Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
Tetrahedron 61 (2005) 5895–5905

Synthesis of polyanionic glycopolymers for the facile assembly of glycosyl arrays

Hirotaka Uzawa,[a,*] Hiroki Ito,[a] Masayuki Izumi,[a] Hideo Tokuhisa,[b] Kazuhiro Taguchi[b] and Norihiko Minoura[a]

[a]*Research Center of Advanced Bionics, National Institute of Advanced Industrial Science and Technology (AIST), Central 5, 1-1-1 Higashi, Tsukuba 305-8565, Japan*
[b]*Nanoarchitectonics Research Center, National Institute of Advanced Industrial Science and Technology (AIST), Central 5, 1-1-1 Higashi, Tsukuba 305-8565, Japan*

Received 3 March 2005; accepted 25 March 2005

Available online 10 May 2005

Abstract—Polyanionic glycopolymers were synthesized aiming at establishing a simple process for assembling glycosyl arrays. The synthetic glycopolymers carry the key carbohydrate epitopes of α-D-galactobioside (Gb_2), β-lactoside, and α-D-mannopyranoside, each of which serves as a ligand of bacterial toxins and adhesion proteins. The Gb_2 epitope, prepared from penta-*O*-acetyl-D-galactopyranose, was coupled with poly(ethylene-*alt*-maleic anhydride) in a polymer reaction to afford a Gb_2-embedded glycopolymer having also carboxylate (COO^-) polyanions at the side chain. The polyanionic glycopolymer was then applied to a preparation of sugar-coated gold electrodes, which involves an alternating layer-by-layer adsorption based on electrostatic interactions. The presence of the Gb_2-coat on the surface was evidenced by Fourier transform infrared reflection absorption spectroscopy. The Gb_2-coated glyco-chip was stable in 10 mM HEPES buffer containing 150 mM NaCl aq. Other glycopolymers carrying the β-lactoside and α-D-mannopyranoside epitopes were applied to the same assembling process. The derived glycosyl arrays will be useful for detecting Shiga toxins, other pathogenic toxins and viruses when applied as glyco-chips for surface plasmon resonance or quartz crystal microbalance technique.

1. Introduction

The cell-surface oligosaccharides bound to glycoproteins and glycolipids are associated with various biological roles on cell surfaces like cell-adhesion, signal transduction and regulation as well as bacterial and viral infections.[1] Therefore, much attention is paid to the species-specific interactions involved in carbohydrate–protein and carbohydrate–carbohydrate bindings. These interactions are analyzed with artificial models assisted by different techniques of quartz crystal microbalance (QCM), surface plasmon resonance (SPR), Au-nanoparticles, and other biological assays.[2] For all of these analyses, the fabrication of glycosyl arrays, chips, or nanoparticles is of crucial significance.[3] For such purpose, simple and convenient immobilization of carbohydrates to these sensor substrates is indispensable. Conventional immobilization processes apply the natural affinity of biotin and streptavidin and the formation of the self-assembled monolayers between the thiolated compounds and gold surfaces.[4]

Recently, Houseman et al. proposed a method for sugar immobilization, which is based on the Diels–Alder coupling reaction on gold surface between benzoquinone and glycosyl cyclopentadiene.[3a] Wong et al. proposed 1,3-dipolar cycloaddition between azido sugars and alkynes.[3b,e] Park et al. attached sugars onto substrate surfaces by Michel addition between maleimides and thiols.[3f] In these studies, monomeric carbohydrate molecules are routinely utilized. However, with an aim to establish a simple process for assembling glycosyl arrays, the utility of glycopolymers has not yet been fully explored,[5] albeit glycomaterials consisting of the glycopolymers can be generally expected to have a multivalent and cluster effect.[6] This effect can potentially enhance binding interactions with the receptor proteins. We describe herein the synthesis and convenient use of polyanionic glycopolymers for a facile immobilization of carbohydrates to Au-substrates. In this paper, an alternating layer-by-layer adsorption technique was applied, which is clearly distinct from the previous immobilization methods.

Keywords: Glycopolymers; Glycosyl arrays; Carbohydrate epitopes; Layer-by-layer; Polyanions.
* Corresponding author. Tel.: +81 29 861 4431; fax: +81 29 861 4680; e-mail: h.uzawa@aist.go.jp

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.03.102

As part of our ongoing project to assemble a library of various sulfo sugars,[7] we are interested in the artificial glycopolymers carrying Gb_3 and Gb_2 in clusters, which are able to adsorb the pathogenic *Escherichia coli* O-157 bacteria and their produced Shiga toxins.[8] In our preceding study, we applied the Gb_3-glycopolymers[8] as well as alkyl Gb_2-monolayers[9] in our QCM sensors targeting the Shiga toxins.

In the present study, we synthesized various polyanionic glycopolymers involving galactobioside (Gb_2) and other biologically important sugar epitopes for the purpose of simple, rapid and practical immobilization. These glycopolymers can be applicable to the fabrication of glycosyl arrays and glyco-chips. Our approach has an intriguing feature in that the polymer has two different functional groups, carbohydrates like Gb_2 as a toxin–ligand and an anionic species like COO^-. The anions distributed at the sugar-embedded polymer chain can tether the cationic counterpart surfaces coated with, for instance, quaternary ammonium ions through electrostatic interactions as depicted in Figure 1.

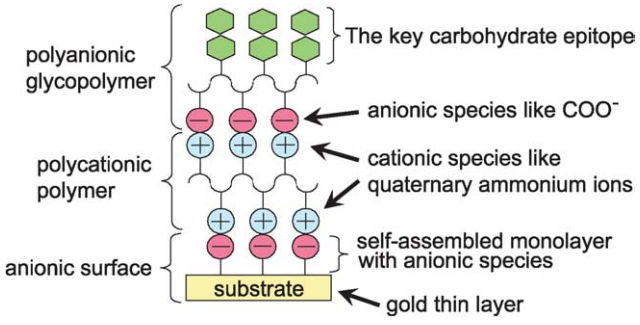


Figure 1. Schematic figure of alternating layer-by-layer membranes onto the substrate surface.

2. Results and discussion

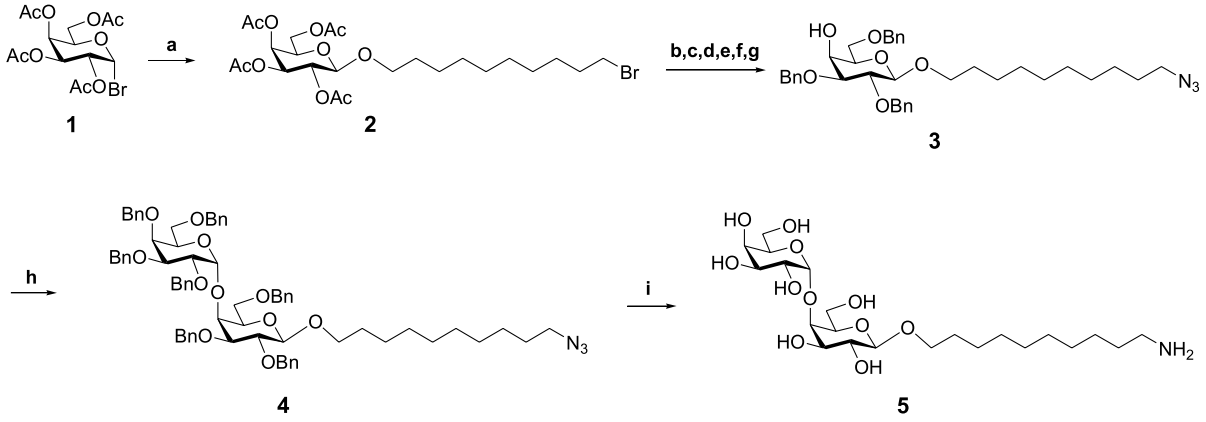
2.1. Synthesis of Gb_2-embedded polyanionic glycopolymers for the detection of *E. coli* O-157 Shiga toxins

Synthesis of the Gb_3-carrying glycopolymers has already been accomplished in the literature and found to show notable activity to block the Shiga toxins-host cell infections.[8] Most of the synthetic studies have focused on the molecular assembly to construct the glycosyl clusters as glycopolymers, glycodendrimers and starfish models.[10] Moreover, substituted polyacrylate-based neoglycoconjugates are reported as versatile chemical tools for biochemical and medicinal applications.[11] In the present approach, we synthesized novel polyanionic glycopolymers to fabricate glycosyl arrays based on the alternating layer-by-layer adsorption method.

For the aimed glycopolymers, the key intermediate **5** was synthesized from the known 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide **1** in a manner similar to our previously reported way (Scheme 1).[12] 10-Bromodecyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside **2** carrying a hydrophobic spacer at the reducing terminal was derived from commercially available **1** in 72% yield. The anomeric configuration in **2** was confirmed by ^1H and ^{13}C NMR spectroscopies. The doublet signal of H-1 with $J=8.0$ Hz appears at δ 4.45 ppm in its ^1H NMR spectrum, showing the β-coupling. The peak at δ 101.2 ppm (C-1) in ^{13}C NMR also supports this result.

Compound **2** was then converted to 10-azidodecyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranoside **3** possessing a reaction point at O-4 in the following way: (1) azide substitution of the terminal bromide by treatment of NaN_3 at 80 °C, (2) de-*O*-acetylation with NaOMe-MeOH, (3) 3,4-*O*-isopropylidenation by treatment with acetone and 2,2-dimethoxypropane in the presence of camphorsulfonic acid (CSA), (4) 2,6-di-*O*-benzylation with BnBr and NaH in DMF, (5) de-*O*-isopropylidenation by treatment of CSA, and (6) selective 3-*O*-benzylation using Bu_2SnO, BnBr and Bu_4NBr (56% in 6 steps).

Stereoselective glycosylation of **3** and 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride[13] was carried out in the presence of $AgClO_4$ as activator in diethylether to obtain α1-4 globobioside **4** and β1-4 disaccharide (α/β=ca. 10:1), which was isolated by silica gel column chromatography, respectively. The ^{13}C NMR signal at δ 100.4 ppm (C-1′) of



Scheme 1. Synthesis of a key intermediate Gb_2 derivative with an amino group at the terminal. Reagents and conditions: (a) 10-bromo-1-decanol, Ag_2CO_3, CH_2Cl_2, MS4A (72%); (b) NaN_3, DMF, 80 °C (99%); (c) NaOMe, MeOH; (d) 2,2-dimethoxypropane, acetone, camphorsulfonic acid (CSA) (73%, two steps); (e) BnBr, NaH (quant.); (f) MeOH, CSA (84%); (g) Bu_2SnO, toluene, reflux, then BnBr, Bu_4NBr (0.5 equiv), 93%; (h) 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride, $AgClO_4$, Et_2O, MS4A, 0 °C–rt, 22 h (α, 67%; β, 7%); (i) $Pd(OH)_2$, H_2, MeOH–AcOEt (2:5, v/v) (85%).

disaccharide **4** shows to be the α-linkage of the newly formed glycosidic bond. COSY and HMQC analyses also confirmed the α1-4 linkage. The FAB-MS spectrum also supports the disaccharide ($[M+Na]^+$ 1176.8). Compound **4** was then subjected to catalytic hydrogenolysis with H_2 and $Pd(OH)_2$ to give the fully deprotected **5** carrying an amino group at the terminal (for **5**; δ 4.97 ppm (d, $J=2.9$ Hz, H-1′) and δ 4.26 ppm (d, $J=7.3$ Hz, H-1)), which gave similar results to the data reported for Galα1-4Gal octadecyl glycoside.[9]

Coupling of 2.0 mol equiv of the key monomeric epitope **5** (0.02 mmol) with a reactive polymer, poly(ethylene-*alt*-maleic anhydride) ($M_w=100{,}000$–$500{,}000$) (0.01 mmol) at 70 °C was performed to produce the Gb_2-embedded anionic polymer **6** containing 43% of the Gb_2 sugar moiety after being dialyzed (M_w 2000 cut-off) in water for 3 days, further purified with a gel-permeation chromatography and lyophilized (Scheme 2). In its IR spectrum, two characteristic signals appeared at 1643 and 1566 cm^{-1}, showing the presence of amide (NHCO) groups in the polymer.

A particularly notable point is that the terminal amino group selectively reacted with an anhydride moiety in the polymer, almost alternately, to generate an amide group and a free carboxylic acid in a ratio of 1:1. Thus, sugar contents do not exceed the 50% value theoretically. The carboxylate anions (COO^-) can effectively serve as the binding sites with the counterpart cationic surfaces or the cationic polymers through electrostatic interactions. The characteristic polymer **6** carrying both Gb_2 carbohydrate and carboxylate anions is advantageous for the immobilization onto sensor surfaces rather than random or block polymers, because the polyanionic glycopolymer **6** distributes carboxylate anions alternately to give almost homogeneous clusters in the polymer chain, while random or block polymerization gave heterogeneous clusters on which carboxylate anions tend to be irregularly distributed in the polymers.

To determine the precise sugar content for the Gb_2 moiety in polymer **6**, the phenol-H_2SO_4 method[14] was carried out. This method is a reliable and sensitive analysis to determine total sugar (Gb_2) amounts after complete hydrolysis of **6**. In a similar approach shown in Scheme 2, various different Gb_2-containing anionic polymers depending on the feed ratio of the key module segment **5** were synthesized, and each sugar content was determined as summarized in Table 1. These polymers can be useful for the fabrication of sensor chips according to varying sugar density.

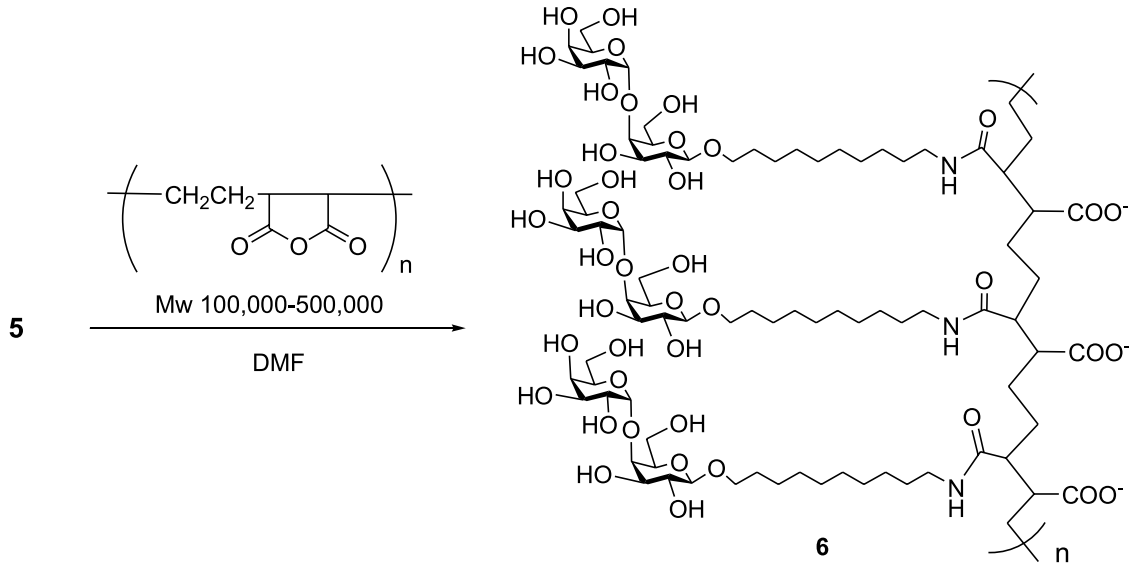
Table 1. Reaction conditions for preparing polyanionic glycopolymer **6** with various Gb_2 contents

Monomer **5** (mol equiv)[a]	Reaction time (h)	Temperature (°C)	Sugar content of **6** (%)[b]	M_w[c]
0.1	35	rt	3	4.0×10^5
1.0	60	rt	13	5.0×10^5
2.0	45	rt	25	6.7×10^5
2.0	32	70	43	7.8×10^5

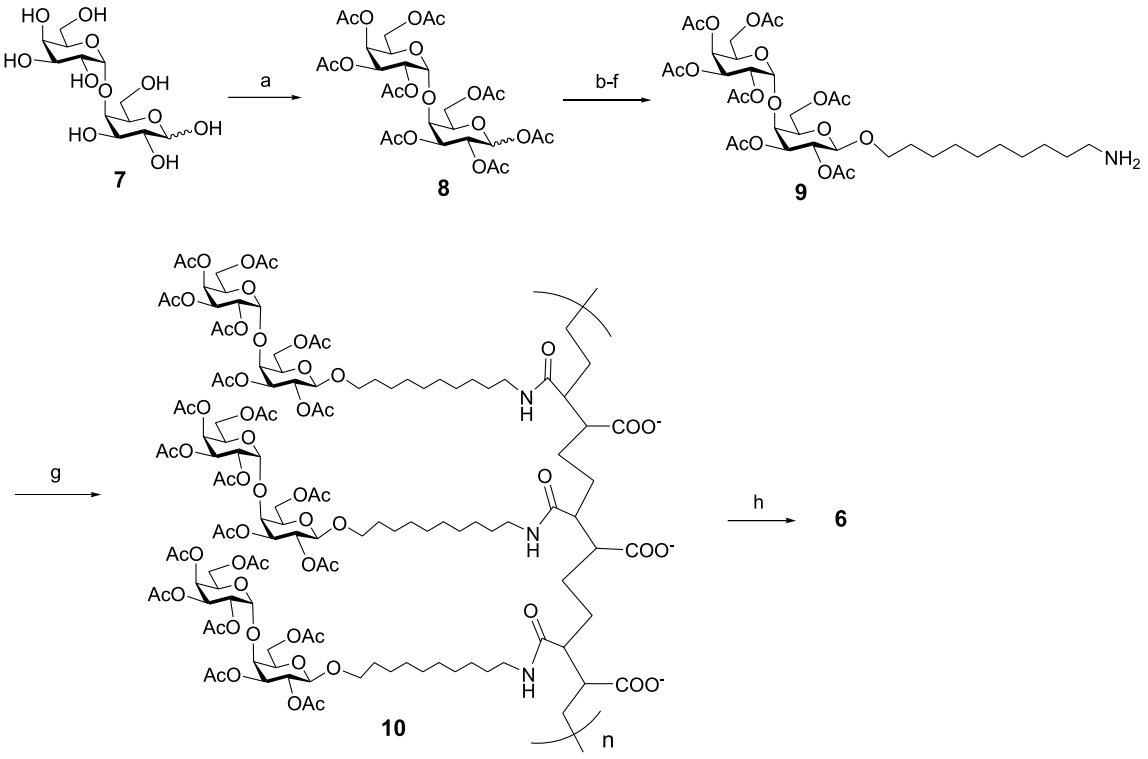
[a] mol equiv to poly(ethylene-*alt*-maleic anhydride).
[b] Determined by the H_2SO_4-phenol method.
[c] Determined by the static light scattering method.

The structure of **6** was further confirmed by an alternative synthesis shown in Scheme 3. Another key carbohydrate intermediate **9** was obtained from commercially available **7** in 6 steps (67% overall yield). Then, compound **9** was reacted with poly(ethylene-*alt*-maleic anhydride) to produce the fully protected Gb_2-embedded polymer **10**. In the ^1H NMR spectrum, broad signals appeared at near δ 7.4 ppm in DMF-d_7, indicating the presence of the amide groups. Deprotection of **10** was achieved in DMF containing excess amount of 1 M NaOH aq to give polyanionic glycopolymer **6**. In the ^1H NMR spectrum, none of the Ac groups was observed. The ^1H NMR of **6** in Scheme 3 is well-consistent with the data for **6** synthesized in Scheme 2. This strategy completely eliminates the possibility of the ester formation by a side reaction between hydroxyl groups like O-6 in the Gb_2 moiety and the active anhydride in the polymer.

When using another polymer, poly(isobutylene-*alt*-maleic



Scheme 2. Synthesis of Gb_2-embedded anionic polymer **6** in the polymer reaction.



Scheme 3. Synthesis of another key module Gb_2 derivative **9** and polyanionic glycopolymer **6**. Reagents and conditions: (a) Ac_2O, DMAP, pyridine (98%); (b) hydrazine–AcOH (93%); (c) Cl_3CCN, DBU (90%; α only); (d) 10-bromo-1-decanol, trimethylsilyltriflate, 0 °C (90%); (e) NaN_3, DMF, 80 °C (99%); (f) $Pd(OH)_2$, H_2, MeOH (91%); (g) poly(ethylene-*alt*-maleic anhydride), DMAP, DMF, then H_2O; (h) 1 M NaOH aq, DMF.

anhydride) (M_w = 60,000), the polymer reaction similar to **6** hardly proceeded. This large difference probably is ascribed to the steric hindrance for the isobutyl group neighboring on the reaction site in the polymer chain.

2.2. Synthesis of other sugar-embedded anionic polymers

One advantage of glycosyl arrays is the potential of concurrent analysis for various viruses, bacteria and other carbohydrate receptor proteins. To this end, several sugar-embedded polymers are required. As model compounds, we newly synthesized two polyanionic polymers carrying β-lactosyl and α-D-mannosyl residues, respectively. These sugar epitopes ubiquitously exist on the cell surfaces.

In this section, we chose the *p*-nitrophenyl (*p*NP) group at the aglycon because the *p*NP group is readily linked to the polymer chains, after transformation to a *p*-aminophenyl (*p*AP) group by chemical reduction.[8,15]

Commercially available *p*NP lactoside **11** was reduced in the presence of Pd under H_2 atmosphere to obtain *p*AP lactoside **12**, quantitatively. Compound **12** was then reacted with poly(ethylene-*alt*-maleic anhydride) in a similar manner to polymer **6** to give **13** carrying lactoside residues. In a similar approach, the mannose-embedded anionic polymer **16** was produced from the corresponding *p*NP α-D-mannoside **14** in Scheme 4. This characterization was carried out as stated above, summarized in Table 2.

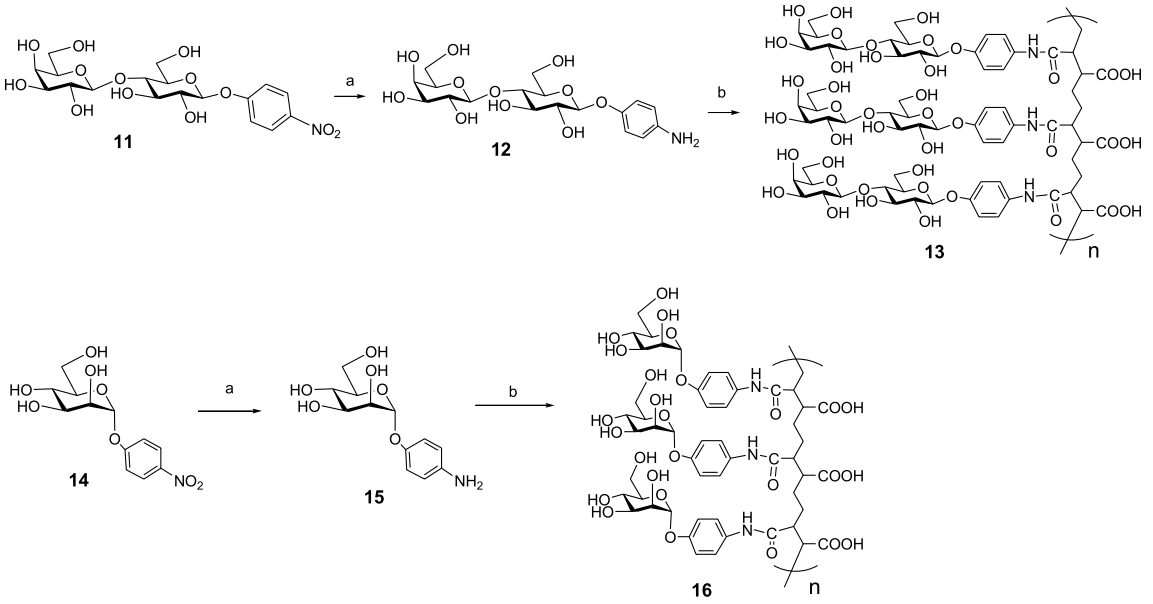
2.3. Alternating layer-by-layer formation of sugar-embedded polyanionic glycopolymer 6 and its confirmation by the FTIR-RAS

The alternating layer-by-layer adsorption technique has provided a convenient way to fabricate thin-layer films onto solid surfaces or polymer-support,[16] and is applied to the study of molecular imprinting and chemical filters.[17] We applied this technique to prepare the glycosyl surfaces.

Mercaptopropionic acid was adsorbed onto a gold surface by a self-assembled monolayer technique[18] to fabricate the surface A in Scheme 5. On this surface A, quaternary ammonium cationic polymer, [poly(diallyldimethylammonium chloride); M_w 400,000–500,000] was adsorbed in 10 mM HEPES buffer (pH 7.4) for 10 min to obtain surface B. Finally, Gb_2-embedded polyanionic polymer **6** was adsorbed by electrostatic interactions to form a three-layer structure (surface C) as illustrated in Scheme 5.

The surface C was analyzed by Fourier transform infrared reflection absorption spectroscopy (FTIR-RAS). Two well-defined adsorptions were observed at 1650 and 1570 cm^{-1}, showing amide groups (Fig. 2). Apparently, this result supports the presence of the Gb_2-sugar epitope on the surface C.

Subsequently, we examined the stability of the Gb_2-containing polyanionic glycopolymer **6** on the surface C. The FTIR-RAS analysis shows that the corresponding amide peaks are nearly completely retained even when

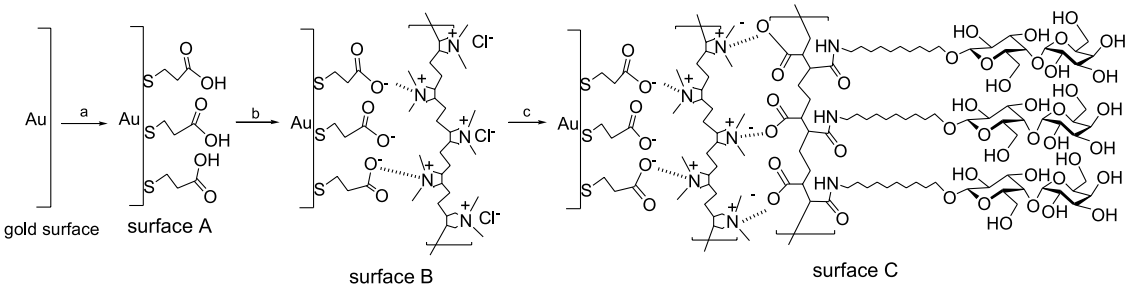


Scheme 4. Synthesis of other sugar-embedded polymers. Reagents and conditions: (a) $Pd(OH)_2$, H_2, H_2O (99% for **12**, 99% for **15**); (b) poly(ethylene-*alt*-maleic anhydride), DMF.

Table 2. Reaction conditions for preparing polyanionic glycopolymer **13** and **16**.

Polymer	Monomer (mol equiv)[a]	Reaction time (h)	Temperature °C	Sugar content (%)[b]	M_w[c]
13	**12** (2.0)	50	rt	40	7.2×10^5
16	**15** (3.6)	22	rt	37	6.0×10^5

[a] mol equiv to poly(ethylene-*alt*-maleic anhydride).
[b] Determined by the H_2SO_4-phenol method.
[c] Determined by the static light scattering method.



Scheme 5. Formation of layer-by-layer adsorption. Reagents and conditions: (a) 3-mercaptopropionic acid in ethanol; (b) poly(diallyldimethylammonium chloride) in 10 mM HEPES buffer (pH 7.4); (c) **6** in the same condition as (b).

washed with 50 mM HCl aq and also exposed to 10 mM HEPES buffer containing 150 mM NaCl aq for at least 24 h (data are not shown), and are stable during the SPR measurements. Those results show that the Gb_2-coated surface C can be usefully applied for toxin detection.

Other surfaces coated with polymers **13** and **16** were also prepared in a similar manner to **6**. More detailed information for surface characterization by the FTIR-RAS, the X-ray photoelectron spectroscopy (XPS) analysis and thickness of each layer by ellipsometry are under study and will be reported in a separate paper.

3. Conclusion

In conclusion, we have synthesized novel polyanionic glycopolymers for the facile fabrication of glycosyl arrays by applying an alternating layer-by-layer method. The present polyanionic glycopolymers are useful to assemble glycosyl arrays or glycosyl chips, for instance, targeting *E. coli* O-157 Shiga toxins. This approach will be a highly practical and promising way to detect toxins, viruses, bacteria and other receptor proteins with the SPR and a quartz crystal microbalance (QCM) method, and the details will be reported in due course.

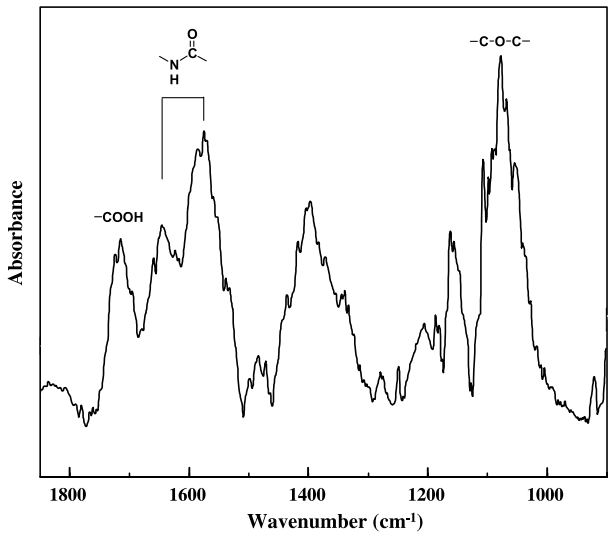


Figure 2. FTIR-RAS spectrum of surface C in Scheme 5.

4. Experimental

4.1. General methods

Globobiose was purchased from Toronto Research Chemicals (Canada). *p*NP β-lactoside and *p*NP α-D-mannopyranoside was purchased from Sigma. 2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl bromide, poly(ethylene-*alt*-maleic anhydride) (M_w=100,000–500,000) and poly(diallyldimethylammonium chloride) (M_w 400,000–500,000) were purchased from Aldrich. Reactions were monitored by thin-layer chromatography (TLC) on Silica Gel 60 F_{254} (E. Merck), which were visualized by UV light and by spraying with 20% H_2SO_4 in EtOH followed by charring at 180 °C. Column chromatography was performed on Silica Gel 60 N (Cica Reagent). NAP™-10 Column (Sephadex G-25) was purchased from Amersham Pharmacia Biotech, Ultrafree-MC filter from Millipore and they were used for a gel permeation chromatography. Optical rotations were measured with JASCO DIP-1000 digital polarimeter at ambient temperature, using a 10-cm micro cell. ^{1}H and ^{13}C NMR spectra were recorded on a Varian INOVA 400 or a JEOL LA-600 spectrometer for solutions in $CDCl_3$, D_2O or DMF-d_7. Chemical shifts are given in ppm and referenced to internal *tert*-butyl alcohol (δ_H 1.23 in D_2O or δ_C 31.2 in D_2O), TMS (δ_H 0.00 in $CDCl_3$ and DMF-d_7), or $CDCl_3$ (δ_C 77.7 in $CDCl_3$). All data are assumed to be first order with apparent doublet and triplets reported as d and t, respectively. Resonances that appear broad are designated b. A digital resolution is ca. 0.4 Hz. FAB mass spectra (FAB-MS) were recorded using a JEOL DX 303 mass spectrometer, and high-resolution mass spectra (HR-MS) were recorded using a Hitachi M 80 mass spectrometer or Mariner Biospectroscopy Workstation ESI-TOF MS. Elemental analyses were performed with a Carlo Elba EA-1108 or Perkin-Elmer EA-2400 instrument. Average molecular weights (M_w) of each glycopolymer were determined by static light scattering using Zetasizer Nano ZS instrument (Malvern Instruments Ltd, Worcestershire, UK), and poly[ethylene-*alt*-maleic acid] was used as the standard polymer (M_w 300,000). Fourier transform infrared reflection absorption spectroscopy (FTIR-RAS) measurements were made using a Digilab FTS-7000 spectrometer equipped with a Harrick Scientific reflection accessory and a liquid-N_2-cooled MCT detector.

4.1.1. Preparation of layer-by-layer substrates by the alternating adsorption. Gold substrates were cleanly washed with a freshly prepared 'piranha solution', H_2SO_4–H_2O_2 (3/1, v/v) to completely remove any organic adsorption, and they were rinsed with milli-Q water several times. The gold substrates were then immersed into ethanolic solution containing mercaptopropionic acid (1 mM). After 16 h, the substrates were rinsed extensively with absolute ethanol and dried under nitrogen atmosphere to give surface A in Scheme 5. On this surface A, quaternary ammonium cationic polymer, [poly(diallyldimethylammonium chloride); M_w 400,000–500,000] (100 μg/mL) was adsorbed in 10 mM HEPES buffer (pH 7.4) for 10 min to obtain surface B. Finally, Gb_2-embedded polyanionic polymer **6** (100 μg/mL) was adsorbed in 10 mM HEPES buffer (pH 7.4) for 10 min to form a three-layer structure (surface C).

4.1.2. FTIR-RAS measurements. FTIR-RAS measurements were carried out using refractor top plate under nitrogen atmosphere.

4.1.3. 10-Bromodecyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside 2. A solution of 10-bromo-1-decanol (123 mg, 0.52 mmol) and Ag_2CO_3 (137.9 mg, 0.5 mmol) in dry CH_2Cl_2 (2 mL) containing freshly activated MS-4 Å (200 mg) was stirred for 1 h at 25 °C under N_2. To the reaction mixture, 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide **1** (206 mg, 0.50 mmol) in dry CH_2Cl_2 (3 mL) was slowly added during 40 min. The reaction mixture was stirred at 25 °C for 20 h and then filtered through a Celite pad. The organic layer was washed with satd aq $NaHCO_3$, and water, respectively. The organic layer was dried ($MgSO_4$), filtered and concentrated. Column chromatography of the product on silica gel with 4:6 EtOAc–hexane afforded compound **2** (209 mg, 72%). $[\alpha]_D$ −9.9° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.39 (dd, H-4, J= 3.2, 0.8 Hz), 5.20 (dd, H-2, J=8.0, 10.4 Hz), 5.02 (dd, H-3, J=3.2, 10.4 Hz), 4.45 (d, H-1, J=8.0 Hz), 4.19 (dd, H-6, J=6.4, 11.2 Hz), 4.13 (dd, H-6′, J=7.2, 11.2 Hz), 3.91–3.85 (m, H-5 and –(CH_2)–, 2H), 3.49–3.44 (m, –(CH_2)–, 1H), 3.41 (t, –CH_2–Br, J=6.8 Hz, 2H), 2.149 (Ac), 2.051 (Ac), 2.049 (Ac), 1.986 (Ac), 1.88–1.81 (m, –(CH_2)–, 2H), 1.62–1.24 (m, –(CH_2)–, 14H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3, 170.2, 170.1, 169.2, 101.2, 70.8, 70.4, 70.1, 68.8, 67.0, 61.2, 33.9, 32.7, 29.30, 29.27, 29.22, 29.1, 28.6, 28.0, 25.6, 20.64, 20.55, 20.47; IR (liquid film): 2931, 2857, 1749, 1648, 1434, 1369, 1223, 1175, 1138, 1079, 1057, 957, 901, 840, 737, 645, 597 cm^{-1}; HRMS calcd for $C_{24}H_{39}BrO_{10}Na$ $[M+Na]^+$ 589.1625, found 589.1635.

4.1.4. 10-Azidodecyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranoside 3.

4.1.4.1. Synthesis of 10-azidodecyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside. 10-Bromodecyl glycoside **2** (1.46 g, 2.58 mmol) and NaN_3 (300 mg, 4.61 mmol) was dissolved in DMF (20 mL). The reaction mixture was stirred at 80 °C for 4 h. The organic layer was diluted with EtOAc, washed with brine solution, dried ($MgSO_4$), filtered and

concentrated. Column chromatography of the product on silica gel with 4:6 EtOAc–hexane gave 10-azidodecyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (1.35 g, 99%). $[\alpha]_D$ −12.9° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.39 (d, H-4, *J*=3.2 Hz), 5.20 (dd, H-2, *J*=8.0, 10.4 Hz), 5.02 (dd, H-3, *J*=3.2, 10.4 Hz), 4.45 (d, H-1, *J*=8.0 Hz), 4.19 (dd, H-6, *J*=6.4, 11.2 Hz), 4.13 (dd, H-6′, *J*=7.2, 11.2 Hz), 3.91–3.85 (m, H-5 and –(CH_2)–, 2H), 3.49–3.44 (m, –(CH_2)–, 1H), 3.26 (t, –CH_2–N_3, *J*=6.8 Hz, 2H), 2.147, 2.051, 2.049 and 1.986 (4×Ac), 1.64–1.24 (m, –(CH_2)–, 16H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3, 170.2, 170.1, 169.2, 101.2, 70.8, 70.4, 70.1, 68.8, 67.0, 61.2, 51.3, 29.3, 29.2, 29.1, 29.0, 28.7, 26.6, 25.7, 20.62, 20.55, 20.47; IR (liquid film): 2932, 2858, 2098, 1756, 1456, 1435, 1370, 1224, 1175, 1135, 1080, 1058, 957, 902, 737, 597 cm^{-1}. Anal. Calcd for $C_{24}H_{39}O_{10}N_3$: C, 54.43; H, 7.42; N, 7.93. Found C, 54.33; H, 7.54; N, 7.90.

4.1.4.2. Synthesis of 10-azidodecyl 3,4-*O*-isopropylidene-β-D-galactopyranoside. Treatment of the above compound (708 mg, 1.34 mmol) with 0.1 M NaOMe for 3 h followed by neutralization with Dowex 50 WXH^+ resin afforded deacetylated product, 10-azidodecyl β-D-galactopyranoside. To a solution of crude 10-azidodecyl β-D-galactopyranoside in dry acetone (20 mL), 2,2-dimethoxypropane (0.86 mL, 7 mmol) and camphorsulfonic acid (255 mg, 1.1 mmol) were added. The reaction mixture was stirred at 25 °C for 2.5 h and then neutralized with Et_3N. The reaction mixture was then diluted with EtOAc. The organic layer was washed with water, dried ($MgSO_4$), filtered and concentrated. Column chromatography on silica gel with EtOAc–hexane (8:2) gave 10-azidodecyl 3,4-*O*-isopropylidene-β-D-galactopyranoside (392 mg, 73%); $[\alpha]_D$ +11.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.18 (d, H-1, *J*=8.4 Hz), 3.25 (t, –CH_2–N_3, *J*=6.8 Hz), 1.52 and 1.35 (2×Me of isopropylidene); ^{13}C NMR (100 MHz, $CDCl_3$): δ 110.9 (Me_2C), 102.9 (C-1), 79.5, 74.5, 74.2, 74.1, 70.7, 62.9, 52.0 (–CH_2–N_3), 30.2, 29.97, 29.95, 29.93, 29.7, 29.4, 28.7 (Me_2C), 27.3, 26.9 (Me_2C), 26.5; IR (liquid film): 3313, 2986, 2933, 2857, 2095, 1456, 1381, 1372, 1241, 1218, 1161, 1144, 1096, 1077, 1036, 964, 904, 891, 873, 808, 738 cm^{-1}; HRMS calcd for $C_{19}H_{35}N_3O_6Na$ [M+Na]$^+$ 424.2424, found 424.2424.

4.1.4.3. Synthesis of 10-azidodecyl 2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranoside. A mixture of isopropylidene compound synthesized in Section 4.1.4.2 (338 mg, 0.84 mmol) and NaH (52 mg, 2.2 mmol) in dry DMF (20 mL) was stirred at 25 °C for 30 min and benzyl bromide (0.26 mL, 2.2 mmol) was added. The reaction mixture was further stirred at 25 °C for 2 h. The reaction mixture was then diluted with EtOAc. The organic layer was washed with water, dried ($MgSO_4$), filtered and concentrated. Column chromatography of the product on silica gel with 4:6 EtOAc–hexane gave 10-azidodecyl 2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (530 mg, quant.); $[\alpha]_D$ +18.7° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz,$CDCl_3$): δ 7.42–7.22 (m, 10H, aromatic), 4.85 (d, CH_2–C_6H_5, 11.6 Hz), 4.78 (d, CH_2–C_6H_5, *J*=11.6 Hz), 4.64 (d, CH_2–C_6H_5, *J*=11.6 Hz), 4.55 (d, CH_2–C_6H_5, *J*=11.6 Hz), 4.3 (d, H-1, *J*=8.0 Hz), 4.16–3.88 (m, H-3, H-4, H-5, –(CH_2)–), 3.82–3.74 (m, H-6 and H-6′), 3.54–3.46 (m, –(CH_2)–, 1H), 3.41–3.35 (m, H-2, 1H), 3.23 (t, –CH_2–N_3, *J*=7.2 Hz, 2H), 1.34 and 1.32 (s, 2×Me), 1.69–1.24 (m, –(CH_2)–, 16H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.3, 138.2, 128.3, 128.1, 127.5, 127.4, 109.8 (Me_2C), 102.9 (C-1), 79.6, 79.0, 73.8, 73.5, 72.2, 69.8, 69.5, 51.4 (–CH_2–N_3), 29.6, 29.4, 29.32, 29.30, 29.1, 28.7, 27.7, 26.6, 26.3, 26.0; IR (liquid film): 3031, 2986, 2931, 2857, 2095, 1497, 1455, 1371, 1243, 1219, 1163, 1097, 1080, 1045, 1029, 871, 806, 736, 697 cm^{-1}; HRMS calcd for $C_{33}H_{47}N_3O_6Na$ [M+Na]$^+$ 604.3363, found 604.3350.

4.1.4.4. Synthesis of 10-azidodecyl 2,6-di-*O*-benzyl-β-D-galactopyranoside. A mixture of the above di-*O*-benzyl compound (508 mg, 0.84 mmol) and camphorsulfonic acid (139 mg, 0.6 mmol) in dry MeOH (10 mL) was stirred at 25 °C for 2 h. The reaction mixture was then neutralized with Et_3N. The reaction mixture was then diluted with EtOAc. The organic layer was washed with water, dried ($MgSO_4$), filtered and concentrated. Column chromatography on silica gel with EtOAc–hexane (4:6) gave 10-azidodecyl 2,6-di-*O*-benzyl-β-D-galactopyranoside (387 mg, 84%); $[\alpha]_D$ +5.2° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38–7.25 (m, aromatics, 10H), 4.97 (d, CH_2–C_6H_5, *J*=11.6 Hz), 4.67 (d, CH_2–C_6H_5, *J*=11.6 Hz), 4.59 (s, CH_2–C_6H_5, 2H), 4.36 (d, H-1, *J*=7.6 Hz), 4.00–3.92 (m, –(CH_2)– and H-4, 2H), 3.82–3.71 (m, H-6 and H-6′, 2H), 3.63–3.46 (m, H-3, H-5, H-2 and –(CH_2)–, 4H), 3.24 (t, –CH_2–N_3, *J*=7.2 Hz, 2H), 1.71–1.22 (m, –(CH_2)–, 16H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 139.1, 138.6, 129.1, 129.0, 128.7, 128.4, 128.33, 128.30, 104.3 (C-1), 79.7, 75.1, 74.2, 73.9, 73.8, 70.5, 70.0, 69.6, 52.0 (–CH_2–N_3), 30.3, 30.03, 29.98, 29.96, 29.7, 29.4, 27.3, 26.7; IR (liquid film): 3401, 3064, 3034, 2929, 2856, 2096, 1454, 1368, 1288, 1260, 1077, 736, 697 cm^{-1}. Anal. Calcd for $C_{30}H_{43}O_6N_3$: C, 66.52; H, 8.00; N, 7.76. Found C, 66.47; H, 8.13; N, 7.57.

4.1.4.5. Synthesis of 3. To a solution of the above de-*O*-isopropylidenated compound (157 mg, 0.29 mmol) in toluene (10 mL), Bu_2SnO (79.7 mg, 0.32 mmol) was added and the mixture was refluxed with azeotropic removal of water for 6 h. The reaction mixture was allowed to 25 °C. Benzyl bromide (0.1 mL, 0.87 mmol) and Bu_4NBr (48 mg, 0.15 mmol) were added and stirred at 25 °C for 3 h. The reaction mixture was then diluted with EtOAc. The organic layer was washed with water, dried ($MgSO_4$), filtered and concentrated. Column chromatography of the product on silica gel with 4:6 EtOAc–hexane gave pure **3** (171 mg, 93%); $[\alpha]_D$ −7.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39–7.25 (m, aromatics, 15H), 4.92 (d, CH_2–C_6H_5, *J*=10.8 Hz), 4.72 (d, CH_2–C_6H_5, *J*=10.8 Hz), 4.72 (s, CH_2–C_6H_5, 2H), 4.59 (s, CH_2–C_6H_5, 2H), 4.35 (d, H-1, *J*=7.6 Hz), 4.02 (br s, H-4), 3.98–3.91 (m, –CH_2O–, 1H), 3.80 (dd, H-6, *J*=6.0, 10.0 Hz), 3.72 (dd, H-6′, *J*=6.0, 10.0 Hz), 3.63 (dd, H-2, *J*=8.0, 9.2 Hz), 3.55 (br t, H-5, *J*=6.0 Hz), 3.53–3.47 (m, –(CH_2)–, 1H), 3.49 (dd, H-3, *J*=3.2, 9.2 Hz), 3.24 (t, –CH_2–N_3, *J*=7.2 Hz, 2H), 1.71–1.22 (m, –(CH_2)–, 16H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 139.3, 138.6, 138.5, 129.03, 129.01, 128.9, 128.7, 128.41, 128.36, 128.32, 128.2, 104.2 (C-1), 81.0, 79.4, 75.6, 74.1, 73.6, 72.8, 70.4, 69.7, 67.4, 52.0 (–CH_2–N_3), 30.3, 30.0, 30.0, 29.7, 29.4, 27.3, 26.7; IR (liquid film): 3536, 3065, 3031, 2929, 2857, 2095, 1454, 1366, 1300, 1264, 1210, 1159, 1096, 1077, 1029, 917, 736, 697 cm^{-1}. Anal. Calcd for

$C_{37}H_{49}O_6N_3$: C, 70.34; H, 7.82; N, 6.65. Found C, 70.60; H, 7.82; N, 6.47.

4.1.5. 10-Azidodecyl *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranoside 4. A solution of compound **3** (116 mg, 0.18 mmol) and tetra-*O*-benzyl-α-D-galactopyranosyl chloride[13] (203 mg, 0.36 mmol) in dry diethyl ether (20 mL) containing freshly activated 4 Å MS (1 g) was stirred under N_2 for 1 h and cooled at 0 °C, $AgClO_4$ (112 mg, 0.54 mmol) was then added. The reaction temperature was gradually elevated to 25 °C, and stirring was continued in the dark for 22 h at the temperature. The reaction mixture was diluted with EtOAc and then filtered through a Celite pad, washed with satd aq $NaHCO_3$, water, respectively. The organic layer was dried ($MgSO_4$), filtered and concentrated. Column chromatography of the product on silica gel with 2:8 EtOAc–hexane afforded disaccharide **4** (134 mg, 67%) and β1-4 isomer (14 mg, 7%). Compound **4**; $[\alpha]_D$ +47.5° (*c* 0.71, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.37–7.16 (m, aromatics, 35H), 5.03 (br s, H-1′), 4.92–4.87 (m, C*H*$_2$–C_6H_5, 3H), 4.80–4.76 (m, C*H*$_2$–C_6H_5, 4H), 4.68 (d, C*H*$_2$–C_6H_5, *J*=11.7 Hz, 1H), 4.55 (d, C*H*$_2$–C_6H_5, *J*=11.4 Hz, 1H), 4.54 (d, C*H*$_2$–C_6H_5, *J*=12.8 Hz, 1H), 4.46–4.42 (m, H-5′, 1H), 4.31 (d, H-1, *J*=7.7 Hz), 4.26 (d, C*H*$_2$-C_6H_5, *J*=12.0 Hz, 1H), 4.22 (d, C*H*$_2$–C_6H_5, *J*=12.0 Hz, 1H), 4.15 (d, C*H*$_2$–C_6H_5, *J*=12.0 Hz), 4.13 (d, C*H*$_2$–C_6H_5, *J*=12.0 Hz), 4.10 (br, H-2′, H-3′, H-4′, 3H), 4.01 (bd, H-4, *J*=2.9 Hz, 1H), 3.98–3.90 (m, H-6a and –OC*H*$_2$–, 2H), 3.66 (dd, H-2, *J*=7.7, 9.9 Hz), 3.56–3.46 (m, H-6′a, H-6b, H-5 and –OC*H*$_2$–, 4H), 3.38 (dd, H-3, *J*=2.9, 9.9 Hz), 3.26–3.22 (m, H-6′b and –C*H*$_2$–N_3, 3H), 1.71–1.26 (m, –(C*H*$_2$)–, 16H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.9, 138.8, 138.7, 138.6, 138.1, 138.0, 128.3, 128.21, 128.16. 128.06, 128.0, 127.8, 127.6, 127.5, 127.4, 127.3, 103.9 (C-1), 100.4 (C-1′), 80.8, 78.9, 76.5, 75.0, 74.8, 74.7, 74.6, 73.7, 73.5, 73.1, 73.0, 72.3, 72.2, 70.1, 69.2, 68.0, 67.9, 51.4 (–C*H*$_2$–N_3), 29.7, 29.4, 29.3, 29.1, 28.8, 26.6, 26.1; IR (liquid film): 3062, 3030, 2929, 2858, 2095, 1497, 1454, 1365, 1269, 1208, 1097, 1056, 1028, 735, 697 cm^{-1}. Anal. Calcd for $C_{71}H_{83}O_{11}N_3$: C, 73.87; H, 7.25; N, 3.64. Found C, 74.05; H, 7.18; N, 3.74; FAB-MS(pos): $[M+Na]^+$ 1176.8. β**1–4** isomer; $[\alpha]_D$ +12° (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47–7.18 (m, aromatics, 35H), 5.12 (d, C*H*$_2$–C_6H_5, *J*=10.8 Hz, 1H), 4.99 (d, C*H*$_2$–C_6H_5, *J*=12.0 Hz, 1H), 4.91 (d, H-1′, *J*=7.6 Hz), 4.81–4.69 (m, C*H*$_2$–C_6H_5, 6H), 4.59–4.49 (m, C*H*$_2$–C_6H_5, 3H), 4.42 (d, C*H*$_2$–C_6H_5, *J*=10.8 Hz, 1H), 4.35 (d, H-1, *J*=8.0 Hz), 4.34 (s, C*H*$_2$–C_6H_5, 2H), 4.24 (br d, H-4′, *J*=2.4 Hz), 3.98–3.93 (m, –O–C*H*$_2$–(CH$_2$)–, 1H), 3.86 (br d, H-4, *J*=2.8 Hz), 3.84–3.67 (m, H-2, H-2′, H-6′a and H-6′b, 4H), 3.56–3.42 (m, H-3, H-3′, H-5, H-5′, H-6a, H-6b and –O–C*H*$_2$–(CH$_2$)–, 7H), 3.23 (t, –C*H*$_2$–N_3, 2H), 1.67–1.25 (m, –(C*H*$_2$)–, 16H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.8, 137.9, 137.7, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9. 127.8, 127.7, 127.6, 127.5, 127.3, 103.9 (C-1), 102.8 (C-1′), 82.0, 81.9, 80.0, 79.9, 75.2, 74.9, 74.5, 74.2, 73.8, 73.5, 73.4, 73.2, 72.7, 70.7, 70.0, 69.9, 68.7, 51.5 (–C*H*$_2$–N_3), 29.8, 29.5, 29.4, 29.3, 28.8, 26.7, 26.2.

4.1.6. 10-Aminodecyl *O*-(α-D-galactopyranosyl)-(1→4)-β-D-galactopyranoside 5. A solution of **4** (49 mg, 0.042 mmol) and a catalytic amount of $Pd(OH)_2$ in EtOAc–MeOH (2:5, 7 mL) was hydrogenated at room temperature under atmospheric pressure for 4 h. The reaction mixture was filtered through a Celite pad and concentrated to give fully deprotected **5** (18 mg, 85%). $[\alpha]_D$ +64.6° (*c* 0.5, CH_3OH); ^{1}H NMR (600 MHz, CD_3OD): δ 4.97 (d, H-1′, *J*=2.9 Hz), 4.29 (t, H-5′, *J*=6.2 Hz), 4.26 (d, H-1, *J*=7.3 Hz), 3.99 (d, H-4, *J*=2.9 Hz), 3.92 (br s, H-4′), 3.87 (dt, –C*H*$_2$O–, *J*=7.0, 9.5 Hz, 1H), 3.83 (dd, H-6a, *J*=7.7, 11.0 Hz), 3.79–3.75 (m, H-2′, H-3′), 3.75–3.70 (m, H-6b, H-6′a), 3.68 (dd, H-6′b, *J*=5.5, 11.0 Hz), 3.60 (t, H-5, *J*=8.4 Hz), 3.56 (dt, –C*H*$_2$O–, *J*=6.6, 9.5 Hz, 1H), 3.53 (dd, H-3, *J*=2.9, 9.9 Hz), 3.46 (dd, H-2, *J*=7.3, 9.9 Hz), 2.90 (t, –C*H*$_2$–NH_2, *J*=7.3 Hz), 1.65–1.28 (m, –(CH$_2$)$_8$–, 16H); ^{13}C NMR (100 MHz, CD_3OD): δ 106.0 (C-1), 103.3 (C-1′), 79.7, 76.9, 75.5, 73.7, 73.4, 72.2, 71.9, 71.6, 63.5, 61.7, 41.7 (–C*H*$_2$–NH_2), 31.7, 31.4, 31.3, 31.2, 31.0, 29.4, 28.3, 27.9; IR (liquid film): 3349, 2930, 2856, 1615, 1376, 1150, 1075, 807 cm^{-1}; HRMS calcd for $C_{22}H_{44}NO_{11}$ $[M+H]^+$ 498.2914, found 498.292.

4.1.7. Polyanionic glycopolymer 6. The reaction conditions are summarized in Tables 1 and 2. A typical procedure is as follows. Sugar monomer, **5** (10 mg, 0.02 mmol) and poly(ethylene-*alt*-maleic anhydride) (1.3 mg, 0.01 mmol as maleic anhydride unit) were dissolved in dry DMF (1 mL), and stirred at 70 °C for 32 h. The reaction mixture was concentrated, the precipitate was dissolved in water (20 mL), dialyzed for 3 days in water (M_w 2000 cut-off) and further purified with NAP™-10 Column (1.3×2.6 cm) to afford the polyanionic glycopolymer **6** (4.1 mg). The sugar content was determined by H_2SO_4-phenol method to be ca. 43%. $[\alpha]_D$ −74.0° (*c* 0.2, H_2O); ^{1}H NMR (400 MHz, D_2O): δ 5.02 (br, H-1′), 4.40 (br, H-1 and H-5′, 2H), 1.7–1.1 (br, –(CH$_2$)–); IR (KBr): 3400, 2930, 2857, 1713, 1643, 1566, 1465, 1406, 1226, 1151, 1075 cm^{-1}.

4.1.8. *O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl)-(1→4)-1,2,3,6-tetra-*O*-acetyl-α/β-D-galactopyranoside 8. *O*-(α-D-galactopyranosyl)-(1→4)-α/β-D-galactopyranoside **7** (50 mg, 0.15 mmol) was dissolved in dry pyridine (4 mL), and acetic anhydride (340 μL, 3.6 mmol) and *N*,*N*-dimethylaminopyridine (8.9 mg, 0.073 mmol) was added. The reaction mixture was stirred at 40 °C for 20 h. The mixture was then diluted with EtOAc, and washed with satd aq $NaHCO_3$, water, respectively. The organic layer was dried ($MgSO_4$), filtered and concentrated. Column chromatography of the product on silica gel with 6:4 EtOAc–hexane afforded peracetylated disaccharide **8** (97.3 mg, 98%) of α/β=ca. 3:2; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.38 (d, $H_α$-1, *J*=3.6 Hz), 5.71 (d, $H_β$-1, *J*=8.0 Hz), 5.59 (dd, $H_β$-4′, *J*=1.2, 3.2 Hz), 5.57 (dd, $H_α$-4′, *J*=1.6, 3.2 Hz), 5.32 (dd, $H_β$-2, *J*=8.0, 10.4 Hz), 5.01 (d, $H_α$-1′, *J*=3.6 Hz), 5.00 (d, $H_β$-1′, *J*=3.6 Hz), 4.87 (dd, $H_β$-3, *J*=2.8, 10.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 99.5, 99.1, 92.0, 89.8; IR (KBr): 2934, 1752, 1650, 1436, 1373, 1231, 1134, 1069, 1015, 938, 905 cm^{-1}. Anal. Calcd for $C_{28}H_{38}O_{19}$: C, 49.56; H, 5.64. Found C, 49.68; H, 5.52.

4.1.9. 10-Aminodecyl *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galactopyranoside 9.

4.1.9.1. Synthesis of *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-α/β-D-galactopyranose. The disaccharide **8** (103 mg, 0.15 mmol)

was treated with hydrazine acetate (27 mg, 0.30 mmol) in DMF (4 mL) at 0 °C for 4 h. The mixture was then diluted with $CHCl_3$, and washed with brine. The organic layer was dried ($MgSO_4$), filtered and concentrated. Column chromatography of the product on silica gel with 8:2 EtOAc–hexane gave *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-α/β-D-galactopyranoside (88.5 mg, 93%). Selective ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.51 (d, H_α−1, J=3.2 Hz), 5.12 (dd, H_β-2, J=8.0, 10.8 Hz), 5.03 (d, H_β-1′, J=3.6 Hz), 5.00 (d, H_α-1′, J=4.0 Hz), 4.91 (dd, H_β-3, J=2.4, 10.8 Hz), 4.70 (br d, H_β-1, J=7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): 98.9 (C-1′), 95.5 (C_β-1), 90.4 (C_α-1); IR (KBr): 3469, 2973, 1751, 1660, 1437, 1374, 1235, 1157, 1131, 1070, 979, 908, 758, 600 cm^{-1}; HRMS calcd for $C_{26}H_{36}O_{18}Na$ $[M+Na]^+$ 659.1800, found 659.1776.

4.1.9.2. Synthesis of *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate. To a mixture of the above selectively deacetylated compound (87 mg, 0.14 mmol) and trichloroacetonitrile (400 μL, 4.0 mmol), 1,8-diazabicyclo [5.4.0]undec-7-ene (30 μL, 0.20 mmol) was added at 0 °C and stirred for 2 h. The reaction mixture was then subjected to a silica gel column chromatography with 4:6 EtOAc–hexane to afford *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate (95 mg, 90%). $[\alpha]_D$ +148.6° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.68 (s, NH), 6.61 (d, H-1, 3.6 Hz), 5.57 (dd, H-4′, J=1.2, 3.2 Hz), 5.42 (dd, H-2, J=3.6, 11.2 Hz), 5.38 (dd, H-3′, J=3.2, 11.2 Hz), 5.31 (dd, H-3, J=2.8, 11.2 Hz), 5.24 (dd, H-2′, J=3.6, 11.2 Hz), 5.03 (d, H-1′, 3.2 Hz), 4.55–4.51 (m, H-5′), 4.37–4.31 (m, H-5 and H-6′), 4.28 (br d, H-4, J=2.4 Hz), 4.16–4.08 (m, H-6a, H-6b and H-6′), 2.15 (Ac), 2.12 (2×Ac), 2.04 (Ac), 2.03 (2×Ac), 1.99 (Ac); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.3, 170.1, 169.8, 169.7, 160.8 (C=NH), 109.7 (C-1), 98.9 (C-1′), 93.6 (CCl_3), 70.6, 69.4, 68.2, 67.7, 67.3, 67.1, 66.7, 61.8, 60.7, 20.9, 20.7, 20.6, 20.5, 20.4; IR (liquid film): 3320, 2977, 1748, 1677, 1434, 1372, 1225, 1134, 1068, 971, 904, 837, 797, 752, 644 cm^{-1}.

4.1.9.3. Synthesis of 10-bromodecyl *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galactopyranoside. A solution of the above trichloroacetimidate compound (61 mg, 0.077 mmol) and 10-bromo-1-decanol (182 mg, 0.76 mmol) in dry CH_2Cl_2 (2 mL) containing freshly activated 4 Å-MS (400 mg) was stirred for 1 h at 25 °C under N_2. The reaction mixture was then cooled to 0 °C and Me_3SiOTf (9 μL, 0.05 mmol) was added. The mixture was stirred at 0 °C for 2.5 h and then triethylamine was added. The mixture was filtered through a Celite pad, then diluted with $CHCl_3$ and washed with satd aq $NaHCO_3$, water, respectively. The organic layer was dried ($MgSO_4$), filtered and concentrated. Column chromatography of the product on silica gel with 4:6 EtOAc–hexane afforded 10-bromodecyl glycoside (59 mg, 90%); $[\alpha]_D$ +60.3° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.56 (dd, H-4′, J=1.2, 3.2 Hz), 5.39 (dd, H-3′, J=3.6, 11.2 Hz), 5.21–5.15 (m, H-2′ and H-2), 5.00 (d, H-1′, J=3.6 Hz), 4.81 (dd, H-3, J=2.8, 10.8 Hz), 4.55–4.52 (m, H-5′), 4.48–4.44 (m, H-6 and H-1), 4.20–4.07 (m, H-6′a, H-6′b and H-6), 4.05 (br d, H-4, J=2.4 Hz), 3.91–3.85 (m, –O–CH_2–(CH$_2$)–, 1H), 3.77 (br t, H-5, J=6.8 Hz), 3.49–3.43 (m, –O–CH_2–(CH$_2$)–, 1H), 3.41 (t, –CH_2–Br, J=6.8 Hz, 2H), 2.13 (Ac), 2.10 (Ac), 2.08 (Ac), 2.07 (Ac), 2.04 (2×Ac), 1.98 (Ac), 1.89–1.81 (m, –CH_2–CH$_2$Br, 2H), 1.61–1.54 (m, –OCH$_2$–CH_2–, 2H), 1.44–1.26 (m, –(CH$_2$)–, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.4, 170.3, 170.2, 169.9, 169.5, 168.9, 101.1 (C-1), 99.2 (C-1′), 72.6, 71.7, 69.9, 68.7, 68.4, 67.7, 67.2, 66.9, 61.8, 60.4, 33.9, 32.6, 29.3, 29.3, 29.2, 29.1, 28.6, 28.0, 25.7, 20.8, 20.6, 20.55, 20.53, 20.48; IR (liquid film): 2931, 2856, 1750, 1434, 1371, 1225, 1180, 1132, 1070, 903, 600 cm^{-1}. Anal. Calcd for $C_{36}H_{55}O_{18}Br$: C, 50.53; H, 6.48. Found C, 50.33; H, 6.35.

4.1.9.4. Synthesis of 10-azidodecyl *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galactopyranoside. A solution of the 10-bromodecyl glycoside (59 mg, 0.069 mmol) and NaN_3 (8 mg, 0.12 mmol) in dry DMF (4 mL) was stirred at 80 °C for 5.5 h. The reaction mixture was then processed in the same way described for the Section 4.1.4.1 to give 10-azidodecyl *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galactopyranoside (55 mg, 99%). $[\alpha]_D$ +70.4° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.56 (dd, H-4′, J=1.2, 3.2 Hz), 5.39 (dd, H-3′, J=3.2, 10.8 Hz), 5.21–5.15 (m, H-2′ and H-2), 5.00 (d, H-1′, J=3.6 Hz), 4.81 (dd, H-3, J=2.4, 11.2 Hz), 4.55–4.52 (m, H-5′), 4.48–4.44 (m, H-6 and H-1), 4.20–4.07 (m, H-6′a, H-6′b and H-6), 4.05 (br d, H-4, J=2.0 Hz), 3.91–3.85 (m, –O–CH_2–(CH$_2$)–, 1H), 3.77 (br t, H-5, J=6.8 Hz), 3.49–3.43 (m, –O–CH_2–(CH$_2$)–, 1H), 3.26 (t, –CH_2–N$_3$, J=6.8 Hz), 2.13 (Ac), 2.10 (Ac), 2.08 (Ac), 2.07 (Ac), 2.04 (2×Ac), 1.98 (Ac), 1.63–1.54 (m, –(CH$_2$)–, 4H), 1.38–1.28 (m, –(CH$_2$)–, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.4, 170.3, 170.2, 169.9, 169.5, 168.9, 101.0 (C-1), 99.2 (C-1′), 72.6, 71.7, 69.8, 68.6, 68.4, 67.7, 67.2, 66.9, 61.8, 60.3, 51.3 (–CH$_2$N$_3$), 29.3, 29.2, 29.1, 28.9, 28.6, 26.5, 25.6, 20.8, 20.6, 20.51, 20.48, 20.43; IR (liquid film): 2933, 2857, 2098, 1751, 1371, 1225, 1133, 1067, 903, 668 cm^{-1}. Anal. Calcd for $C_{36}H_{55}O_{18}N_3$: C, 52.87; H, 6.78; N, 5.14. Found: C, 52.59; H, 6.42; N, 4.77.

4.1.9.5. Synthesis of 9. A mixture of the above azide compound (55 mg, 0.068 mmol) and a catalytic amount of $Pd(OH)_2$ (ca. 5 mg) was stirred in MeOH (5 mL) at room temperature under hydrogen atmosphere for 3 h. The reaction mixture was then processed in the same way described for compound **5** to give **9** (49 mg, 91%). $[\alpha]_D$ +70.4° (*c* 1.0, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD): δ 5.54 (dd, H-4′, J=1.2, 3.2 Hz), 5.40 (dd, H-3′, J=3.2, 11.2 Hz), 5.22–5.14 (m, H-2′ and H-2), 5.06 (d, H-1′, 4.0 Hz), 5.01 (dd, H-3, J=2.4, 10.8 Hz), 4.63 (d, H-1, J=8.0 Hz), 4.58–4.54 (m, H-5′), 4.43 (dd, H-6, J=7.2, 11.2 Hz), 4.21–4.11 (m, H-4, H-6′a, H-6′b and H-6), 4.00–3.96 (m, H-5), 3.90–3.85 (m, –O–CH_2–(CH$_2$)–, 1H), 3.57–3.51 (m, –O–CH_2–(CH$_2$)–, 1H), 2.92 (t, –CH_2–N$_3$, J=7.6 Hz, 2H), 2.13 (Ac), 2.11 (Ac), 2.08 (Ac), 2.05 (Ac), 2.04 (Ac), 2.02 (Ac), 1.96 (Ac), 1.69–1.33 (m, –(CH$_2$)–, 16H); ^{13}C NMR (100 MHz, CD_3OD): δ 172.2, 172.0, 171.9, 171.6, 171.3, 102.3, 100.4, 78.5, 73.8, 73.5, 71.0, 70.6, 69.6, 69.5, 69.0, 68.4, 64.1, 62.0, 40.8, 30.6, 30.5, 30.4, 30.3, 30.2, 28.5, 27.4, 27.0, 21.0, 20.81, 20.76, 20.7, 20.6, 20.5; IR (KBr): 3458, 2935, 2859, 1753, 1631, 1435, 1373, 1230, 1134, 1072, 904, 601 cm^{-1};

HRMS calcd for $C_{36}H_{58}NO_{18}$ $[M+H]^+$ 792.3654, found 792.3629.

4.1.10. Synthesis of acetylated glycopolymer 10. A solution of **9** (33.1 mg, 0.043 mmol) and poly(ethylene-*alt*-maleic anhydride) (2.5 mg, 0.02 mmol as maleic anhydride unit) were dissolved in dry DMF (1 mL), and was stirred at room temperature for 45 h. A few drops of water were added, and the mixture was concentrated and then purified with Ultrafree-MC filter (DMF as an eluent) to afford the acetylated glycopolymer **10** (9.2 mg). $[\alpha]_D$ −38.9° (*c* 0.61, DMF); ^{1}H NMR (400 MHz, DMF-d_7): δ 7.4 (br, N*H*CO), 5.58 (br, H-4′), 5.35 (br, H-3′), 5.25 (br dd, J=3.4, 11.0 Hz, H-2′), 5.2–5.1 (br, H-2, H-1′, H-3), 4.77 (br d, J=6.8 Hz, H-1), 4.62 (br, H-5′), 3.85 (br, –OC*H*$_2$CH$_2$–), 2.17, 2.16, 2.12, 2.09, 2.07, 2.04, 1.99 (7×br s, Ac).

4.1.11. Conversion of acetylated glycopolymer 10 to fully deprotected 6. The above polymer **10** (3 mg) was deacetylated with 1 M NaOH aq. (3 mL) in DMF (1 mL) at room temperature for 3 h. The reaction mixture was then processed in the same way described for the Section 4.1.7 to afford polyanionic glycopolymer **6** (1.3 mg).

4.1.12. Synthesis of glycopolymer 13. A solution of commercially available *p*NP lactoside **11** (683.2 mg, 1.47 mmol) and a catalytic amount of $Pd(OH)_2$ (ca. 5 mg) in H_2O (200 mL) was hydrogenated at room temperature under atmospheric pressure for 15 h. The reaction mixture was filtered through a Celite pad and concentrated to give **12** (636.0 mg, 99%). Then, monomer **12** (86.7 mg, 0.2 mmol) and poly(ethylene-*alt*-maleic anhydride) (12.8 mg, 0.1 mmol as maleic anhydride unit) were dissolved in dry DMF (3 mL), and the mixture was stirred at room temperature for 50 h. The reaction mixture was then processed in the same way described for glycopolymer **6** to give **13** (87 mg). The sugar content was determined by the H_2SO_4-phenol method to be ca. 40%. $[\alpha]_D$ +87° (*c* 0.2, H_2O); ^{1}H NMR (400 MHz, D_2O): δ 7.2 (br, aromatics), 5.12 (br, H-1), 4.48 (br, H-1′), 3.9–3.5 (br, 12H), 2.7 (br, –C*H*(COO)–C*H*(CONH)–), 1.6 (br, –(CH$_2$)–); IR (KBr): 3339, 2903, 1703, 1657, 1549, 1510, 1429, 1373, 1318, 1235, 1163, 1061, 897 cm^{-1}.

4.1.13. Synthesis of glycopolymer 16. A solution of commercially available *p*NP α-D-mannopyranoside **14** (1 g, 3.3 mmol) and a catalytic amount of $Pd(OH)_2$ (ca. 5 mg) in H_2O (200 mL) was hydrogenated at room temperature under atmospheric pressure for 15 h. The reaction mixture was filtered through a Celite pad and concentrated to give **15** (890 mg, 99%). Then, monomer **15** (100 mg, 0.36 mmol) and poly(ethylene-*alt*-maleic anhydride) (12.8 mg, 0.1 mmol as maleic anhydride unit) were dissolved in dry DMF (3 mL), and the mixture was stirred at room temperature for 22 h. The reaction mixture was then processed in the same way described for glycopolymer **6** to give **16** (32 mg). The sugar content was determined by the H_2SO_4-phenol method to be ca. 37%. $[\alpha]_D$ +47° (*c* 0.2, H_2O); ^{1}H NMR (400 MHz, D_2O): δ 7.12 (br, aromatics), 5.52 (br, H-1), 4.0–3.5 (br, H-2, H-3, H-4, H-5, H-6 and H-6′), 2.6 (br, –C*H*(COO)–C*H*(CONH)–), 1.6 (br, –(CH$_2$)–); IR (KBr): 3439, 2932, 1707, 1648, 1548, 1510, 1454, 1410, 1228, 1123, 1065, 1010, 975, 831 cm^{-1}.

Acknowledgements

This work was partly supported by subsidies from the Ministry of Economy, Trade and Industry of Japan.

References and notes

1. (a) Esko, J. D. Microbial carbohydrate-binding proteins. In *Essentials of glycobiology*; Varki, A., Cummings, R., Esko, J., Freeze, H., Hart, G., Marth, J., Eds.; Cold Spring Harbor Laboratory: Spring Harbor, NY, 1999; pp 429–440. (b) Angata, T.; Varki, A. *Chem. Rev.* **2002**, *102*, 439–470. (c) Smith, A. E.; Helenius, A. *Science* **2004**, *304*, 237–242. (d) Karlsson, K. *Annu. Rev. Biochem* **1989**, *58*, 309–350. (e) Hakomori, S. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 225–232.
2. (a) Rojo, J.; Morales, J. C.; Penadés, S. *Top. Curr. Chem.* **2002**, *218*, 45–92. (b) Marx, K. *Biomacromolecules* **2003**, *4*, 1099–1120. (c) Matsuura, K.; Oda, R.; Kitakouji, H.; Kiso, M.; Kitajima, K.; Kobayashi, K. *Biomacromolecules* **2004**, *5*, 937–941. (d) Barrientos, Á.; Fuente, J. M.; Rojas, T. C.; Fernández, A.; Penadés, S. *Chem. Eur. J.* **2003**, *9*, 1909–1921. (e) Otsuka, H.; Akiyama, Y.; Nagasaki, Y.; Kataoka, K. *J. Am. Chem. Soc.* **2001**, *123*, 8226–8230. (f) Matsuura, K.; Kitakouji, H.; Sawada, N.; Ishida, H.; Kiso, M.; Kitajima, K.; Kobayashi, K. *J. Am. Chem. Soc.* **2000**, *122*, 7406–7407. (g) Lundquist, J. J.; Toone, E. J. *Chem. Rev.* **2002**, *102*, 555–578.
3. (a) Houseman, B. T.; Mrksich, M. *Chem. Biol.* **2002**, *9*, 443–454. (b) Fazio, F.; Bryan, M. C.; Blixt, O.; Paulson, J. C.; Wong, C.-H. *J. Am. Chem. Soc.* **2002**, *124*, 14397–14402. (c) Love, K. R.; Seeberger, P. H. *Angew. Chem., Int. Ed.* **2002**, *41*, 3583–3586. (d) Flitsch, S. L.; Ulijn, R. V. *Nature* **2003**, *421*, 219–220. (e) Fazio, F.; Bryan, M. C.; Lee, H.-K.; Chang, A.; Wong, C.-H. *Tetrahedron Lett.* **2004**, *45*, 2689–2692. (f) Park, S.; Shin, I. *Angew. Chem., Int. Ed.* **2002**, *41*, 3180–3182.
4. (a) Wilchek, M.; Bayer, E. A. *Anal. Biochem.* **1988**, *171*, 1–32. (b) Ulman, A. *Chem. Rev.* **1996**, *96*, 1533–1554.
5. Some chemical and hydrophobic adsorption techniques of artificial polymers onto plate surfaces were reported. See the following references; (a) Suzuki, N.; Quesenberry, M. S.; Wang, J. K.; Lee, R. T.; Kobayashi, K.; Lee, Y. C. *Anal. Biochem.* **1997**, *247*, 412–416. (b) Matsuura, K.; Tsuchida, A.; Okahata, Y.; Akaike, T.; Kobayashi, K. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 2973–2977.
6. Mammen, M.; Choi, S.-K.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2754–2794.
7. (a) Uzawa, H.; Nishida, Y.; Sasaki, K.; Minoura, N.; Kobayashi, K. *ChemBioChem.* **2003**, *4*, 640–647. (b) Uzawa, H.; Zeng, X.; Minoura, N. *Chem. Commun.* **2003**, 100–101. (c) Uzawa, H.; Toba, T.; Nishida, Y.; Kobayashi, K.; Minoura, N.; Hiratani, K. *Chem. Commun.* **1998**, 2311–2312. (d) Uzawa, H.; Nishida, Y.; Sasaki, K.; Nagatsuka, T.; Hiramatsu, H.; Kobayashi, K. *Carbohydr. Res.* **2004**, *339*, 1597–1602.
8. (a) Nishida, Y.; Dohi, H.; Uzawa, H.; Kobayashi, K. *Tetrahedron Lett.* **1998**, *39*, 8681–8684. (b) Dohi, H.; Nishida, Y.; Mizuno, M.; Shinkai, M.; Kobayashi, T.; Takeda, T.; Uzawa, H.; Kobayashi, K. *Bioorg. Med. Chem.* **1999**, *7*, 2053–2062. (c) Dohi, H.; Nishida, Y.; Furuta, Y.; Uzawa, H.; Yokoyama, S.-I.; Ito, S.; Mori, H.; Kobayashi, K. *Org. Lett.* **2002**, *4*, 355–357.

9. Uzawa, H.; Kamiya, S.; Minoura, N.; Dohi, H.; Nishida, Y.; Taguchi, K.; Yokoyama, S.; Mori, H.; Shimizu, T.; Kobayashi, K. *Biomacromolecules* **2002**, *3*, 411–414.
10. (a) Matsuoka, K.; Terabatake, M.; Esumi, Y.; Terunuma, D.; Kuzuhara, H. *Tetrahedron Lett.* **1999**, *40*, 7839–7842. (b) Kitov, P. I.; Sadowska, J. M.; Mulvey, G.; Armstrong, G. D.; Ling, H.; Pannu, N. S.; Read, R. J.; Bundle, D. R. *Nature* **2000**, *403*, 669–672. (c) Lundquist, J. J.; Debenham, S. D.; Toone, E. J. *J. Org. Chem.* **2000**, *65*, 8245–8250.
11. Bovin, N. V.; Gabius, H.-J. *Chem. Soc. Rev.* **1995**, *24*, 413–421.
12. Choudhury, I.; Minoura, N.; Uzawa, H. *Carbohydr. Res.* **2003**, *338*, 1265–1270.
13. Austin, P. W.; Hardy, F. E.; Buchanan, J. G.; Baddiley, J. *J. Chem. Soc.* **1965**, 1419–1424.
14. Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. *Anal. Chem.* **1956**, *28*, 350–356.
15. (a) Nishida, Y.; Uzawa, H.; Toba, T.; Sasaki, K.; Kondo, H.; Kobayashi, K. *Biomacromolecules* **2000**, *1*, 68–74. (b) Sasaki, K.; Nishida, Y.; Tsurumi, T.; Uzawa, H.; Kondo, H.; Kobayashi, K. *Angew. Chem., Int. Ed.* **2002**, *41*, 4463–4467.
16. (a) Decher, G. *Science* **1997**, *277*, 1232–1237. (b) Decher, G.; Lvov, Y.; Schmitt, J. *Thin Solid Films* **1994**, *244*, 772–777.
17. (a) Kanekiyo, Y.; Inoue, K.; Ono, Y.; Sano, M.; Shinkai, S.; Reinhoudt, D. N. *J. Chem. Soc., Perkin Trans. 2* **1999**, 2719–2722. (b) Onda, M.; Lvov, Y.; Ariga, K.; Kunitake, T. *Biotechnol. Bioeng.* **1996**, *51*, 163–167.
18. Nuzzo, R. G.; Allara, D. L. *J. Am. Chem. Soc.* **1983**, *105*, 4481–4483.