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Convenient use of non-malodorous thioglycosyl donors for the assembly of multivalent globo- and isoglobosyl trisaccharides

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Abstract

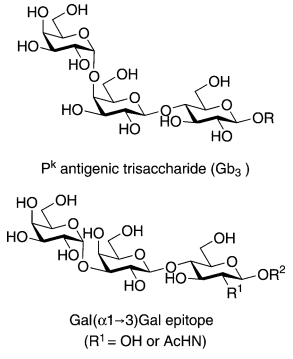
New thioglycosyl donors (*o*-methoxycarbonylphenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside and its 6-*O*-acetyl analogue) were designed and used for the synthesis of glycoconjugate polymers carrying Gb_3 [Gal(α1→4)Gal(β1→4)Glc] and $isoGb_3$ [Gal(α1→3)Gal(β1→4)Glc] clusters as side chains. These donors scarcely evolved the unpleasant odor of thiophenols and showed a high α-anomeric selectivity in the galactosylation of *p*-nitrophenyl β-lactoside derivatives, although in moderate yields. The derived trisaccharides were converted to multivalent carbohydrate ligands and were subjected to a biological assay with Shiga toxins. The multivalent Gb_3 ligand was highly active in inhibiting the toxicity, while the $isoGb_3$ ligand showed no activity, indicating that Stx-I discriminates between the carbohydrate structures.

Keywords: Multivalent oligosaccharides; Stereo- and regioselective glycosylations; Glycoconjugate polymers; Gal(α1→3)Gal epitope; P^k antigenic trisaccharide; Shiga toxins

1. Introduction

Cell surface oligosaccharides with a terminal Gal(α1→3)Gal or Gal(α1→4)Gal linkage are known as ligands of various bacterial toxins (Scheme 1).[1] For example, a P^k antigenic globotrioside [Gb_3: Gal(α1→4)Gal(β1→4)Glc] is recognized by Shiga toxins (Stx-I and Stx-II) produced by *Escherichia. coli* O-157:H-7, which cause a serious hemolytic uremic syndrome in humans.[2] The isomeric isoglobotrioside [$isoGb_3$: Gal(α1→3)Gal(β1→4)Glc] is the ligand of Toxin A produced by *Clostridium difficile*, and the toxin plays a major role in causing antibiotic-associated diarrhea.[3] It is also well known that the analogous Gal(α1→3)Gal(β1→4)GlcNAc linkage is the key determinant of animal antigens responsible for hyperacute rejection upon xenotransplantation.[4]

Because of their biological significance, Gb_3 and $isoGb_3$ trisaccharides and other related α-galactoside epitopes have resulted in a number of synthetic[5,6] and biomedical[7] studies. We already reported the synthesis of Gb_3 and a multivalent model and showed that these ligands displayed an inhibitory activity against Shiga



Scheme 1. Structures of P^k antigenic trisaccharide and Gal(α1→3)Gal epitope.

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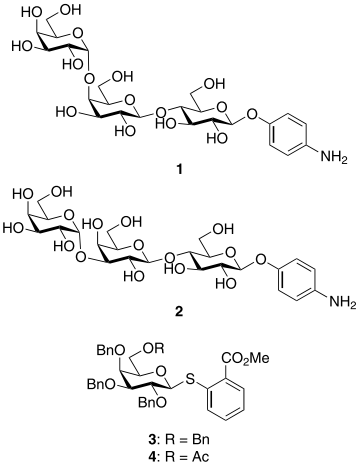
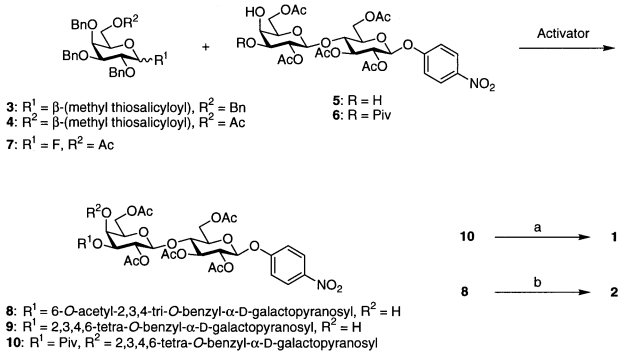


Scheme 2. Structures of the target molecules **1** and **2**, and novel thiogalactosyl donors **3** and **4**.



Scheme 3. Synthesis of target molecules **1** and **2**. Reaction conditions: (a) (i) NaOMe, MeOH, rt, 6 h, 93%; (ii) $Pd(OH)_2$/C, HCl, H_2, MeOH, rt, 6 h, 92%; (b) NaOMe, MeOH, rt, 6 h, 97%; (ii) $Pd(OH)_2$/C, HCl, H_2, MeOH, rt, 6 h, 97%.

toxin-I (CD_{50} $10^{-8} \sim 10^{-9}$ M).[8] A variety of multivalent Gb_3 structures have been also designed by other groups which showed a potent activity against Shiga toxins.[9] These studies clearly demonstrate the significance of carbohydrate clusters as effective ligands of bacterial toxins and other binding proteins and let expect possible applications in the biomedical field. This paper reports a convenient synthesis of *p*-aminophenyl (*p*AP) Gb_3 glycoside **1** and $isoGb_3$ glycoside **2** as precursors of multivalent Gb_3 and $isoGb_3$ ligands (Scheme 2). The synthesis was conducted efficiently by using the novel thiogalactosyl donors **3** and **4**, which were designed as non-malodorous thioglycosyl donors.

2. Results and discussion

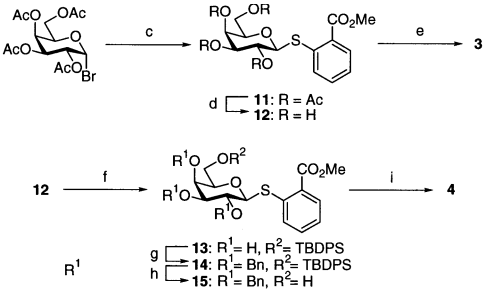
Design of non-malodorous thiogalactosyl donors and its application to α-anomeric selective galactosylation.— The synthetic methods,[5,6] already reported for glycosyl ceramides, could not be applied conveniently to the synthesis of glycoconjugate polymers because of their lack of a suitable functional group for the grafting and polymerization step. Thus, we adopted our previous pathway[8] starting from *p*-nitrophenyl β-lactoside leading to glycosyl acceptors **5** and **6** for the synthesis of **1** and **2** (Scheme 3). At first, a conventional glycosylation step between **5** and glycosyl fluoride **7** was examined[8] (Table 1). Since glycosyl donors with an electron-withdrawing group at O-6 have been reported to promote an α-anomeric specificity,[10] donor **7** was derivatized with a 6-*O*-acetyl group. Glycosylation of fluoride **7** with glycosyl acceptor **5** using tin(II) chloride and silver perchlorate as activators[11] proceeded with the expected α-anomeric selectivity to give the desired product **8** (38% isolated yield, $\alpha/\beta \geqslant 19{:}1$, ^{1}H NMR analysis). Despite the fact that the yield of this reaction could be increased by further optimization, we decided to try another route towards the desired trisaccharide **8**.

Next, we designed thioglycosyl donors **3**[12] and **4** for the construction of the α-(1→3) galactosyl linkages and tested their reactivity. It was expected that the electron-withdrawing *o*-methoxycarbonyl group might decrease the reactivity of thiophenyl donors to improve the stereo- and regioselectivity. The donors were obtained from an S_N2 reaction of methyl thiosalicylate with

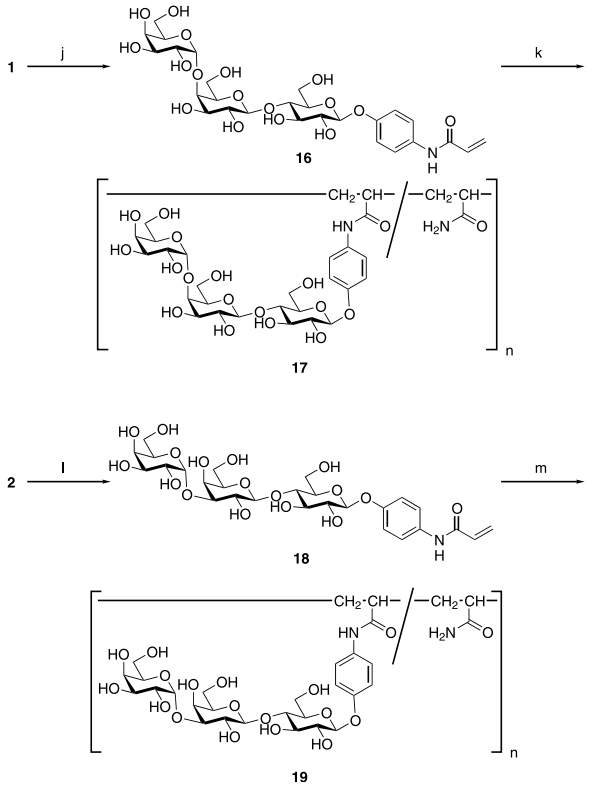
Table 1
Glycosylation of **5** and **6** with various glycosyl donors

Run	Acceptor	Donor equiv [a]	Activator equiv [b]	Temp (°C)	Time (h)	Yield [c] (%)
1	**5**	**7**, 1.5	$AgClO_4$ (1.5), $SnCl_2$ (1.5)	0→rt	12	**8**, 38
2	**5**	**3**, 1.5	NIS (2.0), TfOH (ca.)	−10	2	**9**, 60 [d]
3	**5**	**4**, 1.5	NIS (2.0), TfOH (ca.)	−10	1	**8**, 65
4	**6**	**3**, 2.0	NIS (2.0), TfOH (ca.)	−10	3	**10**, 79

[a] Against acceptor.
[b] Against donor.
[c] Isolated yield.
[d] 4′-*O*-Galactosylated byproduct was generated (17% isolated yield, α/β 82:18).



Scheme 4. Synthesis of novel thioglycosyl donors **3** and **4**. Reaction conditions: (c) methyl thiosalicylate, K_2CO_3, DMF, rt, 2 h, 90%; (d) NaOMe, MeOH, rt, 3 h, 99%; (e) NaH, BnBr, DMF, 16 h, 91%; (f) TBDPSCl, Et_3N, DMAP, Py, rt, 8 h, 85%; (g) BnBr, NaH, DMF, rt, 12 h, 92%; (h) TBAF, THF, rt, 12 h; (i) Ac_2O, Py, rt, 7 h, 94% (2 steps).



Scheme 5. Synthesis of glycoconjugate polymers **17** and **19**. Reaction conditions: (j) acryloyl chloride, Et_3N, CH_2Cl_2, MeOH, 0 °C→rt, 1.5 h, 92%; (k) acrylamide, 2,2-azobis(2-amidinopropane) dihydrochloride, H_2O, 60 °C, 12 h, 70%; (l) acryloyl chloride, Et_3N, CH_2Cl_2, MeOH, 0 °C→rt, 1.5 h, 93%; (m) acrylamide, 2,2-azobis(2-amidinopropane) dihydrochloride, H_2O, 60 °C, 12 h, 76%.

per-*O*-acetyl galactosyl bromide and isolated as crystalline solids (Scheme 4). Though the thiosalicylate smells weakly like a blend of methyl salicylate and thiophenol, the reaction does not emit the unpleasant odor of mercapto alcohol and phenol. Glycosylation between **5** and **4** gave the desired product **8** in satisfactory yield and α-anomeric selectivity (65% isolated yield, α/β ratio ⩾19:1, ^{1}H NMR analysis), while the donor **3** without the 6-*O*-acetyl group tended to show higher reactivity but lower regioselectivity (**9** 60%, α(1→4) isomer in 17% yield). The utility of these new donors could be verified in the reactions with the less reactive acceptor **6** carrying a bulky pivaloyl group in the vicinity. The reaction between **3** and **6** gave the desired globotrioside **10** in high yield (79%, α/β ⩾19:1) by using a mixture of ethyl ether and dichloromethane as solvent.

The Gb_3 and $isoGb_3$ derivatives **10** and **8**, thus derived, were quantitatively converted to the target compounds **1** and **2**, respectively, via *O*-deacetylation with sodium methoxide in methanol followed by catalytic hydrogenation (palladium hydroxide in methanol) to reduce the *p*NP group and cleave the benzyl groups.

Syntheses of multivalent Gb_3 and $isoGb_3$ ligands and their inhibiting activity towards Shiga toxins.—Globosyl- and isoglobosyl amines **1** and **2** were converted to the artificial glycoconjugate polymers **17** and **19** via *N*-acryloylation at the terminal amino group[13] followed by radical copolymerization with acrylamide (feed molar ratio 1:4) in the presence of 2,2-azobis(2-amidinopropane) dihydrochloride (AAPD)[14] (Scheme 5). After dialysis in water (M_w 8000 cut-off) and freeze-drying, **17** and **19** bearing respective Gb_3 and $isoGb_3$ clusters at the side chain were obtained as water-soluble colorless powders (molar ratio carbohydrate–acrylamide ca. 1:8 as determined by ^{1}H NMR spectroscopy).

The polymeric ligands were subjected to a biological assay involving neutralization of the toxicity of Shiga

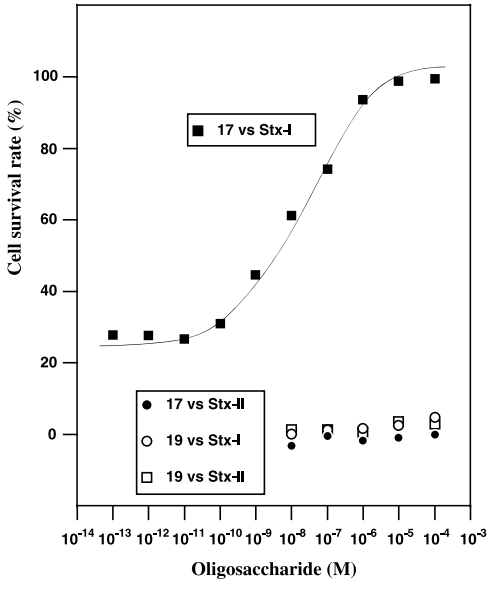


Fig. 1. Neutralization effect of copolymer **17** and **19** against Stx-I and Stx-II. Each symbol indicates as follow: ■, Gb_3 copolymer **17** and Stx-I assay; ●, copolymer **17** and Stx-II assay; □, $isoGb_3$ copolymer **19** and Stx-I assay; ○, copolymer **19** and Stx-II assay.

toxins against human ACHN cells (Fig. 1). Under the conditions that each of Stx-I and Stx-II gave fatal damage to the cells, the Gb_3 multivalent model **17** showed a highly neutralizing activity against Stx-I (CD_{50} $10^{-8} \sim 10^{-9}$ M), but no activity against Stx-II. This result accorded with our previous results, which showed a critical difference in the carbohydrate recognition between the two toxins. On the other hand, the $isoGb_3$ multivalent model **19** did not show any activity against both toxins. This means that the strong activity of **17** against Stx-I can be ascribed to a highly specific interaction between the Gb_3 cluster and the toxin. The toxin strictly discriminates between Gb_3 and $isoGb_3$ structures.

Until recently, most conventional oligosaccharide syntheses have targeted an oligosaccharide of its repetitive sequence. It is now recognized that biological activities of cell surface oligosaccharides are associated with multivalent binding to receptor proteins that are composed of multiple binding subunits. For example, the Shiga toxins are composed of five B-subunits for the tight binding to the Gb_3 cluster on the cell surface.[15] Anthrax toxins consist of heptameric structures for internalization into the host cells, though the contribution of carbohydrate–protein interactions is not clear.[16] This means that a single oligosaccharide sequence cannot bind these toxins effectively, while the multivalent model can. The potential use of these glycosides has been demonstrated by synthesis and biological evaluation of multivalent Gb_3 and $isoGb_3$ models.

In summary, we have accomplished an efficient synthesis of *p*-aminophenyl Gb_3 and $isoGb_3$ glycosides using novel thioglycosyl donors. These glycosides could be easily converted into their multivalent models via radical copolymerization. The synthetic pathway, as well as the biological data presented in this paper, may extend the scope for a practical use of cell surface oligosaccharides.

3. Experimental

General methods.—Reagents of the highest commercial quality were purchased and used without further purification. Anhydrous solvents were purchased from Kanto Chemical Co., Inc. All reactions, except polymerization, were carried out under a dry nitrogen atmosphere, and monitored by thin-layer chromatography (TLC) on E. Merck aluminium roll Silica Gel 60-F_{254} with visualization with UV light and ethanoic phosphomolybdic acid or *p*-anisaldehyde solution. E. Merck Silica Gel 60 (particle size 0.040–0.063 mm) was employed for column chromatography using toluene–EtOAc and $CHCl_3$–MeOH as eluents.

^{1}H NMR (500 MHz) spectra were recorded with a Varian Inova 500 spectrometer equipped with Sun workstation. Unless otherwise stated, ^{1}H NMR spectra were recorded at 25 °C in $CDCl_3$ using an internal Me_4Si standard at 0 ppm. Optical rotations were determined with JASCO DIP-1000 digital polarimeter using a water-jacketed 100-mm cell at 25 °C. IR spectra were recorded on a JASCO FT/IR-230 Fourier transform infrared spectrometer in the form of a KBr disc. Size-exclusion chromatography was performed with a JASCO 800 high-performance liquid chromatograph on Shodex B804 + B805 columns using PBS as eluent.

o-*Methoxycarbonylphenyl 6*-O-*(*tert-*butyldiphenylsilyl)-1-thio-β*-D-*galactopyranoside* (**13**).—To a solution of alcohol **12** (2.00 g, 6.05 mmol), Et_3N (2.48 mL, 18.2 mmol), and DMAP (79 mg, 0.650 mmol) in pyridine (50 mL) was added *tert*-butylchlorodiphenylsilane (2.33 mL, 9.08 mmol) gradually at 0 °C, and the reaction mixture was stirred at rt for 8 h. The reaction mixture was quenched by addition of 1 N HCl and extracted with $CHCl_3$. The combined organic layer was washed with 1 N HCl, satd $NaHCO_3$, and water, dried over $MgSO_4$, and concentrated under diminished pressure. The residue was purified by chromatography (silica gel, 1:1 toluene–EtOAc) to give silyl ether **13** (2.92 g, 85%) as a colorless crystals: mp 112–114 °C; $[\alpha]_D^{24}$ −13.2° (*c* 1.0, $CHCl_3$); IR (disc); *v* 3412 (OH), 1723 and 1251 cm^{-1} (CO_2Me); ^{1}H NMR ($CDCl_3$): δ 7.13–7.86 (m, 14 H, ArH), 5.02 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.05 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.0 Hz, H-2), 4.07 (d, 1 H, $J_{3,4}$ 2.6 Hz, H-4), 3.90 (s, 3 H, CO_2Me), 3.63–3.75 (m, 4 H, H-3, H-5, and H-$6_{R,S}$), 1.03 (s, 9 H, *t*-Bu); FAB$^-$MS: *m*/*z* 567 $[M-1]^-$: Anal. Calcd for $C_{30}H_{36}O_7SSi$: C, 63.35; H, 6.38. Found: C, 63.36; H, 6.36.

o-*Methoxycarbonylphenyl 2,3,4-tri*-O-*benzyl-6*-O-*(*tert-*butyldiphenylsilyl)-1-thio-β*-D-*galactopyranoside* (**14**).—To a solution of the silyl ether **13** (2.50 g, 4.40 mmol) in DMF (100 mL) was added NaH (475 mg, 19.8 mmol) and BnBr (2.35 mL, 19.8 mmol) slowly at 0 °C, and the reaction mixture was stirred at rt for 12 h. The reaction mixture was quenched by addition of MeOH, and to the mixture was added EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under diminished pressure. The residue was purified by chromatography (silica gel, 10:1 toluene–EtOAc) to afford the benzyl ether **14** (3.40 g, 92%) as a white powder: $[\alpha]_D^{24}$ −24.7° (*c* 1.0, $CHCl_3$); IR (disc); *v* 1722 and 1250 (CO_2Me), 1294 and 1132 cm^{-1} (OBn); ^{1}H NMR ($CDCl_3$): δ 7.08–7.83 (m, 29 H, ArH), 4.66 (d, 1 H, $J_{1,2}$ 9.5 Hz, H-1), 4.63–4.99 (m, 6 H, $ArCH_2O$), 4.03 (dd, 1 H, $J_{1,2}$ 9.5, $J_{2,3}$ 9.0 Hz H-2), 3.99 (bd, $J_{3,4}$ 2.5 Hz, H-4), 3.84 (s, 3 H, CO_2Me), 3.85 (dd, 1 H, H-6_S), 3.78 (dd, 1 H, H-6_R), 3.59 (dd, 1 H, $J_{2,3}$ 9.0, $J_{3,4}$ 2.5 Hz, H-3), 3.48 (dd, 1 H, H-5), 1.05 (s, 9 H, *t*-Bu); FAB$^-$MS: *m*/*z* 838 $[M-1]^-$: Anal. Calcd for $C_{51}H_{54}O_7SSi$: C, 73.00; H, 6.49. Found: C, 73.10; H, 6.47.

o-*Methoxycarbonylphenyl 6-O-acetyl-2,3,4-tri-O-benzyl-1-thio-β-D-galactopyranoside* (**4**).—To a solution of benzyl ether **14** (3.00 g, 3.58 mmol) in THF (100 mL) was added TBAF (7.15 mL, 7.15 mmol, 1 M in THF) and the reaction mixture was stirred at rt for 12 h. The reaction mixture was concentrated under diminished pressure, and the residue was diluted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under diminished pressure. The residue containing alcohol **15** was dissolved in pyridine (50 mL), and to the stirred mixture was added Ac_2O (25 mL). After 7 h, the reaction mixture was quenched by addition of EtOH, and the mixture was azeotropically repeatedly dried with toluene. The residue was dissolved in $CHCl_3$, and the organic layer was washed with 1 N HCl, satd $NaHCO_3$, and brine, dried over $MgSO_4$, and concentrated under diminished pressure. The residue was purified by chromatography (silica gel, 5:1 toluene–EtOAc) to give acetate **4** (2.16 g, 94%, 2 steps) as a white powder; $[\alpha]_D^{24}$ −39.6° (*c* 1.0, $CHCl_3$); IR (film); *v* 1743 and 1230 (Ac), 1720 and 1250 (CO_2Me), 1301 and 1136 cm^{-1} (OBn); ^1H NMR ($CDCl_3$): δ 7.18–7.87 (m, 19 H, ArH), 4.65–5.01 (m, 6 H, $ArCH_2O$), 4.74 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.26 (dd, 1 H, H-6_S), 4.10 (dd, 1 H, H-6_S), 4.06 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.0 Hz, H-2), 3.88 (bs, 1 H, H-4), 3.87 (s, 3 H, CO_2Me), 3.67 (ddd, 1 H, H-5), 3.64 (dd, 1 H, $J_{2,3}$ 9.0, $J_{3,4}$ 3.0 Hz, H-3), 2.01 (s, 3 H, Ac); FAB$^-$MS: *m*/*z* 641 $[M-1]^-$: Anal. Calcd for $C_{37}H_{38}O_8S$: C, 69.14; H, 5.96. Found: C, 69.01; H, 5.95.

p-*Nitrophenyl (6-O-acetyl-2,3,4-tri-O-benzyl-α-D-galactopyranosyl)-(1→3)-(2,6-di-O-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside* (**8**)

Method A. A suspension of flame-dried MS 4 Å, $AgClO_4$ (218 mg, 1.05 mmol), and $SnCl_2$ (199 mg, 1.05 mmol) in dry CH_2Cl_2 (6 mL) was stirred at rt for 16 h. The stirred mixture was added to a solution of diol **5** (215 mg, 0.350 mmol) in dry CH_2Cl_2 (2 mL) at 0 °C, and the mixture was stirred at rt for 6 h. The mixture was added to a solution of fluoride **7** (400 mg, 0.702 mmol) in dry CH_2Cl_2 (2 mL) gradually at 0 °C, and the reaction mixture was stirred at the same temperature for 12 h. The reaction mixture was filtered through a pad of Celite, and the filtrate was washed with satd $NaHCO_3$ and water, dried over $MgSO_4$, and concentrated under diminished pressure. The residue was purified by chromatography (silica gel, 5:1 toluene–EtOAc) to furnish trisaccharide **8** (124 mg, 38%) as a white powder.

Method B. A suspension of flame-dried MS 4 Å and NIS (322 mg, 1.43 mmol) in dry CH_2Cl_2 (6 mL) was stirred for 16 h. The stirred mixture was added to a solution of diol **5** (292 mg, 0.476 mmol) and acetate **4** (459 mg, 0.714 mmol) in dry CH_2Cl_2 (4 mL), and the mixture was stirred at rt for 6 h. To the mixture was added TfOH (10 μL) at −10 °C, and the reaction mixture was stirred at the same temperature for 2 h. The reaction mixture was filtered through a pad of Celite, and the filtrate was washed with 5% aq $Na_2S_2O_3$, satd $NaHCO_3$ and water, dried over $MgSO_4$, and concentrated under diminished pressure. The residue was purified by chromatography (silica gel, 5:1 toluene–EtOAc) to furnish the trisaccharide **8** as a white powder (533 mg, 65%): $[\alpha]_D^{24}$ −62.6° (*c* 1.0, $CHCl_3$); IR (film); *v* 1747 and 1228 (Ac), 1519 cm^{-1} (NO_2); ^1H NMR ($CDCl_3$): δ 8.20 and 7.04 (2 × d, 4 H, *p*NP), 7.15–7.44 (m, 15 H, ArH), 5.15 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1Glc), 4.60–4.94 (d × 6, 6 H, $ArCH_2O$), 4.72 (d, 1 H, $J_{1',2'}$ 1.6 Hz, H-1′Gal), 4.32 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1Gal), 3.75 (bs, 1 H, GalH-4), 3.61 (dd, 1 H, $J_{2,3}$ 9.5, $J_{3,4}$ 3.5 Hz, GalH-3), 1.97–2.14 (6 × s, 18 H, Ac); FAB$^-$MS: *m*/*z* 1147 $[M-1]^-$: Anal. Calcd for $C_{57}H_{65}NO_{24}$: C, 59.63; H, 5.71; N, 1.22. Found: C, 59.57; H, 5.72; N, 1.22.

p-*Aminophenyl (α-D-galactopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside* (**2**).—To a solution of trisaccharide **8** (60 mg, 50 mmol) in MeOH (3 mL) was added NaOMe (10 mg), and the reaction mixture was stirred at rt for 6 h. The reaction mixture was quenched by addition of Amberlyst 15E-ion exchange resin, filtered, and concentrated under diminished pressure. The residue was purified by chromatography (silica gel, 30:1 $CHCl_3$–MeOH) to afford a deacetylated compound as a white powder (29 mg, 97%). The powder (60 mg, 50 mmol) was dissolved in MeOH (5 mL), and the mixture was added to 10 N HCl (10 μL, 0.10 mmol) and 10% $Pd(OH)_2$/C. The reaction mixture was stirred vigorously under H_2 atmosphere at rt for 6 h. The reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated under diminished pressure to give amine **2** (29 mg) as a brown amorphous solid which was used for the next step without further purification: $[\alpha]_D^{24}$ −16.9° (*c* 1.0, water); IR (film); *v* 3419 (OH), 1228 and 1076 cm^{-1} (NH_2); ^1H NMR (D_2O): δ 6.83 and 6.64 (2 × d, 4 H, *p*AP), 4.82 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1Glc), 4.77 (d, 1 H, $J_{1',2'}$ 3.2 Hz, H-1′Gal), 4.35 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1Gal).

p-N-*Acryloylamidophenyl (α-D-galactopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside* (**18**).—To a solution of amine **2** (25 mg, 0.04 mmol) and Et_3N (22 μL, 0.120 mmol) in MeOH (3 mL) was added a solution of acryloyl chloride (4 μL, 0.05 mmol) in CH_2Cl_2 (0.5 mL) slowly at 0 °C, and the reaction mixture was stirred at rt for 1.5 h. The reaction mixture was concentrated under diminished pressure and the residue was purified by chromatography (TSKgel HW-40S, water) to furnish monomer **18** (31 mg, 93%) as a white powder: $[\alpha]_D^{24}$ −5.8° (*c* 1.0, water); IR (disc); *v* 3298 (OH), 1660 (amide), 1033 and 827

cm^{-1} (olefin); ^1H NMR (D_2O): δ 7.32 and 7.02 (2 × d, 4 H, *p*AP), 6.28 (dd, 1 H, H-olefin), 6.20 (dd, 1 H, H-trans), 5.75 (dd, 1 H, H-cis), 5.01 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1Glc), 4.99 (d, 1 H, $J_{1',2'}$ 3.6 Hz, H-1′Gal), 4.42 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1Gal), 4.07 (bt, 1 H, H-5Gal); FAB$^-$MS: m/z 648 $[M-1]^-$: Anal. Calcd for $C_{27}H_{39}NO_{17}$: C, 49.92; H, 6.05; N, 2.16. Found: C, 49.90; H, 6.05; N, 2.16.

Glycoconjugate polymer (**19**).—To a solution of monomer **18** (30 mg, 0.046 mmol) and acrylamide (13 mg, 0.184 mmol) in degassed water (0.1 mL) was added 2,2-azobis(2-amidinopropane) dihydrochloride (1 mol% of monomers, 2.3 μmol in degassed water). The reaction mixture was frozen and degassed repeatedly under reduced pressure. The polymerization tube was sealed under reduced pressure, and incubated at 60 °C for 12 h. The reaction mixture was dialyzed in water (M_w 8000 cut off) for 3 days, and lyophilized to give **19** (34 mg, 76%) as a white powder: M_n 3.2×10^5 (SEC analysis, pullulan standard); mol fraction of sugar unit = 0.12 (as determined by ^1H NMR spectrum); IR (disc); ν 3248 (OH), 1662 cm^{-1} (amide); ^1H NMR (D_2O, 80 °C): δ 7.02–7.50 (br, ArH), 5.10 (bs, H-1Glc), 4.92 (bs, H-1′Gal), 4.31 (bs, H-1Gal), 3.61–4.23 (m, sugar-H), 2.39–2.61 (br, methyne of main chain), 1.66–2.03 (br, methylene of main chain). In the same manner as described above, polymer **17** was prepared from monomer **16**. **17**: M_n 4.3×10^5 (SEC analysis, pullulan standard); mol fraction of sugar unit = 0.13 as determined by ^1H NMR spectrum); IR (disc); ν 3225 (OH), 1661 cm^{-1} (amide); ^1H NMR (D_2O, 80 °C): δ 7.05–7.48 (br, ArH), 5.22 (bs, H-1Glc), 5.05 (bs, H-1′Gal), 4.40 (bs, H-1Gal), 3.56–4.31 (m, sugar-H), 2.32–2.65 (br, methyne of main chain), 1.72–1.93 (br, methylene of main chain).

Inhibitory activity of polymers against Stx-I and Stx-II.—A mixture of each Stx (250 pg/mL in PBS) and the diluted polymer solution ($10^{-2} \sim 10^{-14}$ μg/mL) was preincubated in a well at 37 °C for 1 h. To the well were added ACHN cells (65 μL of $8 \times (10^5 \times$ cells/mL) in Eagle's medium containing 10% FCS), and the mixture was incubated for 3 days at 37 °C. The mixture was treated with 0.014% neutral red solution (100 μL) for 1 h at 37 °C, washed with PBS (2×200 μL), and then mixed with 0.5 N HCl–35% EtOH (100 μL). The absorbance at 540 nm was measured at different concentrations.

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