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Synthesis of a multivalent display of a CD22-binding trisaccharide

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Abstract

Multivalent interactions have been implicated in the binding of B-cell surface glycoprotein CD22 to its physiological ligands. Because CD22 can influence B-cell antigen receptor (BCR) signaling, multivalent ligands that cluster CD22 may influence B-cell responses. Here, we report an efficient synthesis of a fluorophore-labeled multivalent display of a CD22-binding trisaccharide, Neu5Acα2,6Galβ1,4Glc, using the ring-opening metathesis polymerization (ROMP). Our synthetic strategy involves the modification of an *N*-hydroxysuccinimide (NHS) ester-substituted polymer generated by ROMP with the aminopropyl glycoside of the trisaccharide. The conjugation efficiency for the coupling is high; when 0.3 equiv of the trisaccharide derivative were used relative to NHS ester groups, the mole fraction (χ) of trisaccharide ligand incorporated onto the backbone was 0.3. A fluorescein-labeled version of the multivalent ligand binds to cells expressing CD22. © 2002 Published by Elsevier Science Ltd.

Keywords: CD22; BCR; Multivalency; Sialic acid; Ring-opening metathesis polymerization (ROMP); Clustering

1. Introduction

CD22 (Siglec-2) is a cell-surface glycoprotein located on mature B-cells and B-cell derived lymphoma cells.[1,2] As a member of siglec family of proteins, CD22 recognizes and binds glycoproteins displaying sialic acid residues.[3] Specifically, the extracellular domain of CD22 binds to the trisaccharide, Neu5Acα-2,6Galβ1,4Glc/GlcNAc, but not to oligosaccharides containing α-(2→3)-linked sialic acid residues.[4–8] In analogy to many protein–carbohydrate interactions,[9–15] it has been proposed that CD22 utilizes multivalency to achieve high affinity ligand binding.[7,16]

CD22 functions in conjunction with the B-cell surface antigen receptor (BCR) to alter the thresholds for responses, thereby fine-tuning signals.[17] Upon BCR cross-linking, CD22 is rapidly phosphorylated at cytoplasmic tyrosine residues.[18–20] These modifications lead to the recruitment of the tyrosine phosphatase SHP-1, a negative regulator of B-cell activation,[21] to the BCR complex. Studies using CD22-deficient mice indicate that CD22 negatively regulates antigen receptor signaling.[22–24] Thus, its production in B-cells may serve to raise the antigen dose threshold for B lymphocyte activation.[22–24]

We sought to investigate the role of CD22 clustering on B-cell activation using multivalent ligands. Our long-term goal is to determine whether the extent of CD22 clustering influences signaling. To this end, we planned to synthesize multivalent ligands that display a trisaccharide-binding epitope for CD22. The ring-opening metathesis polymerization (ROMP) can be used to generate multivalent ligands with different abilities to cluster receptors based on varying lengths and ligand density.[25–30] Unlike traditional synthetic methods for polymer production, such as polyacrylamide polymerizations, ROMP provides a means to generate polymers of narrow polydispersities and of defined molecular mass ranges. Our synthetic strategy involved the modification of a well-defined polymer generated by ROMP that displays *N*-hydroxysuccinimide (NHS) ester groups[31] with amine-containing trisaccharide derivative **1** (Scheme 1) to generate multivalent ligands for CD22.

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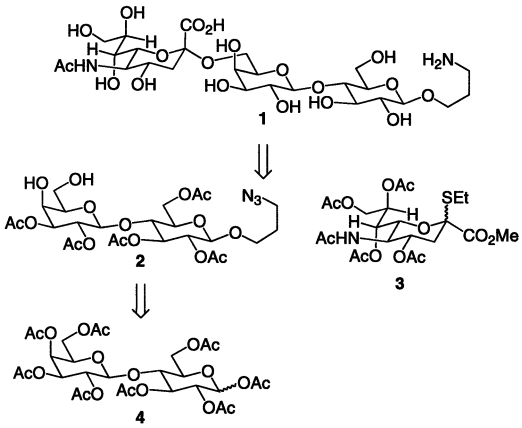
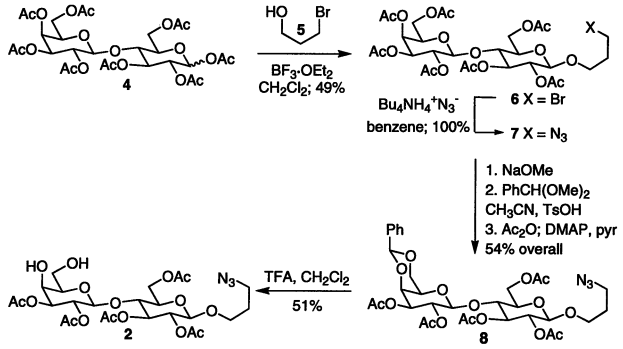


Scheme 1. Retrosynthetic analysis of the aminopropyl trisaccharide glycoside.



Scheme 2. Synthesis of disaccharide acceptor for sialylation.

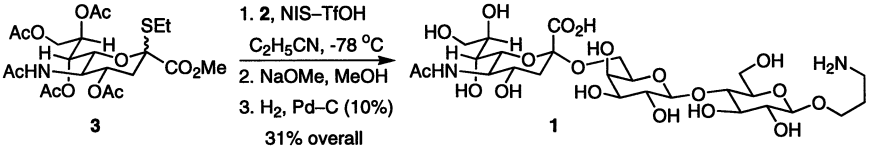
2. Results and discussion

Synthesis of aminopropyl glycoside of the trisaccharide ligand for CD22

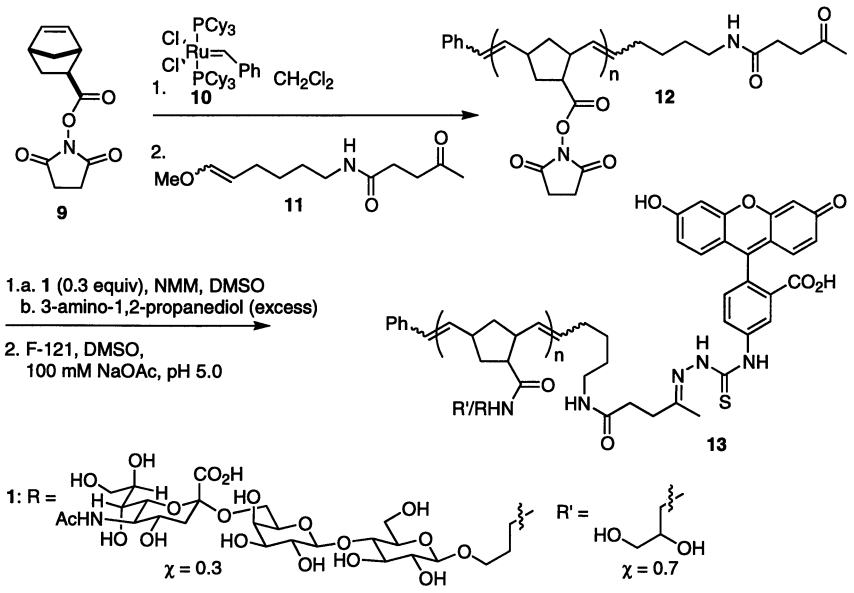
(a) Retrosynthetic analysis. We chose the aminopropyl glycoside of the CD22 trisaccharide, Neu5Acα2,6Galβ1,4Glc, (**1**) as our target (Scheme 1). Several reports incorporate various aminoalkyl linkers at C-1 of the trisaccharide, Neu5Acα2,6Galβ1, 4GlcNAc.[32–34] The aminoalkyl aglycone linker has been previously employed in the construction of multivalent displays of carbohydrate epitopes.[35–37] In addition, conditions have been developed for the coupling of aminoalkyl glycosides to NHS ester-substituted monomers and polymers generated by ROMP.[31,36] To synthesize the Neu5Acα2,6Galβ1,4Glc sequence of **1**, we utilized a strategy similar to those previously reported.[16,38,39] In our retrosynthetic plan, we envisioned that the target trisaccharide aminopropyl glycoside **1** would arise from a precursor containing an azido group (Scheme 1). This precursor could be constructed by sialylation of a lactose acceptor **2**, which bears an azidopropyl linker at the anomeric position, with ethyl thioglycoside donor **3**.[40] We anticipated that the 3-azidopropyl lactoside **2** could be generated from the readily accessible peracetylated lactose **4** utilizing a previously reported route.[39]

(b) Synthesis of the disaccharide acceptor. The disaccharide acceptor **4** could be readily generated, as outlined in Scheme 2. Treatment of β-lactose peracetate **4** and 3-bromo propanol **5** with boron trifluoride etherate afforded bromopropyl β-glycoside **6** in moderate yield.[41] Subsequently, the nucleophilic displacement of the bromide **6** by azide was achieved using tetrabutylammonium azide in benzene[42] to afford the corresponding azidopropyl glycoside **7**[43] in quantitative yield. Compound **7** was subjected to Zemplén conditions to remove the acetate groups.[44] To differentiate the 6-position of the galactose residue, the resulting compound was treated with benzene aldehyde dimethyl acetal to afford the 4′,6′-*O*-benzylidene-protected derivative. The remaining hydroxyl groups were masked by acetylation to afford compound **8** in 54% overall yield (three steps from **7**). Subsequent removal of the benzylidene group catalyzed by trifluoroacetic acid afforded the desired lactose acceptor **2**. Precursors such as these have been shown to undergo selective sialylation at the 6-position.[38,39]

(c) Synthesis of the target trisaccharide aminopropyl glycoside. With the desired disaccharide acceptor **2** in hand, we assembled the target trisaccharide using ethyl thioglycoside **3** as the sialyl donor (Scheme 3).[45] The sialylation reaction was promoted by *N*-iodosuccinimide–triflic acid[46] in propionitrile at −78 °C.[47] Although the azide group is sensitive to strongly acidic promoters, it is stable to these sialylation conditions.[16] The corresponding trisaccharide was isolated and partially purified by flash chromatography, and the resulting mixture contained some sialyl glycal. To generate the target aminopropyl glycoside **1**, the acetate groups were removed using Zemplén conditions.[44] Subsequent saponification of the methyl ester, followed by reduction of the azide, afforded the target trisaccharide **1** as a single anomer in 31% overall yield from **2**. The configuration of the Neu5Ac linkage was determined to



Scheme 3. Synthesis of the target aminopropyl trisaccharide glycoside **1**.



Scheme 4. Synthesis of the NHS ester-substituted polymer by ROMP and its conversion to a fluorescein-labeled multivalent display of CD22 binding epitope.

be α based on gated proton-decoupled ^{13}C NMR,[48] which indicated that the anomeric acid carbon is coupled to H-3ax (J 3.8 Hz). The chemical shift of the H-3eq (δ 2.60) is also consistent with literature values for the α anomer.[49,50]

Synthesis of multivalent displays of the trisaccharide-binding epitope for CD22

(a) Synthesis of NHS ester-substituted polymers by ROMP. With the trisaccharide in hand, we turned to the assembly of the multivalent ligands using ROMP. ROMP provides the means to systematically alter the polymer length and valency; thus, polymers with different abilities to cluster CD22 can be generated. By modifying an NHS ester-substituted polymer generated by ROMP[31] with amine-containing trisaccharide **1** (Scheme 4), we hoped to circumvent difficulties associated with polymerizing a hydrophilic monomer using a hydrophobic carbene initiator, such as **10** (Scheme 4). The requisite polymers were assembled from the NHS ester derived from norbornene carboxylic acid, **9**, using ruthenium initiator **10**[51,52] in dichloromethane at room temperature. For these initial synthetic investigations, a monomer to initiator ratio of 100:1 was employed, but, for future studies, the ratio could be increased or decreased to afford longer or shorter polymers, respectively.[31] Termination with a bifunctional enol ether can allow subsequent selective attachment of a reporter group to the terminus of the polymer.[53–55] The addition of enol ether **11** (Scheme 4) terminated the polymerization reaction by generating an unreactive alkoxy-substituted carbene and transferring the ketone functional group to the terminus of the resulting polymer **12**.[55] This termination strategy was used to allow for selective installation of a fluorescein reporter group via the ketone functionality[56–58] after appending the CD22-binding carbohydrate epitopes to the backbone.

(b) Generation of the multivalent display of the trisaccharide CD22-binding epitopes. We have shown previously that NHS ester-substituted polymers generated by ROMP can be functionalized with a simple amine-containing carbohydrate derivative.[31,54] The attachment of a complex carbohydrate derivative, such as the sialic acid-containing oligosaccharide **1**, to this type of polymer, however, has not been reported. Difficulties in obtaining a high coupling efficiency could arise because, although the NHS ester-containing polymer is extremely soluble in organic solutions, the conjugated polymer should be much less soluble. To maximize the solubility of all the components, the coupling reaction was carried out in dimethyl sulfoxide (DMSO) in the presence of the proton scavenger, *N*-methylmorpholine (NMM). Under these conditions, thin-layer chromatography (TLC) indicated that the efficiency of coupling is high. Specifically, when 0.3 equiv of trisaccharide amine **1** are added to polymer **12**, the final product contains the expected mole fraction (0.3) of trisaccharide epitopes (vide infra). To convert the unreacted NHS ester groups to neutral functionality, the coupling reaction was quenched with an excess of 3-amino-1,2-propanediol to afford the substituted polymer. After extensive dialysis (molecular weight cut-off 1000) to remove low-molecular-weight species, the desired multivalent display of the trisaccharide was isolated as a white, flocculent solid. Analysis by ^1H NMR spectroscopy and TLC indicated that there was no contamination by unconjugated trisaccharide. The mole fraction (χ) of trisaccharide incorporated (0.3) was calculated by comparing the integration of the signals due to anomeric

protons of the trisaccharide group to the alkene protons from the polymer backbone. To convert the resulting putative CD22 ligand into a reporter for visualization of cell surface binding, the multivalent trisaccharide display was labeled with fluorescein-5-thiosemicarbazide, which presumably reacts with the single ketone functional group at the polymer terminus. Unconjugated fluorescein was removed using size-exclusion chromatography (Sephadex G-25 medium) to afford the desired probe. These results demonstrate the flexibility of our synthetic strategy for generating multivalent carbohydrate displays labeled with a reporter group.

(c) Selective binding of the multivalent display of CD22-binding trisaccharide to a periodate treated human B-cell lymphoma. We sought to determine whether our putative multivalent ligand would interact with cell surface CD22. It has been reported previously that CD22 is masked in vivo, presumably because it binds to sialoglycoproteins such as CD45 on the B-cell surface.[59] Mild periodate oxidation is often used to truncate side chains of cell surface sialic acid residues, and this treatment has been shown to unmask CD22 on the B-cell surface.[59] Thus, we treated Raji human B lymphoma cells, which display CD22 on their cell surfaces (Fig. 1B), with sodium periodate to expose CD22 binding sites. Although no specific binding was observed in the absence of periodate treatment, the fluorescein-labeled multivalent display **13** bound to the treated Raji cells (Fig. 1D). The significant increase in fluorescence intensity after incubation with the fluorescein-labeled multivalent ligand (Fig. 1D) suggests that the functionalized polymer **13** interacts with CD22 when it is unmasked. To determine whether the observed cell binding depends on the presence of CD22, we assessed multivalent ligand binding to Jurkat human T lymphoma cells, which do not express CD22 (Fig. 1A). When Jurkat cells were subjected to periodate treatment and incubation with the fluorescein-labeled multivalent ligand, no significant binding was observed (Fig. 1C). Thus, selective cell-surface binding depends on the presence of CD22.

3. Conclusions

To address the consequences of ligand-induced clustering of CD22, we have developed an efficient strategy for generating multivalent ligands that display a trisaccharide epitope designed to bind CD22. Our studies demonstrate that the ROMP can be used to generate highly functionalized polymers, such as the CD22 ligands described here, via the post-polymerization modification strategy. We have demonstrated that the resulting polymers can be converted into tools for visualizing ligand interactions with CD22-producing

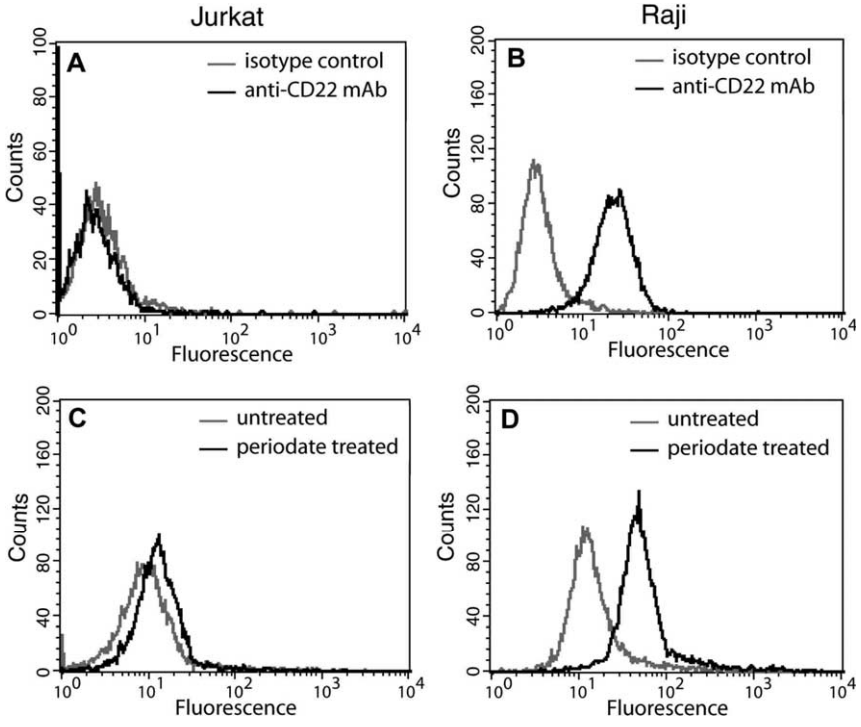


Fig. 1. Selective binding of fluorescein-labeled multivalent CD22 binding epitope **13** to periodate treated cells that express surface CD22. CD22 surface expression was monitored by flow cytometry in Jurkat T cells (A) and Raji B cells (B) using HIB22 fluorescein-labeled anti-CD22 mAb. Binding of 5 μM fluorescein-labeled compound **13** to untreated and periodate treated Jurkat T cells (C) and Raji B cells (D) by flow cytometric analysis. Histograms are representative of three sets of samples.

cells. Our results provide the routes to assemble synthetic multivalent ligands that can be used to address the role of multivalency in CD22 binding. Moreover, the chemistry we have described can be used to generate tools with which to elucidate the importance of CD22 clustering in modulating immune responses.

4. Experimental

General.—All non-aq reactions were run in oven- or flame-dried glassware under inert atmosphere of nitrogen or argon and monitored by TLC. All materials, unless otherwise noted, were obtained from commercial suppliers and used as provided. Fluorescein-5-thiosemicarbazide (F-121) was purchased from Molecular Probes. Anhydrous reaction solvents were distilled as follows: Et_2O, THF, and benzene from sodium–benzophenone ketyl; toluene from sodium–anthracene; MeOH from Mg metal and CH_2Cl_2, Et_3N and pyridine from CaH_2. Analytical TLC was performed on 0.25 pre-coated E. Merck Silica Gel 60 F_{254} plates, and compounds were visualized with ultraviolet light, phosphomolybdic acid stain, *p*-anisaldehyde stain, ninhydrin stain or potassium permanganate stain. Flash column chromatography was performed on SAI silica gel 60 (32–63 μm), with eluents generated using distilled reagent-grade hexanes, CH_2Cl_2, ACS-grade EtOAc, MeOH or $CHCl_3$. PD-10 Desalting Columns (Sephadex G-25 Medium) were purchased from Amersham Biosciences. Dialysis was performed using Spectrum dialysis tubing (MWCO 1000).

3-Bromopropyl 2,3,4,6-tetra-O-*acetyl-β*-D-*galactopyranosyl-(1→4)-2,3,6-tri*-O-*acetyl-β*-D-*glucopyranoside* (**6**).—Peracetylated lactose **4** (550 mg, 0.811 mmol) was combined with dry powdered 4 Å MS and taken up into dry CH_2Cl_2 (4 mL), followed by the addition of 3-bromo-1-propanol **5** (0.11 mL, 1.2 mmol). After stirring at room temperature for 0.5 h, the reaction was cooled to 0 °C, and boron trifluoride etherate (0.41 mL, 3.2 mmol) was added dropwise. The resulting mixture was stirred at room temperature for 12 h. The reaction was quenched with a solution of satd $NaHCO_3$ (10 mL), diluted with EtOAc (30 mL), and separated. The aq layer was washed with EtOAc (2×10 mL). The combined organic phases were dried (Na_2SO_4), filtered, concentrated and purified by flash chromatography (silica gel, 1:1 EtOAc–hexanes) to give the product **6** (304 mg, 49%) as a white foam. ^{1}H NMR (250 MHz, $CDCl_3$): δ 5.35 (d, 1 H, *J* 2.8 Hz, H-4′), 5.21 (t, 1 H, *J* 9.5 Hz, H-3), 5.11 (dd, 1 H, *J* 10.2, 7.5 Hz, H-2′), 4.96 (dd, 1 H, *J* 10.3, 3.3 Hz, H-3′), 4.89 (dd, 1 H, *J* 9.5, 7.8 Hz, H-2), 4.55–4.42 (m, 3 H, H-1,1′,6′a), 4.17–4.03 (m, 3 H, H-5′,6a,6′b), 3.99–3.89 (m, 2 H, H-6b, OCH_2^a), 3.81 (t, 1 H, *J* 9.6, H-4), 3.71–3.55 (m, 2 H, H-5, OCH_2^b), 3.50–3.40 (m, 2 H, CH_2N_3), 2.16, 2.13, 2.04, 1.95 (s, 21 H, $7 \times OCOCH_3$), 2.06–1.90 (m, 2 H, -CH_2-). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 170.2, 170.1, 170.0, 169.9, 169.6, 169.5, 168.9, 100.8, 100.6, 76.0, 72.5, 72.4, 71.4, 70.8, 70.4, 68.9, 67.1, 66.4, 61.8, 60.7, 32.1, 29.9, 20.7, 20.6, 20.5, 20.4, 20.3. MALDIMS (positive-ion mode, α-cyano-4-hydroxycinnamic acid matrix): Calcd for $C_{29}H_{41}BrNaO_{18}$ ($M + Na^+$), 779.1; found, 778.8.

3-Azidopropyl 2,3,4,6-tetra-O-*acetyl-β*-D-*galactopyranosyl-(1→4)-2,3,6-tri*-O-*acetyl-β*-D-*glucopyranoside* (**7**).—Lactosyl bromide **6** (300 mg, 0.396 mmol) was combined with tetrabutylammonium azide (225 mg, 0.792 mmol) and suspended in dry benzene (2 mL). After stirring at room temperature for 12 h, the mixture was concentrated under reduced pressure to remove most of the benzene. The residue was then purified by flash chromatography (silica gel, 2:1 EtOAc–hexanes) to give the product **7** (285 mg, 100%).^{1}H NMR (300 MHz, $CDCl_3$): δ 5.35 (d, 1 H, *J* 2.7 Hz, H-4′), 5.20 (t, 1 H, *J* 9.6 Hz, H-3), 5.11 (dd, 1 H, *J* 10.2, 7.5 Hz, H-2′), 4.97 (dd, 1 H, *J* 10.2, 3.3 Hz, H-3′), 4.88 (dd, 1 H, *J* 9.6, 8.1 Hz, H-2), 4.53–4.47 (m, 3 H, H-1,1′,6′a), 4.15–4.08 (m, 3 H, H-5′,6a,6′b), 3.99–3.89 (m, 2 H, H-6b, OCH_2^a), 3.81 (t, 1 H, *J* 9.6, H-4), 3.65–3.55 (m, 2 H, H-5, OCH_2^b), 3.38–3.32 (m, 2 H, CH_2N_3), 2.15, 2.12, 2.06, 2.05, 2.04, 1.97 (s, 21 H, $7 \times OCOCH_3$), 2.06–1.90 (m, 2 H, -CH_2-). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.1, 170.0, 169.9, 169.8, 169.5, 169.4, 168.8, 100.8, 100.5, 76.0, 72.5, 72.4, 71.4, 70.7, 70.4, 68.9, 67.1, 66.5, 66.1, 61.8, 60.6, 47.8, 28.9, 20.6, 20.5, 20.4, 20.3. MALDIMS (positive-ion mode, α-cyano-4-hydroxycinnamic acid matrix): Calcd for $C_{29}H_{41}N_3NaO_{18}$ ($M + Na^+$), 742.2; found, 742.1.

3-Azidopropyl 4,6-O-*benzylidene-β*-D-*galactopyranosyl-(1→4)-β*-D-*glucopyranoside.*—Azide **7** (2.6 g, 3.6 mmol) was dissolved in dry MeOH (40 mL), and a solution of NaOMe in MeOH (25 wt%, 2.8 mL) was added. The resulting mixture was stirred at room temperature for 12 h. The reaction mixture was then neutralized with Amberlite IR-120 (H^+) resin, filtered and concentrated to give the deacetylated product as a white solid (1.48 g, 96%).

The above product (1.48 g, 3.48 mmol) was suspended in dry CH_3CN (20 mL), and a catalytic amount of *p*-TsOH·H_2O (66 mg, 0.35 mmol) and benzaldehyde dimethyl acetal (1.1 mL, 7.0 mmol) were added. The resulting mixture was stirred at room temperature for 12 h. Et_3N (0.5 mL) was then added to neutralize the reaction mixture. Concentration and purification by flash chromatography (silica gel, 10:1 EtOAc–MeOH) gave the product as a white solid (1.0 g, 56%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.44–7.35 (m, 2 H, Ar), 7.25–7.15 (m, 3 H, Ar), 5.51 (s, 1 H, >C*H*Ph), 4.37 (d, 1 H, *J* 6.9 Hz, H-1), 4.18 (d, 1 H, *J* 7.8, H-1′), 4.12–4.01 (m, 3 H), 3.85–3.71 (m, 3 H), 3.61–3.41 (m, 6 H), 3.35–3.25 (m, 2 H), 3.21–3.11 (m, 2 H, CH_2N_3), 1.84–1.61 (m, 2 H, -CH_2-). ^{13}C NMR (75 MHz,

$CDCl_3$): δ 139.5, 129.9, 129.0, 127.5, 104.8, 104.3, 102.2, 79.9, 77.3, 76.4, 76.2, 74.8, 73.4, 71.7, 70.2, 68.3, 67.6, 61.6, 49.5, 30.2; MALDIMS (positive-ion mode, 2′,4′,6′-trihydroxyacetophenone matrix): Calcd for $C_{22}H_{31}N_3NaO_{11}$ ($M + Na^+$): 536.1; found 536.0; Calcd for $C_{22}H_{31}KN_3O_{11}$ ($M + K^+$), 552.1; found, 551.9.

*3-Azidopropyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-β-*D*-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-*D*-glucopyranoside* (**8**).—The 4′,6′-*O*-benzylidenated, free-OH disaccharide generated in the previous reaction (10.3 g, 19.5 mmol) was dissolved in dry pyridine (150 mL), followed by addition of Ac_2O (90 mL) and *N*,*N*-dimethylaminopyridine (DMAP, 52 mg, 0.43). The mixture was stirred at room temperature for 12 h. The solvents were removed by concentration, and the residue was subjected to co-evaporation with toluene. The residue was purified by flash chromatography (silica gel, 2:1 EtOAc–hexanes) to give the product **8** as a white foam (14.1 g, 100%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.49–7.33 (m, 5 H, Ar), 5.47 (s, 1 H, >C*H*Ph), 5.29–5.19 (m, 2 H, H-2′,3), 4.95–4.86 (m, 2 H, H-2,3′), 4.55–4.44 (m, 3 H, H-1,1′,6a), 4.31 (d, 1 H, *J* 2.8 Hz, H-4′), 4.22 (d, 1 H, *J* 13.0 Hz, H-6′a), 4.11 (dd, 1 H, *J* 13.0, 6.5 Hz), 4.05 (d, 1 H, *J* 13.0 Hz, H-6′b), 3.96–3.86 (m, 1 H, OCH_2^a), 3.78 (t, 1 H, *J* 9.5, H-4), 3.65–3.55 (m, 2 H, H-5, OCH_2^b), 3.46 (s, 1 H, H-5′), 3.38–3.32 (m, 2 H, CH_2N_3), 2.12, 2.10, 2.03 (s, 15 H, 5 × $OCOCH_3$), 1.86–1.76 (m, 2 H, -CH_2-). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.5, 170.2, 170.0, 169.5, 168.7, 137.3, 129.0, 128.0, 126.3, 101.1, 100.8, 100.5, 75.8, 72.9, 72.7, 72.2, 71.8, 71.3, 68.8, 68.2, 66.3, 66.2, 61.8, 60.2, 47.7, 28.8, 20.8, 20.7, 20.6, 20.5. ESIMS: Calcd for $C_{32}H_{41}N_3NaO_{16}$ ($M + Na^+$), 746.2; found, 746.2.

*3-Azidopropyl 2,3-di-*O*-acetyl-β-*D*-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-*D*-glucopyranoside* (**2**).—The 4′,6′-*O*-benzylidene protected lactoside **8** (14.1 g, 19.5 mmol) was dissolved in CH_2Cl_2 (400 mL) and cooled to 0 °C. An aq trifluoroacetic acid (TFA) solution (90%) was then added (40 mL). After stirring for 3 h at 0 °C, TLC indicated incomplete starting material consumption. Additional TFA solution (20 mL) was added, and stirred another 3 h at 0 °C. After this treatment, TLC indicated most of the starting material had been consumed. The reaction mixture was washed with water (2 × 400 mL), satd $NaHCO_3$ (2 × 400 mL), dried (Na_2SO_4), filtered and the solvent was removed under reduced pressure. The resulting light-yellow solid was dissolved in EtOAc (~ 50 mL), hexanes (~ 50 mL) were added, and the product precipitated as a white solid. The solid was filtered and washed with more hexanes to provide the product **2** as a white solid (6.6 g, 51%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.26–5.12 (m, 2 H, H-2′,3), 4.96–4.61 (m, 2 H, H-2,3′), 4.56–4.43 (m, 3 H, H-1,1′,6a), 4.15–4.01 (m, 2 H, H-4′,6b), 3.96–3.72 (m, 3 H, H-4,5′, OCH_2^a), 3.66–3.51 (m, 4 H, H-5,6′a,6′b, OCH_2^b), 3.41–3.30 (m, 2 H, CH_2N_3), 1.85, 1.86, 1.87 (s, 15 H, 5 × $OCOCH_3$), 1.92–1.72 (m, 2 H, -CH_2-). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.6, 170.5, 170.2, 169.5, 101.0, 100.8, 76.3, 74.4, 73.5, 72.6, 71.6, 69.6, 67.8, 66.4, 62.1, 47.9, 28.9, 20.9, 20.8, 20.7. MALDIMS (positive-ion mode, 2′,4′,6′-trihydroxyacetophenone matrix): Calcd for $C_{25}H_{37}N_3NaO_{16}$ ($M + Na^+$), 658.1; found, 658.1.

*3-Aminopropyl (5-acetamido-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosylonic acid)-(2→6)-β-*D*-galactopyranosyl-(1→4)-β-*D*-glucopyranoside* (**1**).—Disaccharide diol **2** (2.0 g, 3.2 mmol) and ethyl thioglycoside **3** (2.54 g, 4.75 mmol) were co-evaporated with dry toluene (2 × 10 mL) and dried under vacuum for 48 h. To this residue dry powdered 4 Å MS and dry propionitrile (16 mL) were added. The resulting suspension was stirred at room temperature for 30 min. After cooling to −78 °C, *N*-iodosuccinimide (2.15 g, 9.28 mmol, recrystallized from 1:1 dioxane–CCl_4) was added in one portion, and the reaction mixture was stirred for 30 min. Triflic acid (0.420 mL, 1.34 mmol) was added dropwise to the reaction solution, which first turned yellow and then gradually turned orange. After 2 h at −78 °C, the reaction was quenched with Et_3N (5 mL), diluted with EtOAc (100 mL), washed with aq $Na_2S_2O_3$ (0.5 M, 2 × 20 mL), dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography (silica gel, 2:1 toluene–acetone) to give the product (3-azidopropyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→6)-2,3-di-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside) as a brown foam (3.5 g) containing some glycal side product as indicated by the ^{1}H NMR spectrum. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.59 (d, 2 H, *J* 9.6 Hz, NHAc), 5.40–5.15 (m, 5 H, H-2′,3,4″,7″,8″), 4.98–4.80 (m, 3 H, H-2,3′,9a″), 4.58–4.42 (m, 4 H, H-1,1′,6a,6″), 4.21–3.99 (m, 5 H, H-4′,5″,6a,6″, OCH_2^a), 3.80 (s, 3 H, $COOCH_3$), 3.88–3.50 (m, 6 H, H-4,5,5′,6′a,6′b, OCH_2^b), 3.40–3.30 (m, 2 H, -CH_2N_3), 2.60 (dd, 1 H, *J* 12.3, 4.2 Hz, H-3*eq*), 2.15, 2.14, 2.12, 2.11, 2.10, 2.07, 2.06, 2.05, 2.04 (s, 29 H, 10 × $OCOCH_3$), 1.97 (t, 1 H, *J* 12.0 Hz, H-3*ax*), 1.89 (s, $NHCOCH_3$), 2.06–1.90 (m, 2 H, -CH_2-). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.8, 170.6, 170.3, 170.2, 170.1, 170.0, 169.5, 169.3, 167.8, 100.7, 100.5, 98.9, 76.1, 73.4, 72.7, 72.3, 71.5, 69.6, 68.8, 67.2, 66.3, 66.1, 62.4, 62.1, 62.0, 53.8, 49.1, 47.9, 37.1, 28.9, 23.0, 20.9, 20.7, 20.6, 20.5. MALDIMS (positive-ion mode, 2′,4′,6′-trihydroxyacetophenone matrix): Calcd for $C_{45}H_{64}N_4NaO_{28}$ ($M + Na^+$), 1131.3; found, 1131.3; Calcd for $C_{45}H_{65}N_4O_{28}$ ($M + H^+$), 1109.4; found, 1109.3.

Without further purification, the trisaccharide generated in the previous reaction (236.6 mg, 0.219 mmol) was dissolved in dry MeOH, and a solution of NaOMe in MeOH (2.0 M, 0.11 mL) was added to the mixture.

The solution was stirred at room temperature for 2.5 h, and water (0.5 mL) was then added. The resulting reaction mixture was stirred at room temperature for 12 h. The reaction was neutralized with Amberlite IR-120 (H^+) resin, and the resin was removed by filtration. The filtrate was concentrated under reduced pressure. The resulting residue was then combined with Pd–C catalyst (10%, 230 mg) and suspended into a mixture of solvents (9 mL, 3:3:1 EtOH–H_2O–AcOH). The suspension was subjected to H_2 at atmosphere pressure for 24 h. The reaction mixture was filtered through a plug of Celite, and the filtrate was concentrated. The residue was purified by flash chromatography (silica gel, 3:3:1:0.1 CH_2Cl_2–MeOH–H_2O–AcOH) to give the product **1** as a white solid (46.5 mg, 31%). ^{1}H NMR (300 MHz, D_2O): δ 4.28 (d, 1 H, J 8.1 Hz, H-1), 4.19 (d, 1 H, J 7.8, H-1′), 3.01–2.91 (m, 2 H, CH_2NH$_2$), 2.49 (dd, 1 H, J 12.3, 4.2 Hz, H-3*eq*), 1.81 (s, 3 H, NHCOCH_3), 1.51 (t, 1 H, J 12.0 Hz, H-3*ax*). ^{13}C NMR (75 MHz, D_2O): δ 177.8, 104.2, 103.0, 101.2, 80.6, 75.6, 74.7, 73.7, 73.5, 73.4, 72.8, 71.8, 69.5, 69.4, 69.3, 68.9, 64.5, 63.7, 61.2, 52.8, 41.1, 38.8, 27.6, 23.0. MALDIMS (positive-ion mode, α-cyano-4-hydroxycinnamic acid matrix): Calcd for $C_{26}H_{47}N_2NaO_{19}$ ($M + H^+$), 691.3; found, 690.9.

NHS ester-substituted polymer **12**.—To a solution of monomer **9** (150.0 mg, 637.6 μmol) in deoxygenated CH_2Cl_2 (5.87 mL) was added a solution of ruthenium initiator **10** (5.2 mg, 6.4 μmol) in deoxygenated CH_2Cl_2 (0.50 mL) such that the monomer concentration of the resulting solution was 0.1 M. The resulting purple solution was stirred at room temperature for 10 min, and TLC showed no remaining monomer. A solution of excess vinyl ether **11** (21.7 mg, 95.6 μmol) in deoxygenated CH_2Cl_2 (0.12 mL) was then added. The resulting brown solution was stirred overnight and then added dropwise to a vortexing solution of 4:1 ether–benzene (2 × 40 mL) to precipitate the polymer. The solution was centrifuged, decanted, and the polymer was collected by filtration with fresh 4:1 ether–benzene to provide polymer **12** (127.9 mg, 85%) as a flocculent grey solid.

Fluorescein labeling of CD22 trisaccharide homopolymer **13**.—To a vial containing NHS ester-substituted polymer **12** (11.4 mg, 48.5 μmol NHS ester groups) was added the trisaccharide amine **1** (10.0 mg, 14.5 μmol) as a solid. Anhyd DMSO (145 μL) was introduced to afford a clear solution to which *N*-methylmorpholine (21.3 μL, 0.194 mmol) was added. The solution was stirred at room temperature for 24 h, and then excess 3-amino-1,2-propanediol (17.7 mg, 0.194 mmol) was added. The resulting mixture was stirred for 12 h. The mixture was added to a PD-10 desalting column (Sephadex G-25) and eluted with water, and the fractions were concentrated in vacuo using a speed vac concentrator. The resulting white powder fractions were dissolved in deionized H_2O and subjected to dialysis (MWCO 1000, 3 × 4 h at 0 °C, then 1 × 8 h, then 1 × 4 h) to provide the trisaccharide homopolymer (16.8 mg) as a white, flocculent solid. Integration of the signals due to polymer alkene protons versus those from the anomeric sugar protons indicated that the mole fraction (χ) of trisaccharide ligand incorporation is 0.3. Selected ^{1}H NMR data (300 MHz, D_2O): δ 5.42–5.10 (m, =CH), 4.40–4.25 (m, H-1,1′).

To a microcentrifuge tube containing trisaccharide homopolymer (16.8 mg, 0.427 μmol ketone) was added aq 100 mM NaOAc at pH 5.0 (100 μL), followed by fluorescein-5-thiosemicarbazide (1.8 mg, 4.3 μmol) in DMSO (10 μL). The tube was mixed by vortexing, and the solution was allowed to stand for about 20 h. The mixture was eluted on a PD-10 desalting column (Sephadex G-25), and the yellow polymer fraction was concentrated by speed vac. The yellow residue was dissolved in deionized H_2O and lyophilized to give a yellow, flocculent solid **13** (14.3 mg).

Mild periodate treatment.—Raji human B lymphoma cells were cultured in RPMI 1640 media supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, and 10% fetal bovine serum (FBS). Jurkat human T lymphoma cells were cultured in RPMI 1640 media supplemented with 2 mM L-glutamine and 10% FBS. For periodate treatment, 2 million cells/mL were washed twice with Dulbecco's phosphate-buffered saline (DPBS), resuspended in DPBS (pH 7.4) containing freshly dissolved 2 mM $NaIO_4$, and then incubated for 30 min at 4 °C in the dark.[59] Excess periodate was quenched by the addition of 25 μL of 20% glycerol.

Flow cytometric analysis.—Periodate-treated cells and untreated cells (2 million cells/mL) were washed several times with DPBS containing 1% bovine serum albumin (pH 7.1 binding buffer) and incubated with fluorescein-labeled multivalent CD22 binding epitope **13** at concentrations between 10 nM and 10 μM for 2 h at 1 °C. Cells were then washed extensively, resuspended in binding buffer, and 10 μg/mL propidium iodide (PI) was added to each sample. The fluorescein emission intensity was analyzed by flow cytometry on a Becton–Dickinson FACScalibur instrument using CellQuest software.

For CD22 detection, Raji and Jurkat cells (300,000 cells/mL) were washed twice with Hanks' balanced salt solution (HBSS) and then incubated with 10 μL anti-human CD22 mAb, clone HIB22, or isotype control (fluorescein-labeled mouse IgG_1) for 30 min at 4 °C. Cells were then washed extensively, resuspended in HBSS, and 10 μg/mL PI was added to each sample. The fluorescein emission intensity was then analyzed by flow cytometry on a Becton Dickinson FACScalibur instrument and with CellQuest software.

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