# Fluorescent Conjugated Polyfluorene with Pendant Lactopyranosyl Ligands for Studies of $Ca^{2+}$-Mediated Carbohydrate−Carbohydrate Interaction

Qi Chen,[†] Yi Cui,[†,‡] Tian-Long Zhang,[†] Jie Cao,[*,‡] and Bao-Hang Han[*,†]

*National Center for Nanoscience and Technology, Beijing 100190, China, and Graduate University of Chinese Academy of Sciences, Beijing 100049, China*

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A well-defined fluorescent conjugated polyfluorene with pendant lactopyranosyl ligands was easily prepared through Cu(I)-catalyzed azide/alkyne "click" ligation and Suzuki coupling polymerization. As a fluorescent multivalent model system of glycoconjugates, the polymer was first used for studies of metal ion-mediated carbohydrate−carbohydrate interaction based on fluorescence spectroscopy. A significant fluorescence quenching of the lactosyl-bearing polyfluorene was observed upon addition of calcium ion, which is attributed to the polymer aggregation derived from $Ca^{2+}$-mediated complex formation. Dynamic light scattering can also prove $Ca^{2+}$-induced aggregation of the polymer based on determination of the corresponding hydrodynamic diameters. The calcium-mediated lactose−lactose interaction was reversible when treated with EDTA. In control studies, $Ca^{2+}$-induced fluorescence quenching can not be observed for cellobiosyl- or galactosyl-functionalized polymer analogues, which show that specific sugar structures are critical for carbohydrate−metal complex formation.

## Introduction

Carbohydrate−carbohydrate interactions between cell surface glycans play crucial roles in many physiological processes such as cell adhesion and cellar recognition.[1] Systematic investigations have demonstrated that most of carbohydrate−carbohydrate interactions, for example, the $Le^X$−$Le^X$ interaction in compaction in embryogenesis,[2] the sulfated disaccharide Glc*p*NAc3S($\beta$1−3)Fuc*p* self-interaction in the cell adhesion of marine sponges,[3] and LacCer−GM3 interaction in the adhesion of mouse B16 melanoma cells to the endothelial cells,[4] are mediated by calcium ion based on multivalent effect. Various multivalent model systems such as glyconanoparticles,[5] glycopolymers,[6] glycodendrimers,[7] and liposomes[8] have been used in studies of $Ca^{2+}$-mediated carbohydrate−carbohydrate interactions. However, the underlying mechanism in these carbohydrate−carbohydrate interactions has not yet been sufficiently clarified.

Synthetic glycopolymers, as multivalent model systems and artificial glycoconjugates, have been demonstrated to be important well-defined tools for investigating carbohydrate-based biological interactions.[9] Moreover, fluorescent conjugated glycopolymers with fluorescent backbones and reporting carbohydrate moieties are attractively employed in carbohydrate−protein interaction studies and biosensor applications because of their intrinsic optical properties, high sensitivities to minor stimuli, and good biocompatibilities.[10] Recently, facile insights into carbohydrate−lectin and carbohydrate−bacteria bindings using fluorescent conjugated glycopolymers have been reported by Bunz's group[11] and Liu's group,[12] respectively.

Metal ion-mediated carbohydrate−carbohydrate interactions have been clearly proven by SPR,[13] TEM,[5] UV−vis spectrum,[5d] NMR,[14] and $\pi$-*A* isotherms.[6b] However, as far as we know, using fluorescent conjugated glycopolymers to study carbohydrate−carbohydrate interactions based on fluorescence spectroscopy has not been reported. Our group have explored the facile prepolymerization and postpolymerization functionalization approaches to prepare well-defined fluorescent conjugated glycopolymers through Cu(I)-catalyzed azide/alkyne "click" ligation.[15] When those methods were used, conjugated glycopolymers containing triphenylamine or fluorene backbones were prepared for applications in detecting biomacromolecules.[16] In this article, D-lactosyl bearing polyfluorene has been synthesized (Scheme 1) and used for investigation of lactose−lactose recognition based on lactose−metal complexes formation. In the presence of specific metal ions, this well-defined fluorescent glycopolymer exhibited exceptional fluorescence quenching or energy transfer efficiency, resulting in amplification of optical signals for transduction of the carbohydrate−carbohydrate recognizing event. We believe it will provide some clues for elucidation of the mechanism underlying in carbohydrate−carbohydrate interactions.

## Experimental Section

**Materials and Measurements.** All chemical reagents were commercially available and used as received unless otherwise stated. Deionized water was purified with a Millipore purification system (Milli-Q water). 9,9-Bis(4-hydroxyphenyl)-2,7-dibromofluorene (**1**)[17] and azido-functionalized sugar derivatives (**3**−**5**)[18] were prepared according to reported methods.

The $^1$H and $^{13}$C NMR spectra were recorded on a Bruker DMX300 NMR spectrometer. The optical rotations were measured with a JASCO DIP-1000 digital polarimeter. Mass spectra were recorded with a VG PLATFORM mass spectrometer using the ESI(+) technique. The molecular weights of the polymers were determined by an Agilent 1100 GPC system in THF. The number-average and weight-average molecular weights ($M_n$ and $M_w$) were estimated by using a calibration curve of polystyrene standard. IR spectra were recorded using a Perkin−Elmer Paragon 1000 FTIR spectrometer. Ultraviolet−visible (UV−vis) spectra were measured using a Perkin−Elmer Lamda 900 UV−vis−NIR

* To whom correspondence should be addressed. Fax: +86-10-82545576. E-mail: hanbh@nanoctr.cn.
[†] National Center for Nanoscience and Technology.
[‡] Graduate University of Chinese Academy of Sciences.

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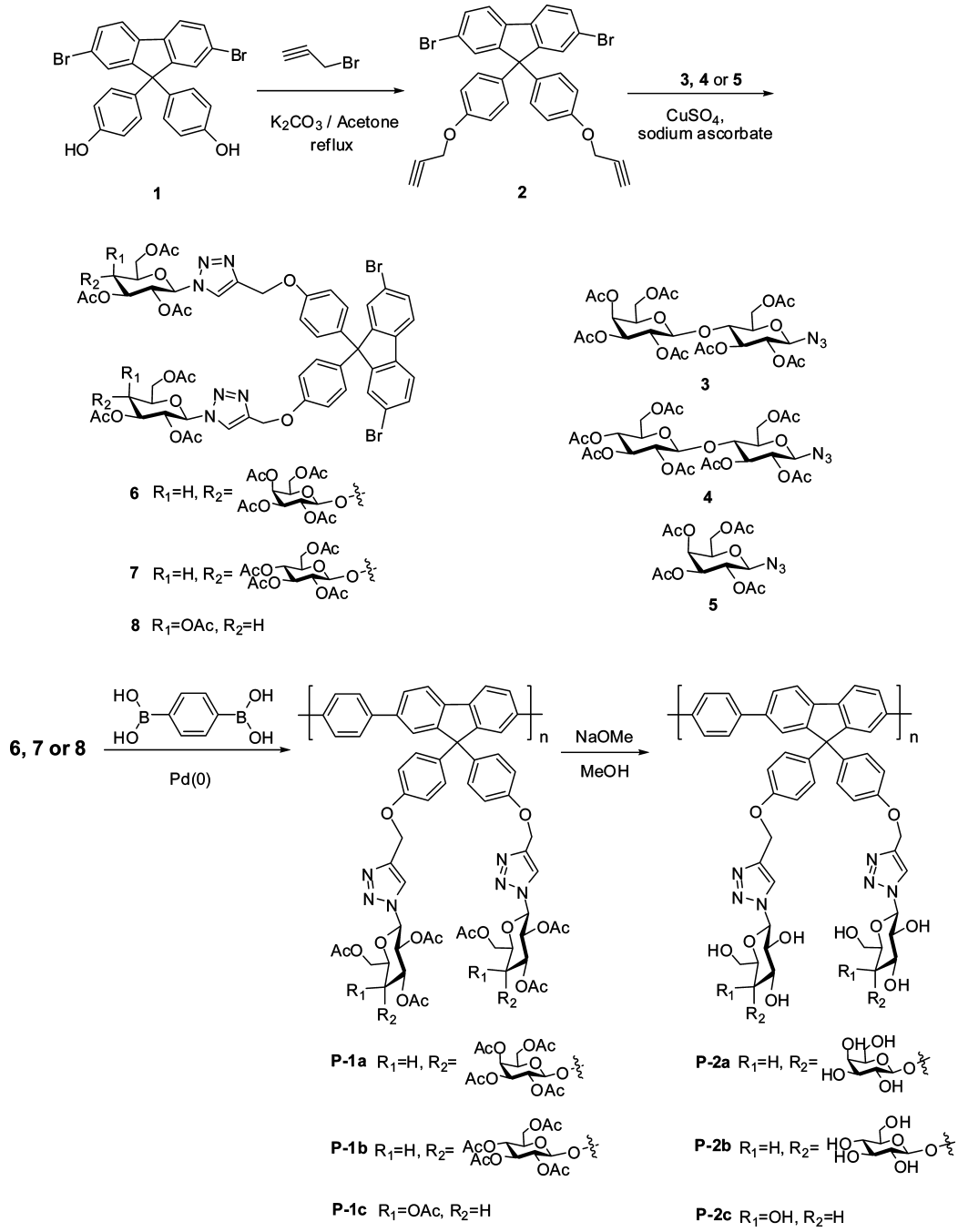
**Scheme 1.** Synthetic Routes to Monomers and Polymers




spectrophotometer and quartz cells with 1 cm path length. The fluorescence spectra were measured in a conventional cell with 1 cm path length using a Perkin−Elmer LS55 luminescence spectrometer. Dynamic light scattering (DLS) measurements were performed on a ZETASIZER Nano-Series instrument.

**Synthesis of Monomers and Polymers.** *9,9-Bis(4-propargyloxyphenyl)-2,7-dibromofluorene* **2**.[16b] To a solution of compound **1** (584 mg, 1.15 mmol) and propargyl bromide (330 mg, 2.76 mmol) in acetone (10 mL) were added anhydrous potassium carbonate (380 mg, 2.76 mmol) and $Bu_4NBr$ (37 mg, 0.115 mmol), and the mixture was refluxed overnight. After removal of acetone, water (15 mL) was added and the product was extracted with ethyl acetate (3 × 20 mL). The combined organic layer was dried over anhydrous $Na_2SO_4$ and concentrated in vacuo. The residue was purified with silica gel column chromatography (ethyl acetate−petroleum ether, 1:6) to give the desired product **2** (544 mg, 81%) as a solid. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.57 (d, $J$ = 8.1 Hz, 2H), 7.53 (s, 2H), 7.48 (d, $J$ = 8.1 Hz, 2H), 7.13 (d, $J$ = 8.6 Hz, 4H), 6.88 (d, $J$ = 8.6 Hz, 4H), 4.64 (s, 4H), 2.54 (s, 2H). ESI(+)-MS calcd for $C_{31}H_{20}Br_2O_2$: 584.3 [M]. Found: 607.5 [M + Na]$^+$.

*Monomer* **6.** To a mixture of **2** (584 mg, 1.0 mmol) and **3** (1.66 g, 2.5 mmol) in $H_2O$−THF (1:1, 15 mL) were added freshly prepared aqueous 1.0 M sodium ascorbate (150 $\mu$L, 0.15 mmol) and $CuSO_4$ (12 mg, 0.075 mmol). The heterogeneous mixture was stirred vigorously in dark room at 50−60 °C until complete consumption of the reactants based on TLC monitoring. After removal of THF under a reduced pressure, water (20 mL) was added and the product was extracted with ethyl acetate (3 × 25 mL). The combined organic layer was dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. The crude product was subjected to column chromatography (ethyl acetate−petroleum ether, 2:1) to give **6** as a foamy solid (1.86 g, 98%). $[\alpha]_D^{25}$ −50° ($c$ 1, $CHCl_3$). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.75 (s, 2H), 7.54 (d, $J$ = 8.4 Hz, 2H), 7.45−7.42 (m, 4H), 7.02 (d, $J$ = 8.7 Hz, 4H), 6.82 (d, $J$ = 8.6 Hz,

4H), 5.82 (d, $J$ = 9.3 Hz, 2H), 5.39−5.34 (m, 6H), 5.12 (s, 4H), 5.08 (d, $J$ = 8.1 Hz, 2H), 4.95 (dd, $J$ = 3.3, 10.5 Hz, 2H), 4.52 (d, $J$ = 7.8 Hz, 2H), 4.45 (d, $J$ = 11.4 Hz, 2H), 4.15−4.05 (m, 6H), 3.94−3.87 (m, 6H), 2.13 (s, 6H), 2.06 (s, 6H), 2.04 (s, 6H), 2.02 (s, 6H), 2.01 (s, 6H), 1.94 (s, 6H), 1.76 (s, 6H). $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 169.3, 169.2, 169.1, 169.0, 168.5, 168.1, 156.3, 152.4, 143.8, 136.8, 136.1, 129.9, 128.2, 128.1, 120.8, 120.6, 120.2, 113.7, 100.1, 84.5, 74.9, 74.6, 71.6, 69.9, 69.8, 69.5, 68.0, 65.6, 63.3, 60.9, 60.8, 59.8, 59.4, 20.0, 19.8, 19.7, 19.6, 19.5, 19.3, 19.1. ESI(+)-MS calcd for $C_{83}H_{90}Br_2N_6O_{36}$: 1907.4 [M]. Found: 1930.3 $[M + Na]^+$.

*Monomer* **7.** Monomer **7** was obtained from compounds **2** and **4** using the same procedure for preparation of monomer **6** as a foamy solid (1.80 g, 96%). $[\alpha]_D^{25}$ −38° ($c$ 1, $CHCl_3$). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.70 (s, 2H), 7.49 (d, $J$ = 8.5 Hz, 2H), 7.38−7.36 (m, 4H), 6.96 (d, $J$ = 8.7 Hz, 4H), 6.75 (d, $J$ = 8.5 Hz, 4H), 5.80 (d, $J$ = 9.1 Hz, 2H), 5.34−5.32 (m, 4H), 5.10 (t, $J$ = 10.5 Hz, 2H), 5.12 (s, 4H), 5.01 (t, $J$ = 10.5 Hz, 2H), 4.87 (t, $J$ = 10.2 Hz, 2H), 4.53 (d, $J$ = 8.2 Hz, 2H), 4.44 (d, $J$ = 11.5 Hz, 2H), 4.30 (dd, $J$ = 3.3, 10.5 Hz, 2H), 4.07 (d, $J$ = 10.5 Hz, 2H), 3.98 (d, $J$ = 11.4 Hz, 2H), 3.92−3.89 (m, 4H), 3.68−3.64 (m, 2H), 2.01 (s, 6H), 2.00 (s, 6H), 1.96 (s, 6H), 1.95 (s, 6H), 1.92 (s, 6H), 1.90 (s, 6H), 1.70 (s, 6H). $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 169.4, 169.1 (2C), 168.5, 168.3, 168.0, 167.9, 156.3, 152.5, 143.7, 136.9, 136.1, 129.9, 128.2, 128.1, 120.8, 120.7, 120.3, 113.8, 99.8, 84.5, 74.9, 74.8, 71.9, 71.3, 71.1, 70.6, 69.5, 66.9, 63.4, 60.9, 60.7, 60.6, 59.3, 20.0, 19.7, 19.6, 19.5 (2C), 19.4, 19.1. ESI(+)-MS calcd for $C_{83}H_{90}Br_2N_6O_{36}$: 1907.4 [M]. Found: 1930.5 $[M + Na]^+$.

*Monomer* **8.** Monomer **8** was obtained from compounds **2** and **5** using the same procedure for preparation of monomer **6** as a foamy solid (1.30 g, 98%). $[\alpha]_D^{25}$ −30° ($c$ 1, $CHCl_3$). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.89 (s, 2H), 7.55 (d, $J$ = 8.5 Hz, 2H), 7.46−7.44 (m, 4H), 7.06 (d, $J$ = 8.7 Hz, 4H), 6.86 (d, $J$ = 8.6 Hz, 4H), 5.85 (d, $J$ = 9.3 Hz, 2H), 5.58−5.51 (m, 4H), 5.24 (dd, $J$ = 3.3, 10.2 Hz, 2H), 5.15 (s, 4H), 4.25−4.21 (m, 2H), 4.17−4.12 (m, 4H), 2.20 (s, 6H), 2.02 (s, 6H), 1.99 (s, 6H), 1.79 (s, 6H). $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 170.2, 169.9, 169.7, 168.9, 157.3, 153.4, 144.7, 137.8, 137.0, 130.8, 129.2, 129.0, 121.7, 121.5, 121.2, 114.7, 86.2, 74.0, 70.7, 67.7, 66.8, 61.8, 61.1, 60.3, 20.5, 20.4, 20.1. ESI(+)-MS calcd for $C_{59}H_{58}Br_2N_6O_{20}$: 1330.9 [M]. Found: 1354.1 $[M + Na]^+$.

*Polymer* **P-1a.** Under a nitrogen atmosphere, sugar-carrying monomer **6** (280 mg, 0.15 mmol), 1,4-phenyldiboronic acid (30 mg, 0.18 mmol), $Pd(PPh_3)_4$ (10 mg), and potassium carbonate (250 mg, 1.8 mmol) were placed in a 50 mL round-bottomed flask, and then THF (15 mL) was added. The mixture was stirred at 70 °C for 36 h under a nitrogen atmosphere. The resulting polymer was purified by precipitation in methanol and washed with methanol−acetone in a Soxhlet apparatus for 48 h. **P-1a** was obtained as a gray powder (214 mg, 78%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.76 (bs, 2H), 7.62−7.55 (m, 6H), 7.52−7.44 (m, 4H), 7.24−7.04 (m, 4H), 6.86−6.84 (m, 4H), 5.82 (br, 2H), 5.40−5.33 (m, 6H), 5.15−5.03 (m, 6H), 4.99−4.95 (m, 2H), 4.53−4.45 (m, 4H), 4.11 (br, 6H), 3.92 (br, 6H), 2.15 (bs, 6H), 2.05 (bs, 24H), 1.96 (bs, 6H), 1.76 (bs, 6H). $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 169.3, 169.2, 169.1, 169.0, 168.5, 168.4, 168.0, 156.3, 152.4, 143.9, 137.6, 136.9, 130.9, 129.9, 128.3, 128.1, 126.4, 126.1, 120.8, 120.2, 113.7, 113.4, 100.1, 84.5, 76.2, 74.9, 74.6, 71.6, 69.9, 69.8, 69.4, 68.0, 65.6, 60.9, 60.7, 59.8, 19.8, 19.6, 19.5, 19.1. GPC (THF, polystyrene standard): $M_n$ = 28300 g/mol; polydispersity = 1.73.

*Polymer* **P-1b.** Polymer **P-1b** was obtained from monomer **7** (280 mg, 0.15 mmol) using the same procedure for preparation of polymer **P-1a** as a gray powder (198 mg, 75%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.79 (bs, 2H), 7.65−7.53 (m, 6H), 7.47−7.40 (m, 4H), 7.23−7.07 (m, 4H), 6.86−6.79 (m, 4H), 5.82 (br, 2H), 5.41−5.37 (m, 4H), 5.16−5.08 (m, 8H), 4.99−4.95 (m, 2H), 4.58−4.49 (m, 4H), 4.39−4.37 (m, 2H), 4.13 (br, 2H), 4.05−4.04 (m, 2H), 3.94−3.92 (m, 4H), 3.69 (br, 2H), 2.15 (bs, 6H), 2.09 (bs, 12H), 2.04 (bs, 12H), 2.01 (bs, 6H), 1.99 (br, 6H), 1.76 (bs, 6H). $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 169.4, 169.2, 169.1, 169.0, 168.8, 168.5, 168.1, 156.5, 152.4, 143.6, 137.7, 136.9, 130.7, 123.0, 128.3, 128.0, 126.3, 120.7, 120.4, 113.8, 113.6, 99.7, 84.5, 76.4, 74.8, 74.6, 71.7, 71.3, 70.9, 69.4, 67.1, 65.6, 61.1, 60.7, 59.5, 19.8, 19.7, 19.5, 19.2. GPC (THF, polystyrene standard): $M_n$ = 27500 g/mol; polydispersity = 1.78.

*Polymer* **P-1c.** Polymer **P-1c** was obtained from monomer **8** (200 mg, 0.15 mmol) using the same procedure for preparation of polymer **P-1a** as a solid (152 mg, 81%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.88 (bs, 2H), 7.80−7.60 (m, 6H), 7.49−7.44 (m, 2H), 7.40−7.22 (m, 6H), 6.96−6.83 (m, 4H), 5.82 (br, 2H), 5.68−5.54 (m, 4H), 5.30−5.15 (m, 6H), 4.18 (br, 6H), 2.19 (bs, 6H), 2.05 (bs, 12H), 1.76 (bs, 6H). $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 169.3, 169.0, 168.8, 168.0, 156.3, 152.4, 143.8, 136.9, 136.1, 131.2, 129.9, 128.2, 128.1, 126.5, 126.3, 120.8, 120.6, 120.3, 113.8, 113.7, 85.2, 73.1, 69.8, 66.9, 66.8, 65.9, 60.9, 60.2, 19.6, 19.5, 19.1. GPC (THF, polystyrene standard): $M_n$ = 21600 g/mol; polydispersity = 1.81.

*Polymer* **P-2a.** Protected glycoplymer **P-1a** (150 mg) was added to a solution of dry $CH_2Cl_2$ (5 mL) and MeOH (10 mL) under a nitrogen atmosphere and followed by 1.0 M $CH_3ONa/CH_3OH$ solution (0.25 mL). The reaction mixture was stirred overnight at room temperature. After removal of the solvent under a reduced pressure, 10 mL of water was added to the residue. The resulting solution was put in a cellulose dialysis tube (cutoff 3500), dialyzed against water for 2 d, and lyophilized to give the desired solid polymer **P-2a** (96.6 mg, 95%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 8.45 (bs, 2H), 8.10−7.76 (m, 6H), 7.69−7.25 (m, 6H), 7.24−7.15 (m, 2H), 7.03−6.94 (m, 4H), 5.67 (br, 2H), 5.11 (s, 4H), 4.28 (br, 2H), 3.87−3.74 (m, 4H), 3.73−3.50 (m, 14H), 3.45−3.30 (m, 6H). $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 157.1, 142.3, 139.2, 139.1, 136.5, 134.3, 131.5, 130.8, 128.9, 128.7, 127.2, 126.5, 125.0, 123.8, 123.7, 114.6, 103.7, 86.9, 79.6, 79.2, 77.8, 75.6, 75.1, 73.3, 71.8, 70.6, 68.1, 60.9, 59.9.

*Polymer* **P-2b.** Polymer **P-2b** was obtained from polymer **P-1b** (150 mg) using the same procedure for preparation of polymer **P-2a** as a solid (93.7 mg, 92%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 8.47 (bs, 2H), 8.12−7.77 (m, 6H), 7.68−7.23 (m, 6H), 7.23−7.13 (m, 2H), 7.03−6.96 (m, 4H), 5.68 (br, 2H), 5.13 (s, 4H), 4.25 (br, 2H), 3.84−3.72 (m, 4H), 3.70−3.49 (m, 14H), 3.46−3.32 (m, 6H). $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 157.5, 142.5, 139.1, 138.9, 136.5, 134.5, 131.4, 131.0, 129.0, 128.8, 127.2, 126.5, 124.9, 123.8, 123.6, 113.5, 99.5, 87.0, 79.5, 79.2, 77.9, 75.5, 74.9, 73.3, 71.7, 70.8, 68.3, 61.0, 59.4.

*Polymer* **P-2c.** Polymer **P-2c** was obtained from polymer **P-1c** (130 mg) using the same procedure for preparation of polymer **P-2a** as a solid (91.1 mg, 96%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 8.39 (bs, 2H), 8.14−7.70 (m, 6H), 7.68−7.40 (m, 6H), 7.28−7.20 (m, 2H), 7.10−6.98 (m, 4H), 5.54 (br, 2H), 5.15 (s, 4H), 4.10 (br, 2H), 3.82−3.70 (m, 4H), 3.65−3.50 (m, 6H). $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 157.2, 142.6, 139.1, 139.0, 136.3, 134.2, 131.5, 128.9, 128.7, 127.3, 126.5, 124.8, 123.8, 123.4, 120.5, 114.6, 88.1, 78.4, 73.7, 69.3, 68.4, 65.3, 60.9, 60.4.

**Studies of Metal Ions-Mediated Carbohydrate−Carbohydrate Interaction Based on Spectrofluorometric Titration.** To a solution of glycopolymer (**P-2a**, **P-2b**, or **P-2c**) in water or $H_2O$−DMSO(9:1, v/v) was added different metal chloride solutions (in the same solvents) individually. The final concentration of glycopolymer is $1.5 \times 10^{-6}$ M, corresponding to the repeating unit. The final concentration for the metal ion is 20 mM. After addition of the metal ion, the sample was allowed to incubate at room temperature overnight prior to recording a spectrum. The excitation wavelength was 370 nm and the emission scan ranged from 370−650 nm.

## Results and Discussion

The synthetic routes to the monomers and glycopolymers are outlined in Scheme 1. Propargyl-attached fluorene derivative **2** was easily prepared from 9,9-bis(4-hydroxyphenyl)-2,7-dibromofluorene **1** by treating with propargyl bromide under a basic condition. The Cu(I)-catalyzed "click reaction"[19] facilitated selective ligation between azido-attached lactose **3** and **2** to furnish lactosyl-bearing monomer **6** smoothly in 98% yield.

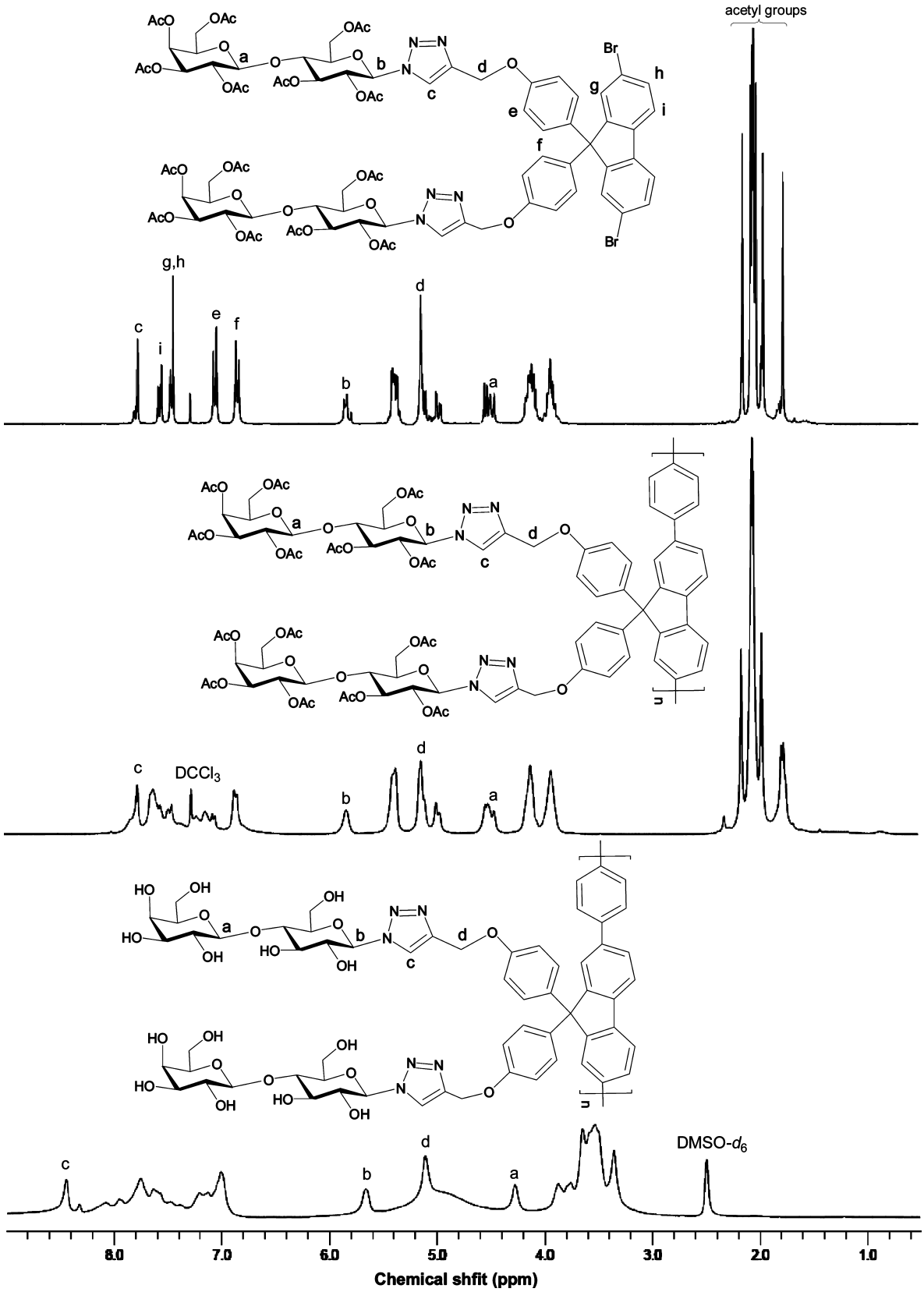



**Figure 1.** $^{1}$H NMR spectra of monomer **6** and polymers **P-1a** and **P-2a**.

Formation of triazole ring is confirmed by chemical shift at 7.75 ppm (single peak, 2H) on $^{1}$H NMR spectrum (Figure 1) and two peaks at 120.8 and 143.8 ppm on $^{13}$C NMR spectrum. A palladium-catalyzed Suzuki coupling polymerization between monomer **6** and 1,4-phenyldiboronic acid gave a well-defined copolymer **P-1a**, poly(fluorene-*alt*-phenylene) containing pendant lactosyl derivative. After removing all the acetyl groups with $CH_3ONa/CH_3OH$, the desired glycopolymer (**P-2a**) was obtained in an excellent yield (95%). According to the same procedures mentioned above, cellobiosyl-functionalized and galactosyl-functionalized polyfluorenes (**P-2b** and **P-2c**) were obtained from monomers **7** and **8**, respectively. Employing an

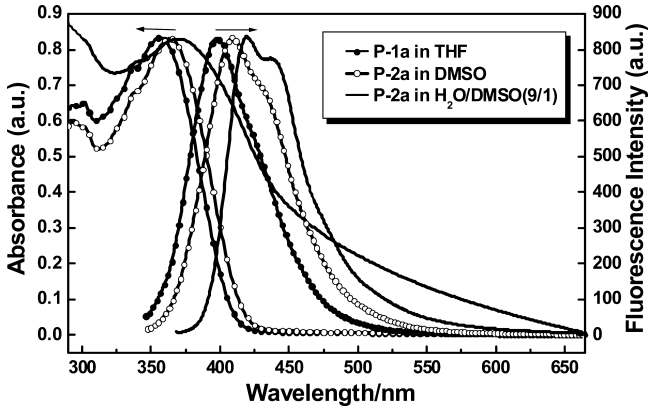



**Figure 2.** UV−vis absorption and fluorescence spectra of polymers **P-1a** and **P-2a**.

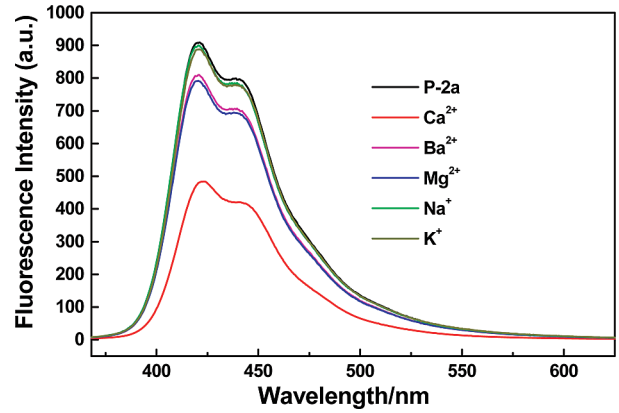



**Figure 3.** Fluorescence spectra of polymer **P-2a** in the absence and presence of various metal ions in water at room temperature. [**P-2a**] = $1.5 \times 10^{-6}$ M, [metal ion] = 20 mM.

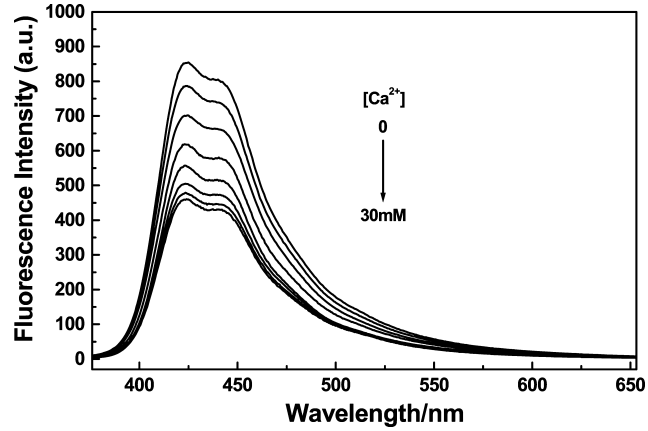



**Figure 4.** Fluorescence spectra of polymer **P-2a** ($1.5 \times 10^{-6}$ M) in the absence and presence of various concentrations of $Ca^{2+}$ in water at room temperature. $Ca^{2+}$ concentrations (mM) from the top downward are 0, 2.0, 5.0, 10.0, 15.0, 17.5, 20.0, and 30.0.

extended 9,9-bis(4-hydroxyphenyl)fluorenyl core as the polymerization monomer is advantageous for providing a more efficient shielding effect on the polyfluorene main chain after insertion of a rigid phenylene spacer between the sugar side chain and the polymer backbone, which would suppress the formation of aggregates or excimers.[20] Figure 1 shows the $^1$H NMR spectra of monomer **6** and polymers **P-1a** and **P-2a**. These data also apparently indicate that polymerization of sugar-bearing monomers is a good approach to prepare well-defined fluorescent conjugated glycopolymers.

Gel-permeation chromatography (GPC) analysis with polystyrene standards shows number-averaged molecular masses ($M_n$) ranged from 28300 to 21600 g/mol for all protected polymers, with polydispersities from 1.73 to 1.81. The protected polymers are readily soluble in common solvents such as methylene chloride, chloroform, and THF, but are insoluble in methanol, ethanol, acetone, and water. The solubility of the resulting polymers is different from their precursor polymers, showing good solubility in DMF, DMSO, and $H_2O$−DMSO (9:1, v/v) as well. The deprotected polymers are not soluble in water freely, only **P-2a** and **P-2b** are slightly soluble in water. Similar results were reported by Takasu for sugar-carrying poly(phenylenevinylene).[10f]

The precursor polymer **P-1a** exhibits an absorption maximum peak at 356 nm and an emission maximum peak at 397 nm in THF solution (Figure 2), which are assigned to the $\pi-\pi^*$ transition of the conjugated polymer backbone. As for the resulting polymer **P-2a**, it exhibits an absorption maximum peak at 365 nm and an emission maximum peak at 409 nm with a vibronic shoulder peak at 431 nm in DMSO solution. Whereas, in $H_2O$−DMSO (9:1, v/v), **P-2a** displays an absorption maximum peak at 370 nm and an emission maximum peak at 420 nm with a vibronic shoulder peak at 436 nm (Figure 2). The obvious bathochromic/red shift in both absorption and emission spectra for polymers **P-2a** could be attributed to the enhanced planar conformation or aggregation of polymer main chain in the solvent with increased polarity.[10h] Fluorescence quantum yields of the polymer were measured in dilute DMSO or aqueous solution, and calculated by using quinine sulfate in 0.1 M sulfuric acid as the reference absolute quantum efficiency (55%). The fluorescence quantum yields for **P-2a** are 68% in DMSO solution and 30% in solution of $H_2O$−DMSO (9:1, v/v), respectively.

Metal ion-mediated interaction of the lactose-bearing polyfluorene **P-2a** was investigated based on the change in fluorescence intensity of **P-2a** in the solution of water upon gradual addition of metal ions. As shown in Figure 3, **P-2a** exhibits almost no change in the fluorescence intensity after addition of $Na^+$ or $K^+$. Addition of $Mg^{2+}$ or $Ba^{2+}$ only caused a slight fluorescence quenching of **P-2a**. However, a significant fluorescence quenching of this glycopolymer was observed when $Ca^{2+}$ was added. It can be inferred that the size of the cation used is important to form a complex with lactose. Previous studies indicated that carbohydrate−metal complexes formed only with cations of ionic radii larger than 0.8 Å, the optimum size being 1.0 Å.[5d,21] Thus, calcium ions with ionic radii of 0.99 Å are ideal for complex formation. Russell's group has reported that, based on SPR measurement, an increased response in aggregation of lactose-coated gold nanoparticles for group 2 metal ions occurred as the ionic radii increased (i.e., Mg < Ca < Ba),[5d] which is different from our results (Mg ≈ Ba < Ca). The discrepancy may be attributed to the different artificial multivalent model systems used. For example, using liposome as the model system, the binding data showing that binding of (KDN)GM3 liposome to Gg3 epitope was much stronger than that of GM3.[22] When glycopolymer was employed as the multivalent carrier, an opposite result was observed.[23]

The preliminary studies of $Ca^{2+}$-mediated lactose−lactose interaction were performed by probing the changes in fluorescence intensity of **P-2a** when titrated with calcium ion. As shown in Figure 4, a substantial fluorescence quenching for **P-2a** ([**P-2a**] = $1.5 \times 10^{-6}$ M) was observed upon adding calcium ion ([$Ca^{2+}$] = 0−30 mM) in water. A possible mechanism for the fluorescence quenching of polymer **P-2a** is attributed to its

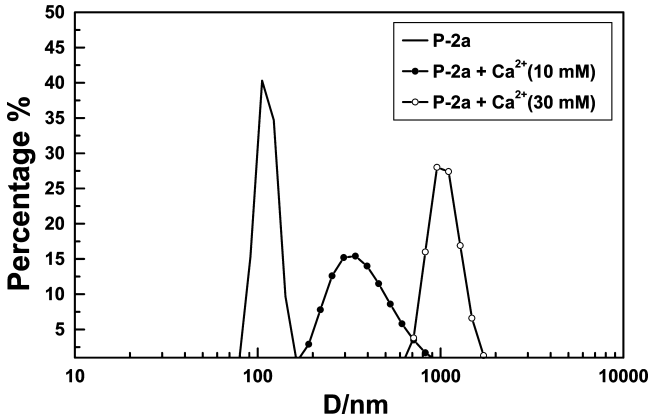



**Figure 5.** Hydrodynamic diameters of polymer **P-2a** in the absence and presence of calcium ion in water determined by DLS at room temperature.

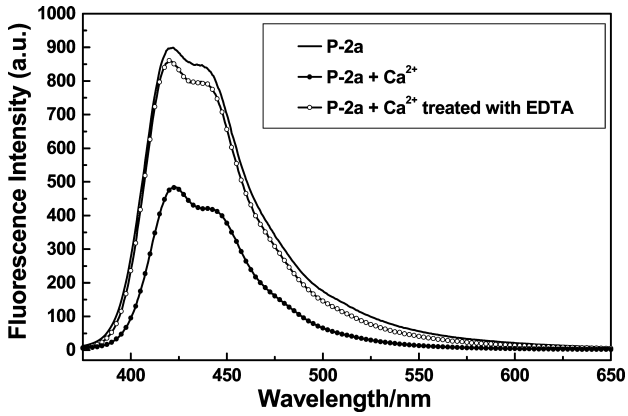



**Figure 6.** Fluorescence spectra of polymer **P-2a**, $Ca^{2+}$ (20 mM)-induced aggregation of polymer **P-2a**, and polymer **P-2a** following addition of $Ca^{2+}$ and subsequent addition of 20 mM EDTA at room temperature.

aggregation derived from **P-2a**−$Ca^{2+}$ complex formation, resulting in self-quenching. Dynamic light scattering can also prove $Ca^{2+}$-induced aggregation of the polymer by determining the corresponding hydrodynamic diameters (Figure 5). It can be seen that the hydrodynamic diameter of the glycopolymer **P-2a** increases from 105 to 1030 nm after addition of calcium ion (0−30 mM). The increment in hydrodynamic diameter is consistent with the spectrofluorometric titration data, which clearly indicates that continuous aggregation occurred during the **P-2a**−$Ca^{2+}$ complex formation. In addition, the hydrodynamic diameter obtained by DLS is related to the solvent. In DMSO solution, polymer **P-2a** shows a good solubility and its diameter in the absence of calcium ion is about 35 nm, which is reasonable, according to the contour length of the polymer (nearly 25 nm). Furthermore, the hydrodynamic diameter is also related to the concentration of the polymer. When the concentration of the polymer **P-2a** is $1.0 \times 10^{-6}$ M, the hydrodynamic diameter of aggregated ensemble in the presence of 30 mM calcium ion decreases to 800 nm, as compared with the 1030 nm at the concentration ($1.5 \times 10^{-6}$ M) of polymer **P-2a**.

The calcium-mediated lactose−lactose interaction is reversible when an EDTA solution (20 mM) was added to the **P-2a**−$Ca^{2+}$ complex aggregate. As shown in Figure 6, the associated fluorescence spectrum almost recovers to the original spectrum of glycopolymer **P-2a** after treated with EDTA. It has been proved that calcium binds with two lactose molecules and four water molecules in an octa-coordinate fashion, in which calcium ion is coordinated to a galactose moiety of one lactose and a

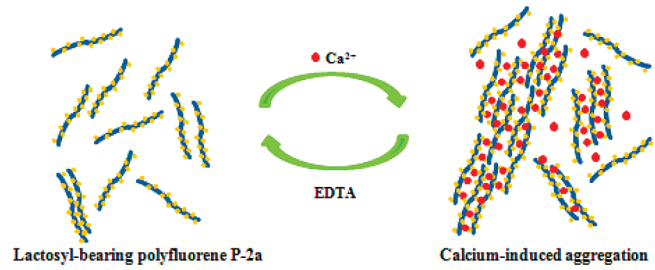



**Figure 7.** Schematic representation of the reversible calcium−lactose polymer aggregation.

glucose moiety of another lactose.[5d,24] Figure 7 shows a schematic representation of the reversible calcium−lactose polymer aggregate formation in which water molecules coordinated with calcium ions are omitted. A control experiment was performed to confirm the importance of the disaccharide component in effecting $Ca^{2+}$ complexation. Glycopolymers **P-2b** or **P-2c**, cellobiosyl- or glactose-bearing polyfluorenes, were designed and prepared for control experiments. Upon addition of 30 mM calcium ions to a solution of the polymer **P-2b** in water or the polymer **P-2c** in $H_2O$−DMSO(9:1, v/v), no significant changes in the fluorescence intensity were observed (Figures S1 and S2), which suggested that specific sugar structures are critical for the formation of carbohydrate−metal complex. These results revealed that conjugated glycopolymer, as a fluorescent multivalent model system of carbohydrate, was expected to have potential applications in detecting specific carbohydrate−carbohydrate interactions.

## Conclusions

In conclusion, a fluorescent conjugated polyfluorene with pendant lactopyranosyl ligands was easily prepared through Cu(I)-catalyzed azide/alkyne "click" ligation and Suzuki coupling polymerization. With fluorescent scaffolding and reporting carbohydrate ligands, this well-defined fluorescent conjugated glycopolymer was first used for studies of metal ion-mediated carbohydrate−carbohydrate interaction through spectrofluorometric titration. Upon addition of calcium ion, a significant fluorescence quenching of the lactosyl-bearing polyfluorene was observed, which is attributed to its aggregation derived from $Ca^{2+}$-mediated complex formation. Additionally, calcium-induced aggregation of the polymer can be confirmed using dynamic light scattering by determination of the corresponding hydrodynamic diameters. It has been shown that not only the cation size but also the sugar structures are critical for carbohydrate−metal complex formation. We believe this kind of fluorescent conjugated glycopolymer can provide an excellent platform for studies of carbohydrate−carbohydrate interactions.

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**Supporting Information Available.** Fluorescence spectra of polymers **P-2b** and **P-2c** in the absence and presence of calcium ion; $^{13}C$ NMR spectra of monomer **6**, polymer **P-1a**, and polymer **P-2a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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