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Synthesis and molecular recognition of carbohydrate-centered multivalent glycoclusters by a plant lectin RCA_{120}

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Abstract—Water soluble and lectin-recognizable carbohydrate-centered glycoclusters were prepared efficiently by the Huisgen 1,3-cycloaddition reaction of methyl-2,3,4,6-tetra-*O*-propargyl β-D-galactopyranoside with 2-azidoethyl glycosides of lactose and *N*-acetyllactosamine. Their binding by a plant lectin RCA_{120} was examined by capillary affinity electrophoresis using fluorescence-labeled asialoglycans from human α1-acid glycoprotein. The glycoclusters showed 400-fold stronger inhibitory effect than free lactose, manifesting strong multivalency effect.
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1. Introduction

Carbohydrate–protein recognition is important in many biological processes in living systems, such as signal transductions, cell adhesion, inflammation, and cancer metastasis, bacterial and viral infection.[1–3] However, in most cases, monosaccharide–protein interactions are very weak (the K_d value being about 10^{-3}–10^{-6} M). In nature, the weak binding of monosaccharides is compensated by multiple and simultaneous binding of many sugar residues,[4] a phenomenon now often referred to as 'glycoside clustering effect'.[5,6] To investigate the carbohydrate recognition with the consideration of glycoside clustering effect, synthetic multivalent glycoconjugates have been used extensively as mimetics of naturally occurring branched carbohydrate ligands. Syntheses of such compounds typically utilize polyfunctional molecules as 'scaffold' to organize structurally well-defined mono- or oligosaccharide units to fit conformation of sugar-binding proteins. For example, monovalent sugars were coupled to such scaffolds as cyclodextrins (CDs),[7] calix[4]-allenes,[8] silsesquioxanes,[9,10] and peptides.[11,12] Applications of the multivalent glycoconjugates include prevention of early adhesion of neutrophils to endothelial surfaces,[13] neutralization of viruses and toxins,[14] and carbohydrate-based anticancer therapy,[15] despite some drawbacks.[16] However, most conventional methods for the preparation of glycoclusters require lengthy and reiterative synthetic steps, requiring protection and deprotection before and after the glycocluster construction.

To obtain simpler and more efficient glycoclusters, we recently reported a new strategy based on the use of thiol radical addition of unprotected ω-thioglycosides to octavinyloligosilsequioxane, eliminating the need to protect and deprotect during glycoclusters construction.[10] In recent years, the Huisgen 1,3-dipolar cycloaddition reaction between an azide and terminal alkyne has been gaining considerable attention as a powerful approach for preparation of peptidotriazoles,[17] fabrication of carbohydrate arrays,[18] synthesis of multivalent neoglycoconjugates,[19] as well as labeling proteins and cell surfaces.[20]

Carbohydrate molecules are innately polyfunctional, and have been widely exploited as multivalent scaffolds for the synthesis of oligosaccharide mimetics with outstanding biological functions. For example, Starfish, a powerful ligand for Shiga-like toxin was developed from a glucoside derivative.[14] We thought that the 1,3-dipolar cycloaddition could be useful for preparation of carbohydrate-centered glycoclusters by the simultaneous

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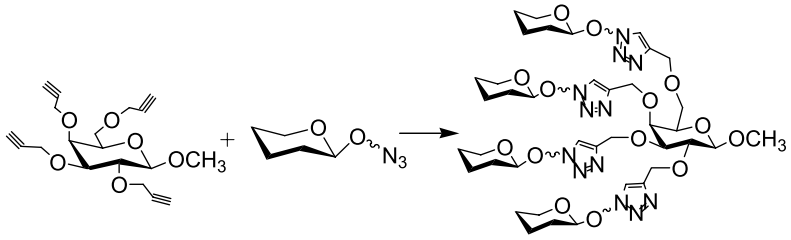


Figure 1. The outline of the synthesis of carbohydrate-centered glycoclusters.

assembly of alkynyl glycoside and azide glycosides. We now describe in this paper, the first synthesis of methyl-2,3,4,6-tetra-*O*-propargyl-β-D-galactopyranoside and its use as a polyfunctional scaffold for construction of glycocluster. Our facile synthesis involves rapid assembling of multivalent glycoclusters in a convergent way from unprotected lactose and *N*-acetyllactosamine derivative using the Huisgen 1,3-cycloaddition reaction as the key step. Our strategy is summarized in Figure 1.

Efficacy of the synthesized glycoclusters was evaluated by capillary affinity electrophoresis (CAE).[21] Inhibition of the binding of the desialylated N-linked oligosaccharides from α1-acid orosomucoid (AGP-asialoglycans) to RCA_{120} by the glycoclusters was quantified and compared with that by lactose.

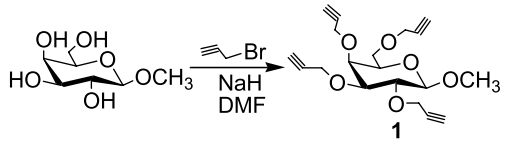
2. Results and discussion

2.1. Synthesis of the tetravalent carbohydrate scaffold

Because of their easy availability, biocompatibility, low toxicity, polyfunctionality, and intrinsic chirality, carbohydrate molecules have been widely used as cores for synthesis of a variety of glycoclusters and glycodendrimes.[22–24] In our current study, we chose the inexpensive commercially available methyl β-D-galactopyranoside as the core molecule. To transform methyl β-D-galactopyranoside into a suitable template for multivalent glycocluster assembly, uniform propargylation of all four hydroxyl groups of methyl β-D-galactopyranoside was accomplished with propargyl bromide and NaH in DMF to give the alkyne cluster **1** in 80% yield (Scheme 1). The product was purified by standard silica gel chromatography.

2.2. Synthesis of azide glycosides

Lactose (Lac) and *N*-acetyllactosamine (LacNAc) are two of the most common structures in a variety of glycoconjugates, and are known to be involved in various types of normal and pathological reactions. In addition, lactosyl group is the primer disaccharide in the biosynthesis of most glycosphingolipids. LacNAc is also a precursor of sialyl Lewis^x, which plays an important role in selectin-mediated adhesion of neutrophils to endothelial cells, the initial event in many inflammatory responses.[25,26] Thus, we chose azide-bearing glycosides of lactose and *N*-acetyllactosamine as monomeric structures for clustering.



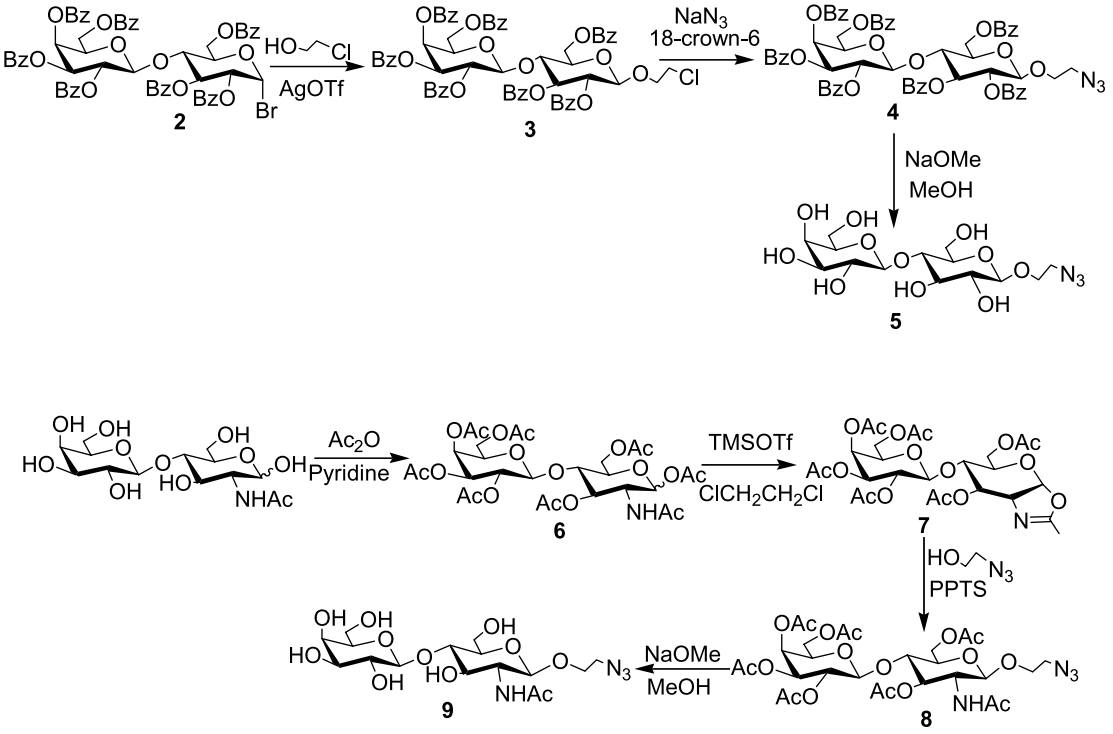
Scheme 1. Synthesis of carbohydrate scaffold.

Although compound **5** has been synthesized before,[27] we used a different route for synthesis as shown in Scheme 2. Per-*O*-benzoylated lactosyl bromide **2**[28] was reacted with 2-chloroethanol under the catalysis of silver triflate (AgOTf) to give the β-lactoside derivative **3** in near quantitative yield. The desired β-anomeric configuration was confirmed by the relatively large coupling constant of the anomeric proton signal ($J_{1,2}$ = 8.0 Hz). It should be noted that Bz-protected lactosyl bromide was chosen over the conventional acetylated counterpart to exclude the possibility of orthoester or α-glycoside formation.[29] The chloroethyl glycoside was then reacted with sodium azide (NaN_3) via an S_N2 type reaction to give 2-azidoethyl glycoside **4** in high yield (90%). The two-step procedure was used instead of direct glycosylation of 2-azidoethanol which is potentially explosive.[30] Subsequent deprotection under the Zemplén condition gave the unprotected glycoside **5**.

The synthesis of the ω-azido-glycoside of *N*-acetyllactosamine, **9**,[31] is shown in Scheme 2. Per-*O*-acetylation of starting material, *N*-acetyllactosamine, produced compound **6**[32] in quantitative yield. This was converted into the oxazoline derivative **7** by treating with TMSOTf.[33] The oxazoline **7** was then allowed to react with 2-azidoethanol using pyridinium *p*-toluenesulfonate (PPTS) as a promoter,[34] giving the corresponding β-glycoside **8**.[35] Subsequent deprotection under the Zemplén condition gave the unprotected glycoside **9**.[31]

2.3. Synthesis of tetravalent carbohydrate-centered glycoclusters

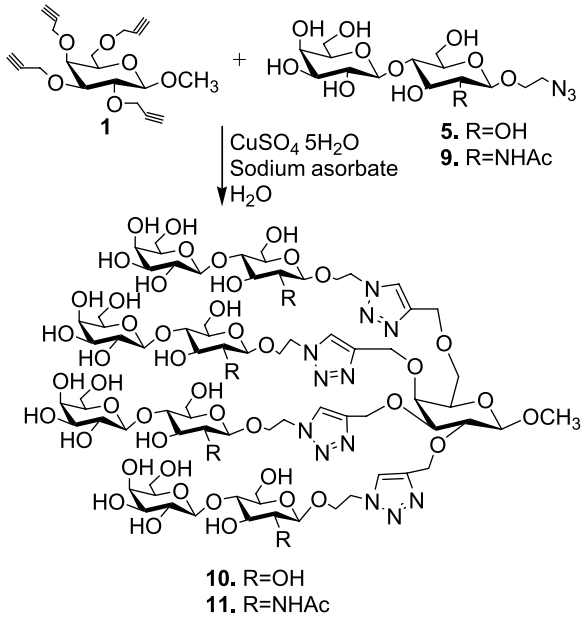
Several neoglycoconjugates have been prepared by copper (I)-catalyzed 1,3-cycloaddition reaction through the use of protected glycosides in organic solvents.[19] We show that unprotected glycoside can be used in this reaction equally efficiently, and this reduces the number of protection and deprotection steps before and after glycocluster assembly. Moreover, our method uses



Scheme 2. Synthesis of 2-azidoethyl glycosides.

aqueous solution and thus is more compatible with environmental protection.

As shown in Scheme 3, direct assembly of glycoclusters was achieved by reacting alkynyl galactoside and unprotected azide glycosides in the presence of $CuSO_4$/sodium ascorbate in water[36] to give the corresponding products **10** and **11**, which are easily isolated in high yields by simple gel filtration. They were fully characterized by ^1H NMR, ^{13}C NMR, and MALDI-TOF mass spectroscopy.



Scheme 3. Assembly of glycoclusters.

2.4. Inhibition of RCA_{120} binding of asialoglycans from human αl-acid glycoprotein by the glycoclusters

RCA_{120} is a bivalent lectin specifically recognizing the exposed β-D-galactose residues. In solution, RCA_{120} binds two galactosides, one by each subunit.[37,38] The binding affinity of glycoclusters **10** and **11** to RCA_{120} was estimated by capillary affinity electrophoresis (CAE) by inhibition of the binding of asialoglycans from human α1-acid glycoprotein (AGP-asialoglycans). AGP contains di-, tri-, and tetra-antennary carbohydrate chains, and the asialoglycans thereof have terminal Galβ1-4GlcNAc on all branches and show high affinity toward RCA_{120}. AGP-asialoglycans showed discrete peaks (A1–A5), identified previously,[39] as shown in Figure 2.

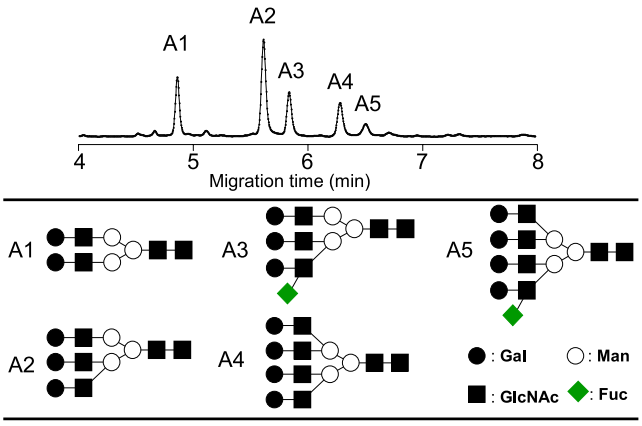


Figure 2. Capillary electrophoresis of AGP-asialoglycans.

In the presence of RCA_{120}, AGP-asialoglycans were bound by RCA_{120} and their peaks were broadened as shown in Figure 3. All peaks broadened as a function of RCA_{120} concentration, but the triantennary glycan (A_3) and tetra-antennary glycans (A_4 and A_5) were most sensitive to lectin RCA_{120}.

The peak broadening was increasingly suppressed when the increasing amounts of the glycocluster were added to the sample. Eventually the peaks became as discrete as the free (unbound) oligosaccharides as shown in Figures 2 and 3 (top profile). For comparison, free lactose was used as monovalent reference under the same experimental conditions. The minimum concentrations of saccharide units to completely inhibit the binding between the AGP-asialoglycans and RCA_{120} (IC_{min}) are summarized in Table 1.

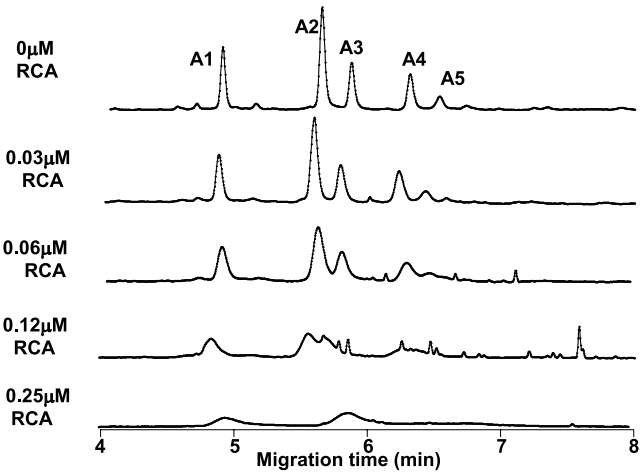


Figure 3. Capillary affinity electrophoresis of AGP-asialoglycans in the presence of various concentrations of RCA_{120}.

Table 1. Inhibition of the binding between RCA_{120} and AGP-asialoglycans by the glycoclusters and lactose

Inhibitor	IC_{min}[a] (M)[b]
Lactose	20×10^{-3}
Glycocluster **10**	50×10^{-6}
Glycocluster **11**	50×10^{-6}

[a] Minimum inhibition concentration.
[b] Molarity of saccharide unit.

These data indicate that glycoclusters **10** and **11** are equally well recognized by RCA_{120} and they are excellent inhibitors for the binding of lectin RCA_{120} to AGP-asialoglycans. Their inhibitory power is much stronger than the free lactose (minimum inhibition concentration of 20 mM) by a factor of 400 (minimum inhibition concentration of 50 μM). From these inhibition experiments, it appears that a significant effect of multivalency is observed for the glycoclusters. A typical inhibitory experiment of glycocluster is illustrated in Figure 4.

3. Conclusion

A simple and effective preparation of carbohydrate-centered glycoclusters based on 1,3-cycloaddition reaction of uniformly alkynylated carbohydrate core with unprotected ω-azido-glycosides was developed. We demonstrated the usefulness of this approach by the preparation glycoclusters of lactose and *N*-acetyllactosamine, which were shown to be bound to RCA_{120} 400-fold more strongly than monovalent lactose. Given the modular nature of this strategy, this approach may offer an alternative avenue for preparing glycoclusters with different densities and spacers, expanding the repertoire of the existing glycoclusters for bioassays and biorecognition.

4. Experimental

4.1. General procedures

Chemicals were from Aldrich (Milwaukee, WI) and used as received. Column chromatography was carried out

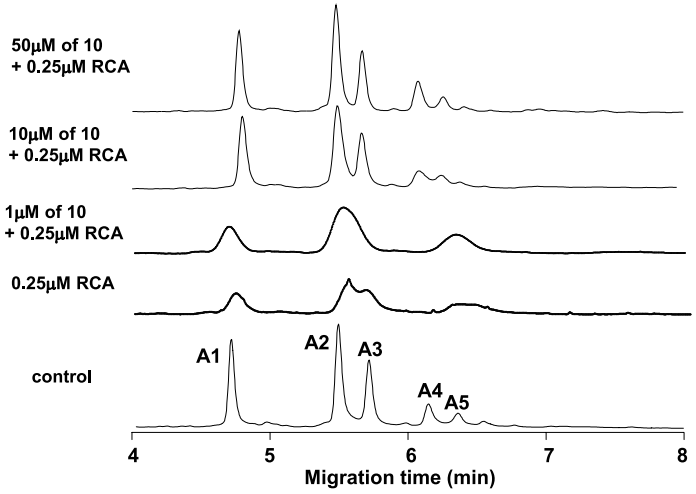


Figure 4. Inhibition effects of glycocluster **10** on the binding between RCA_{120} and AGP-asialoglycans.

using E. Merck silica gel 60F (230–400 mesh). Analytical thin-layer chromatography (TLC) was performed on aluminum sheets coated with silica gel 60 F_{254} (0.25 mm thickness, E. Merck, Darmstadt, Germany). Detection of UV-absorbing compounds on TLC plates was by quenching of the silica gel-imbedded fluorescence and/or charring with 15% H_2SO_4 in ethanol solution followed by heating at ca. 180 °C. The ratios of solvents used for TLC are expressed as vol/vol. Gel filtration chromatography was performed using a column packed with Sephadex G-15 (2×145 cm) eluting with distilled water. Carbohydrates were determined with a version of the phenol–sulfuric acid method.[40] Melting points were determined with a Fisher-Johns apparatus and are not corrected. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Varian-400 NMR spectrometer at nominal resonance frequencies of 400 and 100 MHz, respectively, in $CDCl_3$ (referenced to internal Me_4Si at δ_H 0 ppm, δ_C 0 ppm) and D_2O (referenced to internal acetone at δ_H 2.225 ppm, δ_c 29.8 ppm). The chemical shifts (δ) were expressed in parts per million (ppm). First order J values were given in hertz. Fast atom bombardment mass spectra (FAB MS) data were obtained using *m*-nitrobenzyl alcohol as matrix. Matrix-assisted laser desorption ionization time-of-flight mass spectra (MALDI-TOF MS) were recorded with a MALDI-Compact (Kratos) instrument in the positive mode using 2,5-dihydroxybenzoic acid (DHB) as matrix unless otherwise indicated and an average of 50 laser shots per sample.

4.2. Procedures for capillary affinity electrophoresis (CAE)

Capillary affinity electrophoresis was performed with a P/ACE MDQ glycoprotein system (Beckman Coulter, Fullerton, CA, USA) equipped with an eCAP N-CHO capillary (20 cm effective length, 30 cm total length, 50 μm i.d., Beckman Coulter) using an argon laser-induced fluorescence detector as described previously.[21] Detection was performed by installing a 520 nm filter for emission with a 488 nm argon laser for excitation. Tris–acetate buffer (100 mM, pH 7.4) was used as an electrolyte throughout the work. The sample solution was introduced to the capillary by pressure method (0.5 psi, 5 s). Separation was performed at 25 °C at the applied potential of 18 kV. Data were collected and analyzed with a standard 32 Karat software (Version 4.0, Beckman Coulter) on Microsoft Windows 2000. The procedures are briefly as follows. First, a mixture of fluorescence-labeled oligosaccharides only was analyzed. Then, the capillary was filled with the same electrolyte, but containing the lectin at a specified concentration, and the same mixture of fluorescent oligosaccharides was analyzed.

4.3. Methyl-2,3,4,6-tetra-*O*-propargyl-β-D-galactopyranoside (1)

To a solution of methyl β-D-galactopyranoside (388 mg, 2 mmol) in dry DMF (10 mL) was added NaH (60% w/w in mineral oil, 480 mg, 12 mmol) at 0 °C with frequent venting. After stirring for 20 min, propargyl bromide (80% in toluene, 2.25 mL, 20 mmol) was added slowly and the mixture was stirred at 0 °C for 20 min and maintained at rt for 12 h. TLC indicated the disappearance of the starting material. The reaction mixture was cooled to 0 °C and MeOH (20 mL) was added. The solvents were removed under reduced pressure. The residue was suspended in water (30 mL) and extracted with ethyl acetate (350 mL). The combined organic layer was washed with satd aqueous NaCl (50 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a brown oil which was purified by silica gel chromatography (hexanes/EtOAc 3:1) to give a pale yellow foam (556 mg, 80%). R_f = 0.42 (hexanes/EtOAc 2:1). ^{1}H NMR (400 MHz, $CDCl_3$, rt) δ 4.33–4.49 (4 dd, 8H, J = 14.8 and 2.4 Hz, 4-OCH_2), 4.20–4.26 (m, 3H), 4.04 (br t, 1H), 3.80 (dd, 1H, J = 10 and 2.0 Hz), 3.71 (dd, 1H, J = 10 and 2.0 Hz), 3.58–3.61 (m, 3H), 3.52 (s, 3H, OCH_3), 2.46 (m, 4H, 4-CH); ^{13}C NMR (100 MHz, $CDCl_3$, rt): δ 104.21, 80.79, 80.28, 80.09, 79.52, 79.30, 74.67, 74.49, 74.04, 73.32, 73.20, 68.94, 59.90, 59.51, 58.99, 58.71, 56.92; MALDI-TOF MS: *m*/*z* calcd for $C_{19}H_{22}NaO_6$ (M+Na), 369.1; found 368.9.

4.4. 2-Chloroethyl (2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (3)

A mixture of lactosyl bromide[28] (2.64 g, 2.33 mmol) and 2-chloroethanol (4.0 mL, 59.6 mmol) in dry CH_2C1_2 (20 mL) was stirred under N_2 with MS 4 Å (3 g) for 1 h, then cooled to −15 °C and powdered AgOTf (1.3 g, 5.06 mmol) was added. The reaction was allowed to stir at 0 °C for 2 h until the complete disappearance of the starting material, quenched by the addition of Et_3N (5 mL), filtered through a pad of Celite, diluted with CH_2Cl_2 (100 mL), washed with satd aqueous $NaHCO_3$ (50 mL), 2 M aqueous $Na_2S_2O_3$, water (50 mL), dried (Na_2SO_4), and evaporated to give a residue, which was purified by silica gel chromatography (toluene/acetone 25:1) to afford the product as a white foam (2.49 g, 95%). R_f = 0.29 (toluene/acetone 20:1). ^{1}H NMR (400 MHz, $CDCl_3$, rt) δ 7.92–8.00 (m, 12H), 7.11–7.74 (m, 23H, arom), 5.79 (dd, 1H, J = 9.6 Hz, H-3, Glc), 5.68–5.73 (m, 2H), 5.45 (dd, 1H, J = 9.6 and 7.6 Hz, H-2), 5.37 (dd, 1H, J = 10.4 and 3.6 Hz), 4.86 (d, 1H, J = 8.0 Hz, H-1, Gal), 4.76 (d, 1H, J = 8.0 Hz, H-1, Glc), 4.60 (dd, 1H, J = 12.0 Hz, H-6a, Glc), 4.48 (dd, 1H, J = 4.4 Hz, H-6b, Glc), 4.24 (t, 1H, J = 9.6 Hz, H-4, Glc), 3.98, 3.75 (2 m, 2H, $OCH_2CH_2N_3$), 3.84–3.89 (m, 2H), 3.69–3.75 (m, 2H), 3.49 (m, 2H, $OCH_2CH_2N_3$); ^{13}C NMR (100 MHz, $CDCl_3$, rt): δ 165.83, 165.57, 165.40, 165.38, 165.25, 165.22, 164.79, 133.56, 133.41, 133.28, 133.21, 129.99, 129.87, 129.74, 129.68, 129.64, 129.58, 129.53, 129.44, 129.39, 129.27, 128.82, 128.63, 128.59, 128.52, 128.34, 128.25, 101.34, 100.98, 75.95, 73.11, 72.72, 71.74, 71.53, 71.39, 69.85, 67.50, 62.26, 61.04, 42.18; MALDI-TOF MS: *m*/*z* calcd for $C_{63}H_{53}NaClO_{18}$ (M+Na), 1155.3; found 1156.1.

4.5. 2-Azidoethyl (2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (4)

To a solution of the chloride **3** (2.15 g, 1.90 mmol) in dry DMF (10 mL) were added NaN_3 (1.23 g, 19 mmol) and 18-crown-6 (50 mg, 0.19 mmol). The resulting mixture

was heated at 70 °C with stirring for 36 h. The solvent was removed under reduced pressure, the residue was dissolved in EtOAc (100 mL), and washed with brine (50 mL) and water (50 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was subjected to silica gel column chromatography with toluene–acetone (25:1) as the eluent to give **4** (1.95 g, 90%). $R_f = 0.28$ (toluene/acetone 20:1). ^{1}H NMR (400 MHz, $CDCl_3$, rt) δ 7.95–8.02 (m, 12H), 7.13–7.74 (m, 23H, arom), 5.82 (dd, 1H, $J = 9.6$ Hz, H-3, Glc), 5.71–5.76 (m, 2H), 5.49 (dd, 1H, $J = 9.6$ and 7.6 Hz, H-2), 5.40 (dd, 1H, $J = 10.4$ and 3.6 Hz), 4.90 (d, 1H, $J = 8.0$ Hz, H-1, Gal), 4.78 (d, 1H, $J = 7.6$ Hz, H-1, Glc), 4.63 (dd, 1H, $J = 11$ Hz, H-6a, Glc), 4.49 (dd, 1H, $J = 4.4$ Hz, H-6b, Glc), 4.28 (t, 1H, $J = 9.6$ Hz, H-4, Glc), 3.98, 3.75 (2 m, 2H, $OCH_2CH_2N_3$), 3.85–3.93 (m, 2H), 3.63–3.75 (m, 3H), 3.38, 3.24 (2m, 2H, $OCH_2CH_2N_3$); ^{13}C NMR (100 MHz, $CDCl_3$, rt): δ 165.84, 165.57, 165.40, 165.22, 165.19, 164.80, 133.56, 133.41, 133.28, 133.21, 133.18, 129.99, 129.80, 129.74, 129.68, 129.64, 129.57, 129.53, 129.45, 129.39, 129.31, 128.82, 128.63, 128.58, 128.52, 128.31, 128.25, 101.07, 100.98, 75.93, 73.11, 72.82, 71.75, 71.60, 71.40, 69.88, 68.48, 67.50, 62.22, 61.04, 50.53; MALDI-TOF MS: *m/z* calcd for $C_{63}H_{53}N_3Na_{18}$ (M+Na), 1162.3; found 1162.6.

4.6. 2-Azidoethyl (β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (5)[27]

To a solution of **4** (2.28 g, 2 mmol) in dry methanol (30 mL) was added NaOMe (50 mg). The mixture was stirred for 16 h at room temperature. The solvent was removed by evaporation. The residue was dissolved in water (30 mL) and neutralized with Dowex 50Wx8 (H^+), filtered, and extracted with toluene (2×30 mL) and ethyl acetate (20 mL). The water layer was lyophilized to give a powder (740 mg, 91%). ^{1}H NMR (400 MHz, D_2O, rt) δ 4.53 (d, 1H, $J_{1,2} = 8.4$ Hz, H-1), 4.43 (d, 1H, $J = 8.0$ Hz, H-1′), 3.78, 4.05 (2m, 2H, $OCH_2CH_2N_3$), 3.91 (d, 1H), 3.71–3.86 (m, 5H), 3.65 (m, 3H), 3.60 (br, 1H), 3.55 (m, 3H), 3.34 (t, 1H); ^{13}C NMR (100 MHz, D_2O, rt): δ 102.49, 101.74, 77.88, 74.92, 74.38, 73.90, 72.34, 72.08, 70.51, 68.11, 60.59, 59.61, 50.10; MALDI-TOF MS: *m/z* calcd for $C_{14}H_{25}N_3NaO_{11}$ (M+Na), 434.1; found 434.2.

4.7. 2-Azidoethyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*- 2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (8)[35]

A solution of *N*-acetyllactosamine peracetate **6**[32] (1.8 g, 2.64 mmol) in dry 1,2-dichloroethane (12 mL) was treated with TMSOTf (0.5 mL, 2.63 mmol) under a nitrogen atmosphere, and the mixture was stirred overnight at 50 °C. Triethylamine (1.8 mL) was added and the solvent was evaporated. The resulting residue was purified by silica gel chromatography, eluting with toluene–ethyl acetate–triethylamine (100:200:1), to give the syrupy oxazoline derivative **7** (1.3 g, 80%).

To a solution of oxazoline derivative **7**[33] (1.08 g, 1.75 mmol) in 1,2-dichloroethane (35 mL) were added 2-azidoethanol (1.28 mL, 16.9 mmol, 9.6 equiv) and pyridinium *p*-toluenesulfonate (PPTS)[34] (45 mg, 0.175 mmol). The mixture was stirred under a nitrogen atmosphere for 2 h at 70 °C. The solution was cooled, neutralized with pyridine (3 mL), diluted with chloroform (50 mL), and stirred in ice-water. The organic layer was washed successively with aqueous sodium hydrogen carbonate (30 mL) and water (30 mL), dried over anhydrous Na_2SO_4, and evaporated. The residual syrup was purified by silica gel chromatography (hexanes/acetone 1:1) to give pure product **8** (880 mg, 72%). ^{1}H NMR (400 MHz, $CDCl_3$, rt) δ 5.78 (d, 1H, $J = 9.2$ Hz), 5.36 (d, 1H, $J = 3.2$ Hz), 5.12 (m, 2H), 4.98 (dd, 1H, $J = 10.4$ and 3.2 Hz), 4.58 (d, 1H, $J = 7.6$ Hz), 4.51–4.55 (m, 2H), 4.09–4.16 (m, 4H), 3.97–4.07 (m, 3H), 3.89 (t, 1H, $J = 7.0$ Hz), 3.81 (t, 1H, $J = 8.8$ Hz), 3.67 (m, 2H), 3.48 (m, 1H), 3.26 (m, 1H), 2.18 (s, 3H, CH_3CO), 2.16 (s, 3H, CH_3CO), 2.12 (s, 3H, CH_3CO), 2.08 (s, 3H, CH_3CO), 2.06 (m, 6H, 2 CH_3CO), 1.97 (s, 3H, CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$, rt) δ 170.64, 170.41, 170.14, 170.08, 169.36, 101.02, 100.82, 75.73, 72.75, 72.27, 70.82, 70.73, 69.08, 68.24, 66.60, 62.10, 60.78, 53.26, 50.62, 23.29, 20.89, 20.86, 20.66, 20.53; MALDI-TOF MS: *m/z* calcd for $C_{28}H_{41}N_4NaO_{17}$ (M+Na), 727.2; found 727.3.

4.8. 2-Azidoethyl(β-D-galactopyranosyl)-(1→4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranoside (9)[31]

To a solution of **8** (704 mg, 1 mmol) in dry methanol (15 mL) was added NaOMe (30 mg). The mixture was stirred for 3 h at room temperature. The solution was neutralized with Dowex 50Wx8 (H^+). After removing the resin by filtration, the filtrate was concentrated under reduced pressure to give a white powder (430 mg, 95%). ^{1}H NMR (400 MHz, D_2O, rt) δ 4.60 (d, 1H, $J_{1,2} = 8.0$ Hz), 4.47 (d, 1H, $J_{1',2'} = 8.0$ Hz, H-1′), 3.97 (d, 1H, H-4′), 3.78, 4.06 (2m, 2H, $OCH_2CH_2N_3$), 3.80 (dd, 1H, H-2), 3.74 (dd, 1H, H-3′), 3.72 (dd, 1H, H-4), 3.69 (dd, 1H, H-3′), 3.55 (dd, 1H, H-2′), 3.41–3.51 (2m, 2H, $OCH_2CH_2N_3$), 2.03 (s, 3H, CH_3CO); ^{13}C NMR (100 MHz, D_2O, rt): δ 174.21, 102.44, 100.53, 77.96, 74.92, 74.37, 72.05, 70.52, 68.33, 60.59, 59.58, 54.57, 49.91, 21.83; MALDI-TOF MS: *m/z* calcd for $C_{16}H_{28}N_4NaO_{11}$ (M+Na), 475.1; found 475.0.

4.9. General procedures for synthesis of glycoclusters

Methyl-2,3,4,6-tetra-*O*-propargyl β-D-galactopyranoside **1** (0.06 mmol) and azide glycoside (0.48 mmol) were suspended in water (12 mL). Sodium ascorbate (0.044 mmol, 45 μL of freshly prepared 1 M solution in water) was added, followed by copper (II) sulfate pentahydrate (0.022 mmol, 22 μL of 1 M solution in water). The mixture was stirred vigorously for 24 h, at which point the mixture cleared and TLC analysis indicated complete consumption of the reactants. The reaction mixture was reduced to about 2 mL and applied to a Sephadex G-15 column (2×145 cm) eluting with distilled water. Appropriate fractions were pooled and lyophilized to give white foam.

4.9.1. Glycocluster 10. Yield, 83%; ^{1}H NMR (400 MHz, D_2O, rt) δ 8.12 (m, 4H), 4.6 (m, 15H), 4.44 (m, 9H), 4.28 (m, 6H), 4.12 (m, 6H), 3.92 (m, 8H), 3.76–3.52 (m, 40H), 3.25 (m, 5H); ^{13}C NMR (100 MHz, D_2O, rt): δ 143.48, 143.12, 125.35, 103.12, 102.50, 101.88, 77.87, 74.93, 74.33, 73.81, 72.19, 72.08, 70.51, 68.11, 67.74, 64.70, 62.93, 62.55, 60.59, 59.59, 56.96. 49.93. MALDI-TOF MS: *m/z* calcd for $C_{75}H_{122}N_{12}NaO_{50}$ (M+Na), 2013.7; found, 2014.2.

4.9.2. Glycocluster 11. Yield, 88%; ^{1}H NMR (400 MHz, D_2O, rt) δ 8.11(m, 4H), 4.33 (m, 9H), 4.05 (m, 11H), 3.94 (m, 13H), 3.66–3.75 (m, 43H), 3.55 (m, 12H), 1.89 (br s, 12H); ^{13}C NMR (100 MHz, D_2O, rt): δ 173.6, 140.03, 124.79, 102.80, 102.43, 100.44, 77.91, 74.91, 74.31, 72.06, 71.78, 70.51, 68.10, 67.03, 60.58, 59.56, 54.32, 49.97, 21.72. MALDI-TOF MS: *m/z* calcd for $C_{83}H_{134}N_{16}O_{50}$ (M+H), 2156; found, 2156.

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