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Preparation and characterization of glycopolymers with biphenyl spacers via Suzuki coupling reaction

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1. Reagents

4-Vinylphenylboronic acid (VPBA, Tokyo Chemical Industry Co. Ltd.) was used for polymerization. Blocbuilder MA was provided by Arkema. For the synthesis of *p*-halophenyl glycosides, acetic anhydride, D-mannose, 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide, $NaHCO_3$, Na_2CO_3, $MgSO_4$, and Celite were purchased from FUJIFILM Wako Pure Chemical Corporation. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride, *p*-chlorophenol, *p*-bromophenol, *p*-iodophenol, boron trifluoride diethyl ether complex, tetrabutylammonium bromide, and sodium methoxide were purchased from Tokyo Chemical Industry Co. Ltd. $Pd(OAc)_2$ (FUJIFILM Wako Pure Chemical Corporation) and Pd/C type NX wetted with water (Pd content: 10%, FUJIFILM Wako Pure Chemical Corporation) were used as catalysts, and QuadraSil^{TM} MP (FUJIFILM Wako Pure Chemical Corporation) was used as a scavenger. Cystamine (FUJIFILM Wako Pure Chemical Corporation), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, Tokyo Chemical Industry Co. Ltd.), *N*-hydroxysuccinimide (NHS, Tokyo Chemical Industry Co. Ltd.), and glycolic acid (100 mmol/L, FUJIFILM Wako Pure Chemical Corporation) were used for the amine coupling reaction. Concanavalin A (Con A, J-Oil Mills Inc.), peanut agglutinin (PNA, FUJIFILM Wako Pure Chemical Corporation), and wheat germ agglutinin (WGA, FUJIFILM Wako Pure Chemical Corporation) were used as protein probes. A buffer solution of 4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid (HEPES, Nacalai Tesque, Inc.), containing 10 mmol/L HEPES, 137 mmol/L NaCl, 2.7 mmol/L KCl, 1.8 mmol/L $CaCl_2$, and 0.5 mmol/L $MgCl_2$ $6H_2O$, were prepared. Solvents, such as *N*,*N*-dimethylacetamide (DMAc), dimethylsulfoxide (DMSO), *N*,*N*-dimethylformamide (DMF), pyridine, toluene, chloroform, dichloromethane, ethyl acetate, hexane, super-dehydrated methanol, and 2-

propanol were used without purification. Deuterated solvents (DMSO-d_6 and D_2O, Cambridge Isotope Laboratories, Inc.) were used for nuclear magnetic resonance (NMR) measurements.

2. Synthesis of poly(vinylphenylboronic acid)

The polymer precursor for glycoside incorporation, poly(vinylphenylboronic acid) (poly(VPBA)), was synthesized via nitroxide-mediated polymerization (Fig. S1), as previously reported [1]. VPBA (20 mmol) and Blocbuilder MA (0.4 mmol) were used as the monomer and initiator, respectively, and dissolved in mixture of DMAc and water (95:5, 20 mL). The monomer concentration and feed ratio of the monomer:initiator were adjusted to 1 mol/L and 50:1, respectively. After the mixture was deaerated by three freeze-thaw cycles, polymerization was initiated by heating at 110 °C. After 20 h, air was fed into the mixture to stop the reaction. The products were purified by dialysis (molecular weight cut off: 3500 Da) against water. The solvent was evaporated and dried *in vacuo* to afford poly(VPBA) (yield: 91.9%). The product structure was characterized using NMR spectroscopy and gel permeation chromatography (GPC). NMR spectra were obtained using a spectrometer (JNM-ECZ400, JEOL Ltd.) in a mixture of DMSO-d_6 and D_2O (50:50). NMR spectra were analyzed using a software (Delta v5.3.1, JEOL Ltd.). Before the GPC measurement, phenylboronic acids in the polymer were esterified by the addition of pinacol to prevent interaction with the column matrix [2, 3]. GPC (HLC-8320GPC, Tosoh Corporation) was measured using TSKgel SuperAW guard column and TSKgel SuperAW 4000, 3000, and 2500 columns (Tosoh Corporation) in a mobile phase of DMF

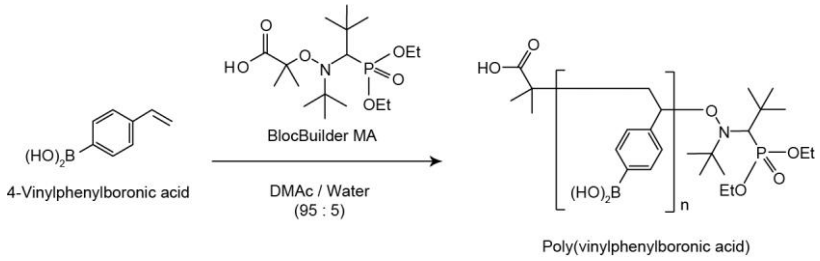


Fig. S1 Schematic illustration of nitroxide-mediated polymerization of 4-vinylphenylboronic acid.

containing 10 mM LiBr at a flow rate of 0.5 mL/min and a temperature of 40 ºC. The calibration curve for GPC was prepared using poly(methyl methacrylate) standards (Showa Denko K.K.).

^{1}H NMR (DMSO-d_6 and D_2O (50:50), 400 MHz): δ = 1.76 ppm (br, polymer main chain), δ = 6.60 and 7.58 ppm (br, aromatic H).

GPC (pinacolate-esterified polymer): M_w = 12400, M_n = 10200, polydispersity index = 1.21.

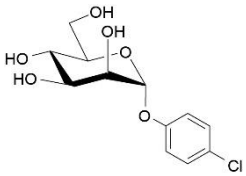
3. Synthesis of *p*-halophenyl glycosides

p-Chlorophenyl-α-D-mannopyranoside

Acetic anhydride (423 mmol) was added to a solution of D-mannose (28.2 mmol) in pyridine (40 mL), and the reaction mixture was stirred overnight. Pyridine was removed first by evaporation, followed by azeotropic distillation with toluene several times. The residue was dissolved in ethyl acetate and washed with 1 mol/L HCl, saturated $NaHCO_3$, and water. Then, $MgSO_4$ was added to the organic layer and filtered. The solvent was evaporated and dried *in vacuo* to afford penta-*O*-acetyl-D-mannopyranoside (yield: 92.4%).

Activated molecular sieves were added to penta-*O*-acetyl-D-mannopyranoside (7.7 mmol) in dried dichloromethane and *p*-chlorophenol (23.1 mmol). The solution was stirred under nitrogen atmosphere for 1 h. After the addition of boron trifluoride diethyl ether complex (42.4 mmol), the resulting solution was stirred overnight under nitrogen. The solution was filtered using Celite, and washed with 5 wt% Na_2CO_3 and water. $MgSO_4$ was then added to the organic layer and filtered. The solvent was evaporated, and the residue was recrystallized from ethyl acetate/hexane to afford *p*-chlorophenyl-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (yield: 51.9%).

Sodium methoxide (0.5 mmol) was added to a solution of *p*-chlorophenyl-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (2.2 mmol) in super-dehydrated methanol (100 mL). The reaction mixture was stirred at room temperature for 1 h. The solution was neutralized using a cationic exchange resin (Amberlyst 15DRY, Organo Co.), and filtered through a filter paper. The solvent was evaporated, and the residue was recrystallized with ethanol to afford *p*-chlorophenyl-α-D-mannopyranoside (pCP-Man).

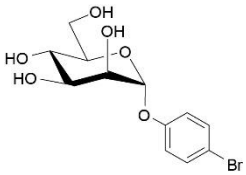


^{1}H NMR (DMSO-d_6 and D_2O (50:50), 400 MHz, TMS): δ = 7.37 ppm (d, 2H, aromatic H), δ = 7.14 ppm (d, 2H, aromatic H), δ = 5.48 ppm (s, 1H, anomeric H), δ = 4.01 ppm (dd, 1H, H-2), δ = 3.86 ppm (dd, 1H, H-3), δ = 3.7-3.5 ppm (m, 4H, H-4, H-5, and H-6) (overlapped with EtOH peak).

_p_-Bromophenyl-α-D-mannopyranoside

Penta-*O*-acetyl-D-mannopyranoside was synthesized using the above-mentioned method. Activated molecular sieves were added to penta-*O*-acetyl-D-mannopyranoside (26.3 mmol) in dried dichloromethane and *p*-bromophenol (78.9 mmol). The solution was stirred under nitrogen atmosphere for 1 h. After the addition of boron trifluoride diethyl ether complex (145 mmol), the resulting solution was stirred overnight under nitrogen. The solution was filtered using Celite, and washed with 5 wt% Na_2CO_3 and water. $MgSO_4$ was then added to the organic layer and filtered. The solvent was evaporated, and the residue was recrystallized from ethyl acetate/hexane to afford *p*-bromophenyl-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (yield: 57.9%).

Sodium methoxide (0.5 mmol) was added to a solution of *p*-bromophenyl-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (2.0 mmol) in super-dehydrated methanol (100 mL). The reaction mixture was stirred for 1 h. The solution was neutralized using Amberlyst 15DRY, filtered using a filter paper, and then concentrated *in vacuo* to afford *p*-bromophenyl-α-D-mannopyranoside (pBP-Man).

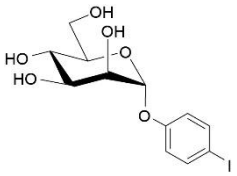


^{1}H NMR (DMSO-d_6 and D_2O (50:50), 400 MHz, TMS): δ = 7.50 ppm (d, 2H, aromatic H), δ = 7.09 ppm (d, 2H, aromatic H), δ = 5.49 ppm (s, 1H, anomeric H), δ = 4.01 ppm (dd, 1H, H-2), δ = 3.86 ppm (dd, 1H, H-3), δ = 3.7-3.6 ppm (m, 3H, H-4 and H-6), δ = 3.53 ppm (m, 1H, H-5).

***p*-Iodophenyl-α-D-mannopyranoside**

Penta-*O*-acetyl-D-mannopyranoside was synthesized using the above-mentioned method. Activated molecular sieves were added to penta-*O*-acetyl-D-mannopyranoside (25.7 mmol) in dried dichloromethane and *p*-iodophenol (51.4 mmol). The solution was stirred under nitrogen atmosphere for 1 h. After the addition of boron trifluoride diethyl ether complex (119 mmol), the resulting solution was stirred overnight under nitrogen. The solution was filtered using Celite, and washed with 5 wt% Na_2CO_3 and water. $MgSO_4$ was then added to the organic layer and filtered. The solvent was evaporated, and the residue was recrystallized from ethyl acetate/hexane to afford *p*-iodophenyl-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (yield: 71.3%).

Sodium methoxide (1.8 mmol) was added to a solution of *p*-iodophenyl-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (1.8 mmol) in super-dehydrated methanol (100 mL). The reaction mixture was stirred at room temperature for 1 h. The solution was neutralized using Amberlyst 15DRY, and filtered through a filter paper. The solvent was evaporated, and the residue was recrystallized with ethanol to afford *p*-iodophenyl-α-D-mannopyranoside (pIP-Man).



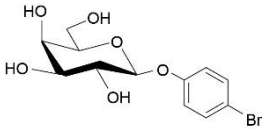
^{1}H NMR (DMSO-d_6 and D_2O (90:10), 400 MHz, TMS): δ = 7.61 ppm (d, 2H, aromatic H), δ = 6.93 ppm (d, 2H, aromatic H), δ = 5.36 ppm (s, 1H, anomeric H), δ = 3.81 ppm (dd, 1H, H-2), δ = 3.65 ppm (dd, 1H, H-3), δ = 3.6-3.4 ppm (m, 3H, H-4 and H-6) (overlapped with H_2O and EtOH peaks), δ = 3.35 ppm (m, 1H, H-5).

***p*-Bromophenyl-β-D-galactopyranoside**

A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (24.3 mmol) in dichloromethane (100 mL) was mixed with *p*-bromophenol (72.9 mmol) and

tetrabutylammonium bromide (26.7 mmol) in 1 mol/L aqueous NaOH (100 mL), and the solution was stirred for 2 h. Product was extracted twice with chloroform. The organic layer was washed with 5 wt% Na_2CO_3 and water. $MgSO_4$ was then added to the organic layer and filtered. The solvent was evaporated, and the residue was recrystallized with 2-propanol to afford *p*-bromophenyl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (yield: 50.0%).

Sodium methoxide (2.4 mmol) was added to a solution of *p*-bromophenyl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (9.4 mmol) in super-dehydrated methanol (80 mL). The reaction mixture was stirred for 1 h. The solution was neutralized using Amberlyst 15DRY, and filtered through a filter paper. The solvent was evaporated, and the residue was recrystallized with 2-propanol to afford *p*-bromophenyl-β-D-galactopyranoside (pBP-Gal).



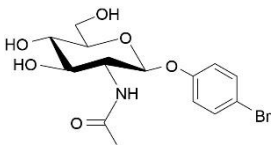
^{1}H NMR (DMSO-d_6 and D_2O (50:50), 400 MHz, TMS): δ = 7.47 ppm (d, 2H, aromatic H), δ = 7.07 ppm (d, 2H, aromatic H), δ = 5.39 ppm (s, 1H, anomeric H), δ = 3.86 ppm (dd, 1H, H-2), δ = 3.70 ppm (dd, 1H, H-3), δ = 3.6-3.5 ppm (m, 3H, H-4 and H-6) (overlapped with 2-propanol peak), δ = 3.38 ppm (m, 1H, H-5).

_p_-Bromophenyl-_N_-acetyl-β-D-glucosaminide

A solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (8.2 mmol) in dichloromethane (30 mL) was mixed with *p*-bromophenol (24.6 mmol) and tetrabutylammonium bromide (9.0 mmol) in 1 mol/L aqueous NaOH (30 mL), and the solution was stirred for 1 h. Product was extracted twice with chloroform. The organic layer was washed with 5 wt% Na_2CO_3 and water. $MgSO_4$ was then added to the organic layer and filtered. The solvent was evaporated, and the residue was recrystallized from

ethanol to afford *p*-bromophenyl-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (yield: 36.0%).

Sodium methoxide (1.5 mmol) was added to a solution of *p*-bromophenyl-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (6.1 mmol) in super-dehydrated methanol (50 mL). The reaction mixture was stirred for 1 h. The solution was neutralized using Amberlyst 15DRY, filtered through a filter paper, and then concentrated *in vacuo* to afford *p*-bromophenyl-*N*-acetyl-β-D-glucosaminide (pBP-GlcNAc).



^{1}H NMR (DMSO-d_6, 400 MHz, TMS): δ = 7.80 ppm (d, 1H, -NH-), δ = 7.46 ppm (d, 2H, aromatic H), δ = 6.94 ppm (d, 2H, aromatic H), δ = 5.11, 5.08, and 4.62 ppm (3H, hydroxyl H), δ = 4.95 ppm (d, 1H, anomeric H), δ = 3.7-3.6 ppm (m, 2H, H-2 and H-3), δ = 3.52-3.26 ppm (m, 3H, H-4 and H-6) (overlapped with H_2O peak), δ = 3.18 ppm (m, 1H, H-5), δ = 1.80 ppm (s, 3H, CH_3- of acetamido).

4. Suzuki coupling reaction between poly(VPBA) and *p*-halophenyl glycosides

Poly(VPBA) was dissolved in a mixture of DMF and water (50:50) to obtain a concentration of 2 g/L ([phenylborate unit] = 13.5 mmol/L). Na_2CO_3 (20 mmol/L) and pBP-Man (20 mmol/L) were added to the polymer solution, and the mixture was heated to 80 °C. The Suzuki coupling reaction was initiated by the addition of $Pd(OAc)_2$ in a small amount of acetone, and the mixture was stirred at 80 °C for 24 h. The concentration of Pd catalyst in the mixture was 0.1 mmol/L. After the mixture solution was cooled, QuadraSil™ MP was added, stirred, and filtered. The products were purified by dialysis (molecular weight cut off: 3500 Da) against DMSO and then water. The solvent was evaporated and dried *in vacuo*. The NMR spectra of the polymer were recorded (Fig. S2). In the spectrum, a remarkable peak corresponding to the anomeric proton of mannoside was observed at 5.38 ppm. The peaks for other glycoside protons were localized at 3.3-4.0 ppm. The incorporation percentage of the glycoside ligand was estimated from the

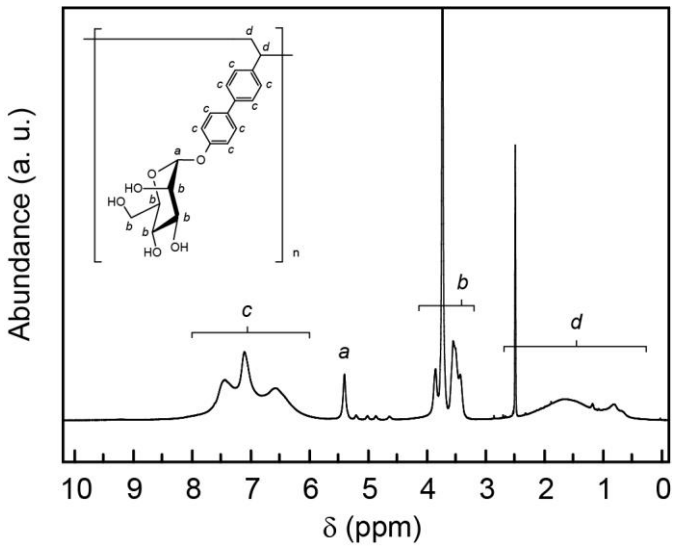


Fig. S2 ^{1}H NMR spectrum of poly(VBiph-Man), prepared using pBP-Man with 20 mmol/L, in the mixture of DMSO-d_6 and D_2O (90:10).

integral ratio of the peaks for the anomeric proton of glycoside and aromatic protons (6.0-8.0 ppm). The incorporation percentage of saccharide ligand in poly(VBiph-Man) prepared using pBP-Man at a concentration of 20 mmol/L was 64 ± 10%.

The presence of saccharides in poly(VBiph-Man) was confirmed by Fourier transform infrared (FT-IR) spectrometry (Alpha spectrometer, Bruker). For FT-IR, pellet samples with 50 mg of KBr and 1 mg of the polymer were prepared using a hydraulic pellet press (60 MPa, Pixie, Pike Technologies). Transmittance spectra were obtained at room temperature in air with a spectral resolution of 1 cm^{-1} and 128 scans. The FT-IR spectra of the polymer before and after the Suzuki coupling reaction are shown in Fig. S3. Peaks for O-H and C-H stretching vibrations were observed at 3400 and 2900 cm^{-1}, respectively. Aromatic C=C peaks were also observed at 1600-1500 cm^{-1}. In particular, the peaks at 1510 and 1495 cm^{-1} increased due to the formation of the biphenyl group. Alternatively, the broad peak for B-O at 1400-1300 cm^{-1} decreased after the Suzuki coupling reaction. There was a sharp peak at 1230 cm^{-1} and multiple peaks in 1110-980 cm^{-1}, which did not

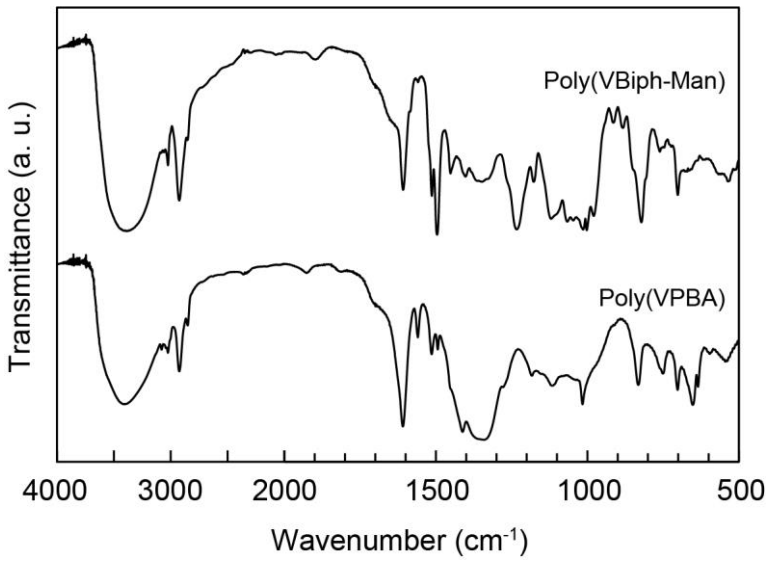


Fig. S3 FT-IR spectra of poly(VPBA) and poly(VBiph-Man) prepared by the Suzuki coupling reaction (incorporation percentage: 70%).

appear in the spectrum of poly(VPBA). These peaks corresponded to C-O-C and C-OH stretching vibrations, respectively.

The Suzuki coupling reaction between poly(VPBA) and pBP-Man was also performed using a heterogenous catalyst system. Man ligands were incorporated into poly(VPBA) under the above conditions, but Pd/C was used instead of $Pd(OAc)_2$. The incorporation percentage of Man using Pd/C was 16 ± 3%. This value was lower than that obtained using $Pd(OAc)_2$. Using the immobilized catalyst, the accessibility of reactants, such as pBP-Man and phenylboronic acid in the polymer, to Pd catalysts on the matrix was limited.

5. QCM measurements for binding of glycopolymers to proteins

To prepare the poly(VBiph-glycoside)s, where the glycoside ligand was incorporated into almost all units, pBP-Man and pBP-Gal were catalytically coupled at a high concentration (150 mmol/L). Poly(VBiph-GlcNAc) was also prepared at a concentration of 150 mmol/L. Other conditions and procedures were the same as those described above. After purification of the polymers using dialysis, the polymer concentration in the solution was adjusted to 100 mg/L. Furthermore, the dissolution of poly(VBiph-glycoside) in water was difficult once the polymer was dried. This low solubility could be attributed to the hydrophobicity of bulky functional group, that is, biphenyl group. The evaluation of the association between biphenyl spacers in the polymer is under investigation.

The binding abilities of the poly(VBiph-glycoside)s to proteins were evaluated using a quartz crystal microbalance (QCM, Affinix Q8, Initium Inc.) with a fundamental resonance frequency of 27 MHz. The gold electrode of the sensor chip (QCM01S-01, Initium Inc.) was cleaned with sodium dodecyl sulfate solution and then piranha solution (3:1 v/v mixture of sulfuric acid and hydrogen peroxide). The surface of the QCM cells was aminated using cystamine (10 mmol/L). EDC (100 mmol/L) and NHS (100 mmol/L) were dissolved in the polymer solution and then incubated for 0.5 h. The resulting mixture was loaded into the aminated QCM cells, and incubated for 2 h. The cells were washed with pure water. Residual amine groups on the surface were blocked by loading an aqueous solution of glycolic acid (100 mmol/L) activated by EDC and NHS.

The sensor cells were subjected to a 10 mmol/L HEPES buffer solution (pH: 7.2), and subsequently kept at 25 °C until the frequency reached a steady state. Protein solutions were injected into the cells, and the QCM frequency change ($-\Delta F$) at equilibrium was

monitored. Protein concentrations in the cells were sequentially enhanced by injection of protein solutions after adsorption equilibrium.

The binding ability of poly(VBiph-Man) to the protein is indicated in the main text. The binding abilities of poly(VBiph-Gal) and poly(VBiph-GlcNAc) to proteins are shown in Fig. S4. The -Δ*F* value of poly(VBiph-Gal) for PNA (galactoside-binding protein) was greater than that for WGA (*N*-acetyl-glucosaminide-binding protein). The adsorption behavior of poly(VBiph-GlcNAc) was reversed: the -Δ*F* value of WGA was greater than that of PNA. Using the Langmuir equation, the apparent dissociation constant (K_D) between the poly(VBiph-Gal) and PNA was 1.0×10^{-7} mol/L. Meanwhile, the K_D between the poly(VBiph-GlcNAc) and WGA was 3.7×10^{-7} mol/L.

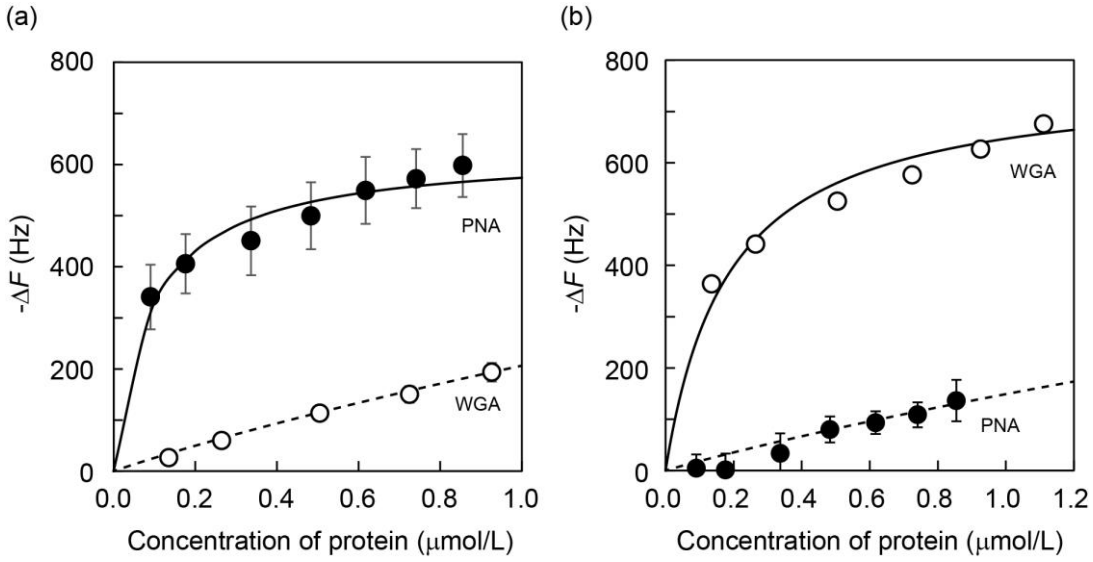


Fig. S4 Frequency changes in QCM for adsorption of proteins to (a) the poly(VBiph-Gal)-immobilized and (b) the poly(VBiph-GlcNAc)-immobilized surfaces at different protein concentrations. Solid line represents curve fitting based on the Langmuir equation (R^2 = 0.912 and 0.933 for poly(VBiph-Gal) and poly(VBiph-GlcNAc), respectively). The error bars were determined from two different measurements; however, the adsorption of WGA to the poly(VBiph-GlcNAc)-immobilized surface was only determined once.

6. Fluorescence measurements of glycopolymers

An aqueous solution of poly(VBiph-glycoside)s and poly(VPBA) with a concentration of 100 mg/L was prepared. The solutions (200 μL) were added to a 96-well black flat-bottom microplate (Corning Incorporated). The fluorescence spectra of the polymers were determined using a microplate reader (Infinite M200, Tecan Japan). The excitation wavelength was set to 270 nm, which is the maximum absorption wavelength of poly(VBiph-glycoside). A comparison of the fluorescence spectra of poly(VPBA) and poly(VBiph-Man) is presented in the main text. Fig. S5 demonstrates the fluorescence spectra of poly(VBiph-Gal) and poly(VBiph-GlcNAc) in water. A fluorescence peak was observed at 340-350 nm in the spectra of poly(VBiph-Gal) and poly(VBiph-GlcNAc).

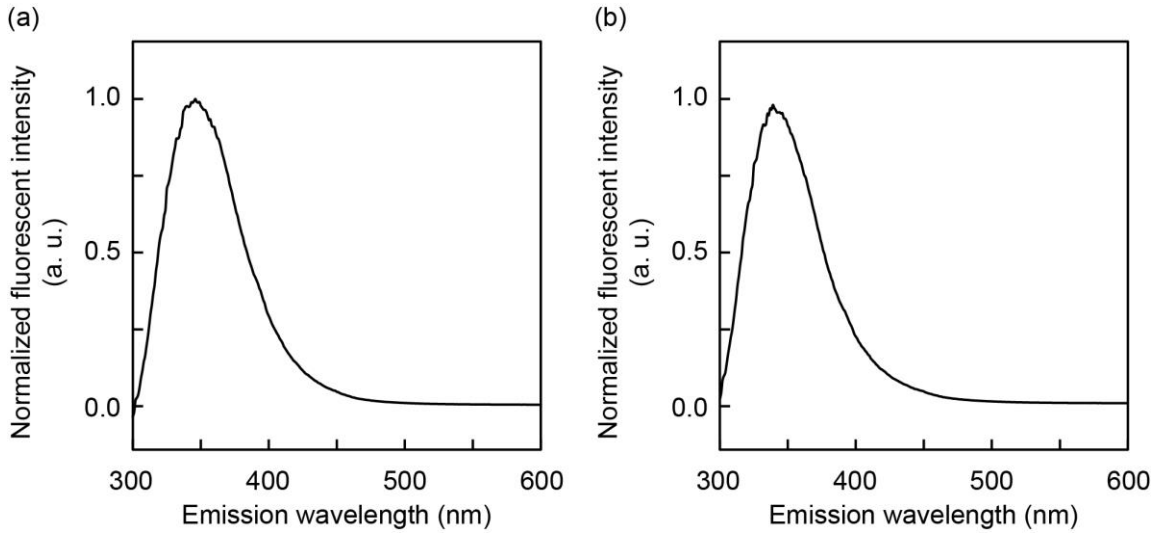


Fig. S5 Fluorescent spectra of (a) poly(VBiph-Gal) and (b) poly(VBiph-GlcNAc) in water.

References

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