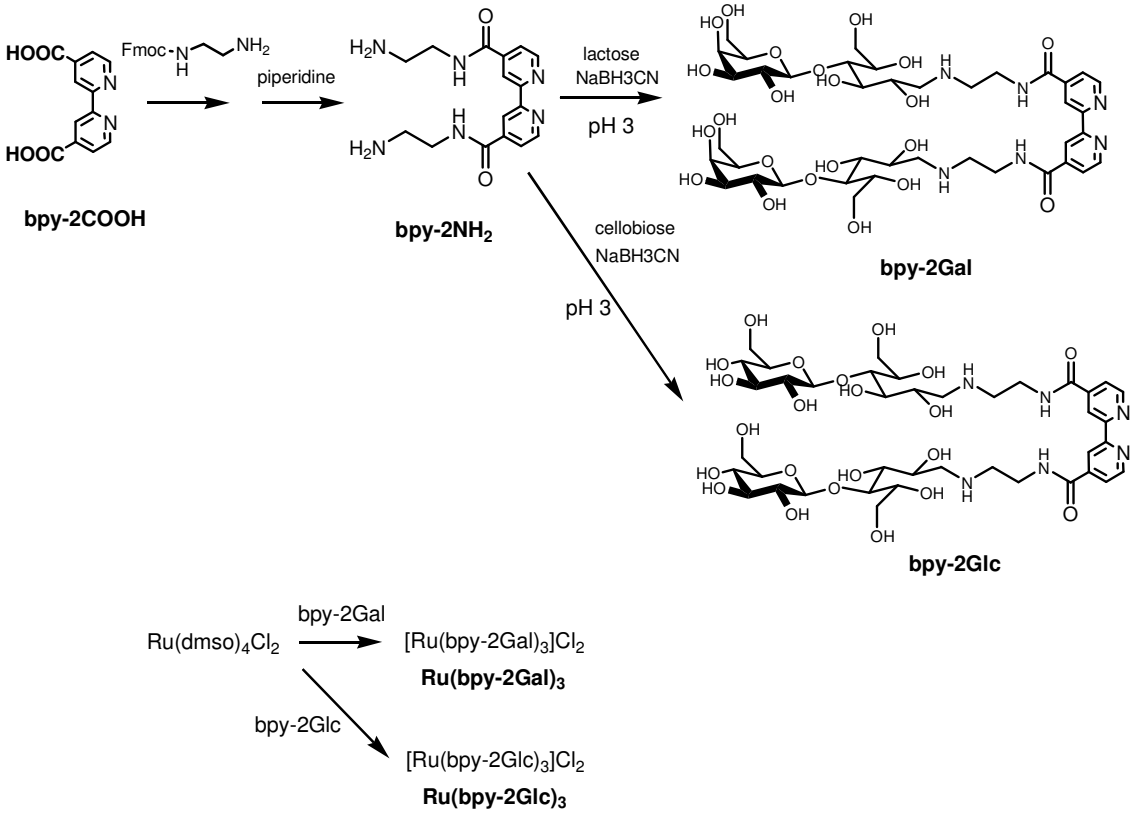
Supporting Information

Syntheses and identification of metalloglycoclusters.

Metalloglycoclusters, $Ru(bpy\text{-}2Gal)_3$ and $Ru(bpy\text{-}2Glc)_3$, were synthesized by following the scheme S1.



Scheme S1 Synthetic scheme of the metalloglycoclusters.

Materials. All chemical reagents were purchased from the chemical companies and used without further purification. Ruthenium(III) chloride, 2, 2'-bipyridine-4, 4'-dicarboxylic acid (bpy-2COOH), lactose and cellobiose were obtained from Sigma-Aldrich. Tetanus toxin c-fragment was purchased from Funakoshi and used without further purification. Reversed phase column chromatography (RP-HPLC) was performed on LC-8A and SPD-M10A (Shimadzu). The purified products were identified by electrospray ionization (ESI) time-of-flight (TOF) mass spectrometry performed on a JMS-T100LC (JEOL) and matrix-assisted laser desorption ionization (MALDI) TOF mass Ultraflex III (Bruker). ^{1}H and ^{13}C NMR spectra were recorded on a FT-NMR (Bruker).

bpy-2NH$_2$. Bpy-2COOH (100 mg, 4.1×10^{-4} mol) was added to 10 mL of thionyl chloride and the mixture was

refluxed for 6 h. After the mixture turned into yellow solution, the solution was evaporated. The yellow solid was dissolved to the mixture of THF (10 mL) and CH_2Cl_2 (10 mL). Fmoc-NH-$(CH_2)_2$-NH_2 (325 mg, 10.2×10^{-4} mol) dissolved in THF (5 mL), CH_2Cl_2 (5 mL), and DIEA (2 mL) was added in dropwise to the yellow solutioin and kept overnight. After quenching the reaction by adding methanol, piperidine (9 mL) was added to the crude product dissolved in methanol and stirred at rt for 12 h. The crude product was purified by a silica-gel column chromatography to give colorless solid (67% in yield). ^{1}H NMR (400 MHz, CD_3OD) δ 8.90 (d, *J* = 5.1 Hz, 2H; C*H(bpy)*), 8.69 (s, 2H; C*H(bpy)*), 7.93 (d, *J* = 5.0 Hz, 2H; C*H(bpy))* 3.70 (t, *J* = 6.1 Hz, 4H; CH_2 *(linker moiety)*), 3.14 (t, *J* = 6.8 Hz, 4H; CH_2); ^{13}C NMR (100 MHz, CD_3OD) δ 167.6, 156.5, 155.8 ,150.3, 149.6, 143.0, 122.1, 121.2, 119.5, 44.5, 39.7, 39.5; ESI-MS *m/z* (M = $C_{16}H_{20}N_6O_2$) 329.1 $[M + H]^+$ (calcd: 329.2), 351.1 $[M + Na]^+$ (calcd: 351.2).

bpy-2Gal. Bpy-2NH_2 (80 mg, 2.4×10^{-4} mol) was dissolved in H_2O (10 mL), then β-lactose (1.66 g, 4.9×10^{-5} mol) and DMAC (10 mL) were added to the solution. The solution was stirred in a water bath at 315 K and acetic acid (2 mL) was added to keep acidity of the solution. Sodium cyanoborohydrate (0.772 g, 0.123 mol) was added to the solution and the mixture was stirred until the MS signal for bpy-2NH_2 disappeared. The crude product was purified by a RP-HPLC with CAPCELL PAK C18 MGII column (4.6 mm × 250 mm) (Shiseido) (11% in yield). ^{1}H NMR (400 MHz, D_2O) δ 8.84 (br, 2H; *CH(bpy)*), 8.45 (br, 2H; *CH(bpy)*), 7.85 (br, 2H; *CH(bpy)*), 4.20 – 3.50 (m, 38H; *CH_2 and CH of carbohydrate and linker moieties*); ^{13}C NMR (100 MHz, D_2O) δ 169.1 169.0 158.6 150.4 142.7 122.1 119.9 102.9 102.8 79.0 78.8 78.3 75.4 75.3 72.4 71.0 70.9 70.8 70.6 70.1 68.7 68.6 67.7 66.4 66.3 66.1 61.9 61.8 61.3 61.2 61.1 49.8 47.3; ESI-MS *m/z* (M = $C_{40}H_{64}N_6O_{22}$) 981.3 $[M + H]^+$ (calcd: 981.4), 491.1 $[M + 2H]^{2+}$ (calcd: 491.2).

bpy-2Glc. Using bpy-NH_2 (41 mg, 1.3×10^{-4} mol), β-cellobiose (171 mg, 4.9×10^{-4} mol), and sodium cyanoborohydrate (316 mg, 5.0×10^{-3} mol), bpy-2Gal was synthesized by the same procedure with bpy-2Gal described above (13% in yield). ^{1}H NMR (400 MHz, CD_3OD) δ 8.76 (br, 1H; C*H(bpy)*), 8.38 (br, 1H; C*H(bpy)*), 7.78 (br, 1H; C*H(bpy))* 3.90 – 3.20 (m, 19H; *CH_2 and CH of carbohydrate and linker moieties*); ^{13}C NMR (100 MHz, CD_3OD) δ 168.9, 155.5, 150.3 ,142.6, 122.1, 119.9, 102.3, 78.7, 76.0, 75.4, 73.2, 70.9, 70.6, 69.5, 67.7, 61.9, 60.6, 49.9, 47.3, 36.7; ESI-MS *m/z* (M = $C_{40}H_{64}N_6O_{22}$) 981.3 $[M + H]^+$ (calcd: 981.4), 491.1 $[M + 2H]^{2+}$ (calcd: 491.2).

Ru(bpy-2Gal)$_3$. $[Ru(dmso)_4Cl_2]$ (0.8 mg, 1.7×10^{-6} mol) and 3 equiv. of bpy-2Gal (5.0 mg, 5.0×10^{-6} mol)

were dissolved in H_2O (0.4 mL), then the reaction mixture was refluxed for 13 hours to give a dark red solution. After removing the solvent, the crude product was purified by a RP-HPLC with CAPCELL PAK C18 MGII column (4.6 mm × 250 mm) (Shiseido) with acetonitrile/H_2O (0.1% HCl) to give the titled compound as dark orange solid (quantitative). The compound was used for the binding analysis without purification of diastereomers (Λ- and Δ- forms). ^{1}H NMR (400 MHz, D_2O) δ 8.97 (br, 6H; *CH(bpy)*), 7.92 (br, 6H; *CH(bpy)*), 7.74 (br, 6H; *CH(bpy)*), 4.00 – 3.16 (m, 114H; *CH_2 and CH of carbohydrate and linker moieties*). MALDI-MS *m/z* (M = $C_{120}H_{192}N_{18}O_{66}Ru$) 3042.1 $[M - H]^+$ (calcd: 3042.1).

Ru(bpy-2Glc)$_3$. Using $[Ru(dmso)_4Cl_2]$ (0.8 mg, 1.7×10^{-6} mol) and 3 equiv. of bpy-2Glc (5.0 mg, 5.0×10^{-6} mol), $Ru(bpy\text{-}2Glc)_3$ was synthesized by the same procedure with $Ru(bpy\text{-}2Gal)_3$ (80% in yield). The compound was used for the binding analysis without purification of diastereomers (Λ- and Δ- forms). ^{1}H NMR (400 MHz, D_2O) δ 9.02 (br, 6H; *CH(bpy)*), 7.93 (br, 6H; *CH(bpy)*), 7.73 (br, 6H; *CH(bpy)*), 4.10 – 3.10 (m, 114H; *CH_2 and CH of carbohydrate and linker moieties*). MALDI-MS *m/z* (M = $C_{120}H_{192}N_{18}O_{66}Ru$) 3042.5 $[M - H]^+$ (calcd: 3042.1).

Ru(bpy)$_2$(bpy-2Gal). Bipyridine (165 mg, 9.6×10^{-4} mol, 2 equiv) and $RuCl_3$ (100 mg, 4.8×10^{-4} mol) were mixed in 5 mL of 1M HCl. The mixture was kept for 1 week, then the precipitation was collected by a suction filtration. The collected dark green solid, $Ru(bpy)_2Cl_2$, (5 mg, 1.0×10^{-5} mol) was suspended in 5 mL of H_2O. After adding bpy-2Gal (10 mg, 1.0×10^{-5} mol) and LiCl (3 mg, 7.3×10^{-5} mol), the reaction mixture was refluxed for 20 h. Solution was removed by evaporation to give a crude product. The residue was chromatographed (RP-HPLC) through CAPCELL PAK C18 MGII column (4.6 mm × 250 mm) (Shiseido) with acetonitrile/H_2O (0.1% HCl) as eluent to obtain the titled compound. ^{1}H NMR (400 MHz, D_2O) δ 8.91 (s, 1H; *CH(bpy)*), 8.71 (d, 2H; *CH(bpy)*), 8.15 (m, 3H; *CH(bpy)*), 7.72 (m, 2H; *CH(bpy)*), 7.66 (br, 1H; *CH(bpy)*), 7.35 (t, 2H; *CH(bpy)*), 3.95 - 3.28 (m, 19H; *CH_2 and CH of carbohydrate and linker moieties*); ESI-MS *m/z* (M = $C_{60}H_{80}N_{10}O_{22}Ru$) 697.2 $[M]^{2+}$ (calcd: 697.2).

UV-vis and ^{1}H NMR titration of metal ions to confirm formation of ML_3 (M = Fe^{II}, L = bpy-2Gal or bpy-2Glc) complex.

(UV-vis titration) $FeCl_2$ was added to an aqueous solution of bpy-2Gal (1.0×10^{-3} mol/L). At every molar ratio (Fe:bpy-2Gal), UV-vis absorption spectrum was recorded using a quartz cell (path length: 10 mm). Fig. S1 summarizes the absorption spectra. and absorption coefficients following addition of Fe^{II} ions. Since the intensity

change plateued when the molar ratio of Fe:bpy-2Gal reached 1:3, the UV-vis titration experiment supports quantitative formation of ML_3 complex in the examined condition.

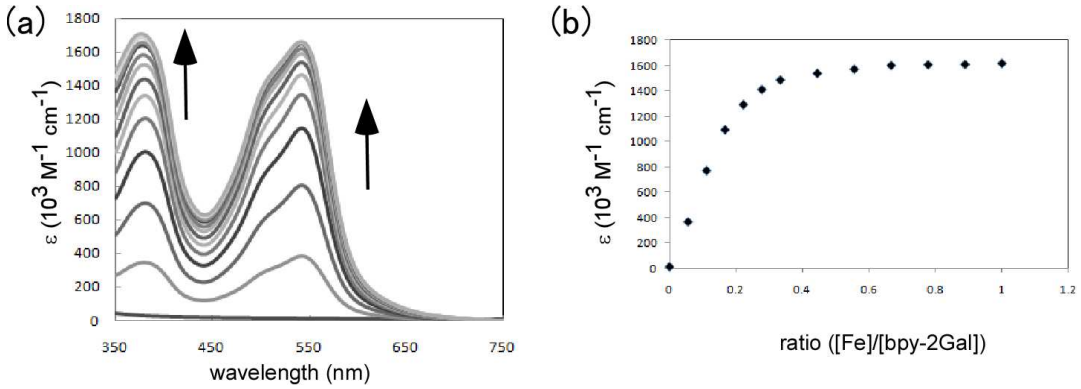


Fig. S1 (a) absorption spectra of bpy-2Gal with Fe^{II} (0 – 1.0×10^{-3} mol/L) and (b) absorption coefficient at 550 nm plotted against the molar ratio (Fe:bpy-2Gal).

(^{1}H NMR titratio) To a solution of bpy-2Gal (1.0×10^{-4} mol/L) in D_2O (0.4 mL), $FeCl_2$ in D_2O was added in stepwise. By addition of $FeCl_2$, a new set of the signals appeared and signals of free ligand decreased. When molar ratio (Fe:bpy-2Gal) reached 1:3, the signals of free ligand disappeared. This titration result supports that bpy-2Gal forms thermodynamically stable ML_3 complex in aqueous solution.

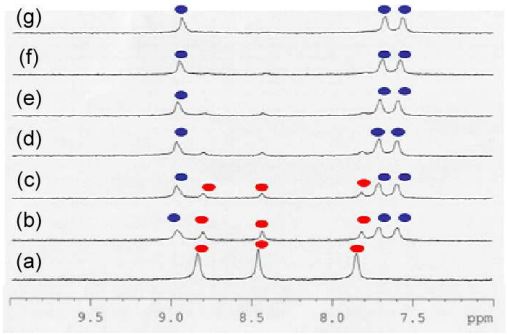


Fig. S2 ^{1}H NMR spectra of bpy-2Gal with Fe^{II} at molar ratio (Fe:bpy-2Gal) (a) 0:3, (b) 1/3:3, (c) 2/3:3, (d) 1:3, (e) 4/3:3, (f) 5/3:3, and (g) 2:3. Red and blue circles represent free bpy-2Gal and $[Fe(bpy\text{-}2Gal)_3]^{2+}$, respectively.

Fluorescence emission intensity and polarization measurements. Emission intensity and fluorescence polarization was recorded on a fluorescence spectrophotometer (HITACHI, F-7000) equipped with an auto-polarization-measurement-system. All spectra of the metalloglycoclusters were measured using 20 μM of the metalloglycocluster with or without 5 μM of lectin in PBS buffer at 298 K. For analyzing binding properties including a dissociation constant, the concentration of the metalloglycocluster was 20 μM and that of PNA or

ConA was varied from 0 – 20 μM. Excitation wavelength was at 468 nm for Ru(bpy-2Gal)$_3$ and Ru(bpy-2Glc)$_3$, and at 450 nm for Ru(bpy)$_3$.

Fluorescence polarization (P) was calculated by the equation, $P = (I_{\parallel} - I_{\perp} \times G)/ (I_{\parallel} + I_{\perp} \times G)$, where I is the fluorescence intensity using the polarizer at 0° for excitation ($I_{\parallel}$ and $I_{\perp}$ were observed using the emission polarization filter at 0° and 90°, respectively). The correction factor G was determined by the following equation, $G = i_{\parallel} / i_{\perp}$, where i is the fluorescence intensity using at 90° for excitation ($i_{\parallel}$ and $i_{\perp}$ were observed using the emission polarization filter at 0° and 90°, respectively). For each sample, G factors were defined by measuring $i_{\parallel}$ and $i_{\perp}$ at individual wavelength.

Emission spectral change caused by addition of ethanol.

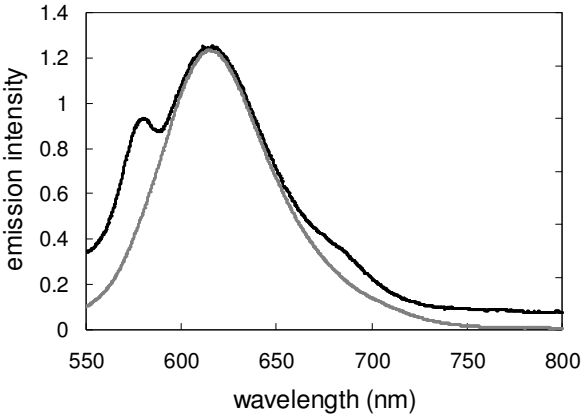


Fig. S3 Emission spectra of 20 μM Ru(bpy-2Gal)$_3$ in PBS (gray) and in PBS with 50% ethanol (black) (λ_{ex}: 468 nm, T: 298 K).

Calculation of the dissociation constant

Dissociation constants (K_d) between PNA and Ru(bpy-2Gal)$_3$, or ConA and Ru(bpy-2Glc)$_3$ were determined with non-linear least squares fitting. Using the fluorescence polarization value, the data was fitted to the following equation, $P/P_{max} = [(K_d + [probe]_{toatal} + [lectin]_{total}) - \{(K_d + [probe]_{total} + [lectin]_{total})^2 - 4[probe]_{total}[lectin]_{total}\}] / 2[probe]_{total}$, where P is the polarization value, P_{max} is the maximum polarization value, $[probe]_{total}$ is the total concentration of Ru(bpy-2Gal)$_3$ or Ru(bpy-2Glc)$_3$, and $[lectin]_{total}$ is the total concentration of PNA or ConA. The simulation results were summarized in Table S1.

Table S1 Results of Non-Linear Squares Fitting using Polarization Value

	P_{max}	K_d (M)	Correlation
Ru(bpy-2Gal)$_3$	0.52	6.1×10^{-6}	0.9996
Ru(bpy-2Glc)$_3$	0.46	1.8×10^{-5}	0.9824

Affinity Evaluation of Tetanus Toxin c-Fragment.

Fluorescence polarization values were recorded on a fluorescence spectrophotometer (HITACHI, F-7000) equipped with an auto-polarization-measurement-system. The metalloglycoclusters, $Ru(bpy\text{-}2Gal)_3$ and $Ru(bpy\text{-}2Glc)_3$, were measured using 2 μM of the PBS solution. Polarization values of the metalloglycocluster with or without 0.05 μM tetanus toxin c-fragment were collected in PBS buffer at 298 K. Excitation wavelength was at 468 nm for $Ru(bpy\text{-}2Gal)_3$ and $Ru(bpy\text{-}2Glc)_3$.

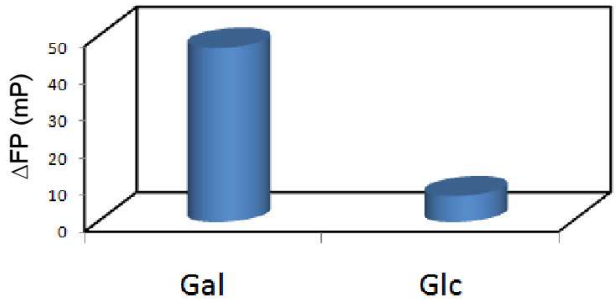


Fig. S4 Changes in fluorescence polarization values of 2 μM $Ru(bpy\text{-}2Gal)_3$ or $Ru(bpy\text{-}2Glc)_3$ in PBS (λ_{ex}: 468 nm, T: 298 K) when 0.05 μM tetanus toxin c-fragment was added.