2$ (3 mL) for 2 h. Compound **6** (0.256 g, 0.424 mmol) was treated with 20% TFA in CH_2Cl_2 (5 mL) for 2 h at room temperature. When the reaction was complete, the solvent was evaporated and the residual TFA was removed by co-evaporating the residue with toluene. Then, the de-protected amine salt was dissolved in CH_2Cl_2 (10 mL) and Et_3N (0.12 mL, 0.848 mmol) was added at 0°C. A solution of 4,4′-bis(chlorocarbonyl)-2,2′-bipyridine in CH_2Cl_2 (5 mL) was added to the reaction mixture through a dropping funnel and the solution was stirred at 0°C for 1 h and at room temperature for another 2 hours. The reaction turned into a pink in color. This solution was concentrated and dissolved in MeOH (10 mL). 1 M NaOMe solution was added to the pinkish solution until pH 9 and it was stirred at room temperature for 3 h. As the reaction proceeded, white precipitates came out from the solution and were filtered through a fritted glass funnel to afford **16b** (0.15 g, 76%) of a white solid; *O*-acetylated **16a**: FAB-MS (pos. *m*/*z*) calcd for $C_{56}H_{78}N_8O_{22}$: 1214.52; found: 1215.86 (M^++1, 6.2%); **16b**: $[\alpha]_D$=+54.5 (*c* 0.2, DMSO); ^{1}H NMR (D_2O) δ 1.47 (quintet, 4H, *J*=7.6 Hz, H-e), 1.72 (quintet, 8H, *J*=7.4 Hz, H-d, H-f), 2.09 (s, 6H, NAc), 2.36 (t, 4H, *J*=7.3 Hz, H-c), 3.33–3.40 (m, 2H, H-b′), 3.46 (t, 4H, *J*=6.8 Hz, H-g), 3.52–3.58 (m, 4H, H-a′, H-b), 3.74–3.83 (m, 6H, H-6′s, H-a), 3.88–3.96 (m, 4H, H-3, H-5), 4.01 (d, 2H, $J_{3,4}$=2.8 Hz, H-4), 4.22 (dd, 2H, $J_{2,3}$=11.0 Hz, H-2), 4.89 (d, 2H, $J_{1,2}$=3.6 Hz, H-1), 7.75 (d, 2H, *J*=4.6 Hz, H-i), 8.29 (s, 2H, H-l), 8.76 (d, 2H, *J*=4.6 Hz, H-j); ^{13}C NMR (D_2O) δ 21.5 (C-e), 24.6 (NAc), 25.2 (C-d), 27.5 (C-f), 35.2 (C-c), 38.4 (C-b), 39.4 (C-g), 49.3 (C-2), 60.7 (C-6), 65.9 (C-3), 67.4 (C-a), 68.0 (C-4), 70.6 (C-5), 96.7 (C-1), 118.9 (C-l), 121.5 (C-i), 142.6 (C-k), 149.7 (C-j), 154.7 (C-h), 167.1, 173.9, 176.4 (C=O's); FAB-HRMS (pos. *m*/*z*) calcd for $C_{44}H_{67}N_8O_{16}$: 963.4675; found: 963.4680 (M^++1, 5.6%). Anal. calcd $C_{44}H_{66}N_8O_{16}$ C, 54.88; H, 6.91; N, 11.64: found: C, 54.98; H, 6.75; N, 11.25.

4.1.16. Coupling of dimer 10 with bipyridine (17b). 4,4′-Bis(chlorocarbonyl)-2,2′-bipyridine **14b** was prepared as above by refluxing 2,2′-bipyridine-4,4′-bicarboxylic acid **14a** (10 mg, 0.041 mmol) with $SOCl_2$ (3 mL) for 2 h. Compound **9** (86 mg, 0.082 mmol) was treated with 20% TFA in CH_2Cl_2 (5 mL) for 3 h. The solvent and the residual TFA were co-evaporated with toluene. To a solution of amine salt and Et_3N (28 mL, 0.205 mmol) in CH_2Cl_2 (2 mL) was added a solution of carbonyl chloride in CH_2Cl_2 (2 mL) at 0°C and stirred for 3 h. The pinkish solution was concentrated and the residue was purified by size exclusion column chromatography (Sephadex LH 20) eluting with MeOH. The purified product was then treated with 1 M NaOMe in pH 9 for 16 h. The solution was concentrated and size exclusion column chromatography (Sephadex LH 20) of the crude product eluting MeOH yielded 61.5 mg (94%) of a white foam; *O*-acetylated compound **17a**: ^{1}H NMR ($CDCl_3$) δ 1.49–1.68 (m, 8H, H-e, H-f), 1.92, 199, 2.10 (3s, 48H, OAc, NAc), 2.55–2.68 (m, 4H, H-d), 3.10–3.25 (m, 8H, H-c), 3.31–3.55 (m, 12H, H-a′, H-b, H-b′), 3.60–3.70 (m, 8H, H-a, H-g), 3.98–4.15 (m, 12H, H-5, H-6′s), 4.52 (ddd, 4H, $J_{2,3}$=11.4 Hz, $J_{2,NH}$=9.4 Hz, H-2), 4.84 (d, 4H, $J_{1,2}$=3.6 Hz, H-1), 5.07 (dd, 4H, $J_{3,4}$=3.1 Hz, H-3), 5.29 (d, 4H, H-4), 6.92 (d, 4H, NHAc), 7.65 (m, 2H, NHCOAr), 7.73

(d, 2H, J=4.6 Hz, H-j), 7.80–7.88 (m, 2H, NH), 8.67 (s, 2H, H-l), 8.75 (d, 2H, J=4.6 Hz, H-i); FAB-MS (pos. m/z) calcd, for $C_{92}H_{132}N_{14}O_{42}$: 2104.86; found: 2106.56 (M^++1, 8.0%); **17b**: $[\alpha]_D$=+81.8 (c 0.22, DMSO); ^{1}H NMR (D_2O) 1.62 (quintet, 4H, J=7.4 Hz, H-f), 1.67–1.74 (m, 4H, H-e), 2.09 (s, 12H, NAc), 2.62–2.72 (m, 4H, H-d), 3.90 (d, 8H, J=3.1 Hz, H-c), 3.44–3.62 (m, 16H, H-a, H-b, H-b′, H-g), 3.76–3.83 (m, 12H, H-6′s, H-a), 3.87–3.93 (m, 8H, H-3, H-5), 4.00 (d, 4H, $J_{3,4}$=3.2 Hz, H-4), 4.22 (dd, 4H, $J_{2,3}$=11.0 Hz, H-2), 4.89 (d, 4H, $J_{1,2}$=3.7 Hz, H-1), 7.85 (d, 2H, J=5.1 Hz, H-i), 8.45 (s, 2H, H-l), 8.87 (d, 2H, J=5.1 Hz, H-j); ^{13}C NMR (D_2O) δ 21. 6 (NAc), 23.6 (C-f), 25.8 (C-e), 38.4 (C-b), 39.3 (C-g), 49.3 (C-2), 54.8 (C-d), 57.9 (C-c), 60.7 (C-6), 66.0 (C-a), 67.4 (C-3), 68.0 (C-4), 70.6 (C-5), 96.9 (C-1), 119.1 (C-l), 121.6 (C-i), 142.9 (C-j), 149.8 (C-k), 155.0 (C-h), 167.4 (C═O, Ar), 173.31, 173.86 (C═O's); FAB-HRMS (pos. m/z) calcd for $C_{68}H_{109}N_{14}O_{30}$: 1601.7434; found: 1601.6491 (M^++1, 3.6%). Anal. calcd $C_{68}H_{108}N_{14}O_{30}$ C, 50.99; H, 6.80; N, 12.24: found: C, 50.82; H, 6.82; N, 12.66.

4.2. General method for the preparation of self-assembling clusters on Cu(II)

To a solution containing two equimolar amount of de-acetylated divalent bipyridine ligands **15b** and **16b**, or tetravalent ligand **17b** in de-ionized water (1 mL) was added one equimolar amount of $CuSO_4{\cdot}5H_2O$. The reaction mixture was then stirred at room temperature for 48 h. The color of the reaction solution was light bluish purple. The solution was then lyophilized to yield Cu(II) coordinated clusters, **18**–**20**.

4.2.1. Tetramer 18. ^{1}H NMR (D_2O) δ 1.82 (s, 12H, NAc), 3.64–3.82 (m, 20H, CH_2N, OC*H*H, H-6′s), 3.85–3.98 (m, 16H, H-3–H-5, OCH*H*), 4.17–5.02 (m, 4H, H-2), 4.80–4.92 (m, 4H, H-1), 7.75–7.78 (m, 4H, Ar), 7.80–7.84 (m, 4H, Ar), 9.08–9.10 (m, 4H, Ar); ^{13}C NMR (D_2O) δ 21.8 (Nac), 39.4, 49.3, 60.6 (C-6), 65.7, 67.3 (C-3), 68.0 (C-4), 70.5 (C-5), 96.1 (C-1), 122.3, 124.6; MALDI-TOF calcd for $C_{64}H_{88}N_{12}O_{28}Cu$: 1535.51; found: 1536.06.

4.2.2. Tetramer 19. ^{1}H NMR (D_2O) δ 1.38 (s, 8H, H-e), 1.65 (s, 16H, H-d, H-f), 2.08 (s, 12H, NAc), 2.31 (s, 8H, H-c), 3.09 (s, 8H, H-g), 3.37 (s, 4H, H-b′), 3.55 (s, 8H, H-a′, H-b), 3.79 (s, 12H, H-6′s, H-a), 3.92, 3.95 (2s, 8H, H-3, H-5), 4.03 (s, 4H, H-4), 4.22 (s, 4H, H-2), 4.87 (s, 4H, H-1), 7.70, 7.80, 9.03 (bs, H-i, H-j, H-l); ^{13}C NMR (D_2O) δ 19.5 (NAc), 22.5 (C-f), 23.1 (C-e), 24.3 (C-d), 33.1 (C-c), 36.4 (C-b), 37.4 (C-g), 47.3 (C-2), 58.6 (C-6), 63.9 (C-a), 65.3 (C-3), 65.9 (C-4), 68.5 (C-5), 94.7 (C-1), 172.0, 174.4 (C═O's), aromatic peaks are not detectable; FAB-MS (pos. m/z) calcd for $C_{88}H_{132}N_{16}O_{32}Cu$: 1987.85; found: 1988.14 (M^++1, 0.8%), 1025.45 (monomer+Cu, 8.1%); MALDI-TOF calcd: 1987.85; found: 1989.39.

4.2.3. Octamer 20. ^{1}H NMR (D_2O) δ 1.56 (s, 8H, H-f), 1.64 (s, 8H, H-e), 2.10 (s, 24H, NAc), 2.63 (s, 8H, H-d), 3.33 (s, 16H, H-c), 3.47–3.63 (m, 32H, H-a′, H-b, H-b′, H-g), 3.81 (s, 24H, H-a, H-6′s), 3.93 (s, 16H, H-3, H-5), 4.02 (s, 8H, H-4), 4.23 (s, 8H, H-2), 4.94 (s, 8H, H-1), aromatic peaks were broadened by the paramagnetic Cu(II) ions; ^{13}C NMR (D_2O) δ 19.6 (NAc), 22.8 (C-f), 22.8 (C-e), 36.4 (C-b), 37.4 (C-g), 47.3 (C-2), 52.7 (C-d), 55.8 (C-c), 58.7 (C-6), 64.0 (C-a), 65.4 (C-3), 66.0 (C-4), 68.6 (C-5), 94.9 (C-1), 171.2, 171.8 (C═O's); MALDI-TOF calcd for $C_{136}H_{216}N_{28}O_{60}Cu$: 3264.41; found: 3267.76.

4.3. Enzyme linked lectin assay (ELLA)

Nunc microtitration plates were coated with asialoglycophorin at 100 μL/well of a stock solution of 5 mg/mL in 0.01 M phosphate buffer (PBS, pH 7.3) for 2 h at 37°C. The wells were then washed three times with 300 μL/well of 0.01 phosphate buffer (pH 7.3) containing 0.05% (v/v) Tween 20 (PBST). This washing procedure was repeated after each incubation period. The wells were then blocked with 150 μL/well of 1% BSA/PBS for 1 h. Inhibitors used include allyl α-D-GalNAc as a reference monovalent compound and synthetic multivalent GalNAc-containing ligands which were used as stock solution of 0.317 μM in PBS. Each inhibitor was added in serial 2- to 10-fold dilutions (60 μL/well) in PBS with appropriate lectin-enzyme conjugate concentration (60 μL/well of 500-fold dilution of a 1 mg/mL stock solution of *V. villosa* in PBS) on Linbro (Titertek) microtiter plates. These inhibitor solutions (100 μL) were then transferred to antigen-coated plates and incubated for another hour at 37°C. The plates were washed and 50 μL/well of 2,2′-azinobis(3-ethylbenzothiazolin-6-sulfonic acid), diammonium salt (ABTS, 1 mg/4 mL) in citrate-phosphate buffer (0.2 M, pH 4.0 with 0.015% H_2O_2) was added. The reaction was stopped after 20 min by adding 50 μL/well of 1 M H_2SO_4 and optical density measured at 410 nm. The percent inhibition was calculated as follows:

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

IC_{50} values were reported as the concentration required for 50% inhibition of the coating antigen. Each test was performed in duplicate.

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