DOI: 10.1002/chem.200801875

VIP

Highly Water-Soluble, Fluorescent, Conjugated Fluorene-Based Glycopolymers with Poly(ethylene glycol)-Tethered Spacers for Sensitive Detection of *Escherichia coli*

Cuihua Xue,[a] Singaravelu Velayudham,[a] Steve Johnson,[a] Ratul Saha,[b] Adrian Smith,[a] Wilbel Brewer,[a] Pushpalatha Murthy,[a] Susan T. Bagley,[b] and Haiying Liu*[a]

Abstract: Two bromide-bearing, fluorene-based, conjugated polymers with oligo(ethylene glycol)- and poly(ethylene glycol)-tethered spacers have been prepared by the Suzuki coupling polymerization of bromide-bearing, fluorene monomers. β-Glucose and α-mannose residues have been covalently attached to the conjugated polymers by post-polymerization functionalization of the precursor polymers with thiol-functionalized carbohydrates under basic conditions through thioether linkage. A glucose-bearing glycopolymer with oligo(ethylene glycol)-tethered spacers (**polymer A**) displays poor water solubility. However, glycopolymers with poly(ethylene glycol)-tethered spacers (**polymers B** and **C**) are highly water-soluble due to their long, flexible, hydrophilic spacers. Incubation of the ORN178 strain of *Escherichia coli* (*E. coli*) with α-mannose-bearing glycopolymer (polymer **C**) results in the formation of fluorescent cell clusters, causing significant red shifts in UV/Vis absorption and fluorescent spectra of the polymer through multivalent cooperative interactions of the polymeric carbohydrates with the bacterial pili. In contrast, polymer **C** displays no interactions with a mutant ORN208 strain of *E. coli*.

Keywords: bacterial detection · bioimaging · biosensors · conjugation · fluorescence · glycopolymers

Introduction

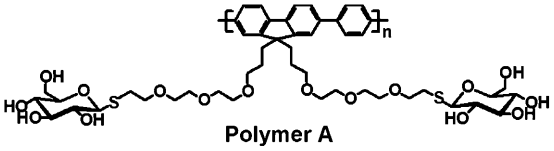
Carbohydrates are the key components of glycolipid, polysaccharide, and glycoprotein cell-surface molecules and play critical roles in cell–cell recognition, cell adhesion, differentiation, trafficking, signaling between cells, cellular metastasis, and viral or bacterial infections.[1–3] Fluorescent, conjugated glycopolymers combine fluorescent scaffolding and carbohydrate reporting functions into one package and thus offer a general and powerful platform for whole-cell applications, because their multivalent display of carbohydrates can mimic multivalent interactions at cell–cell interfaces at which avidity is crucial.[4–9] A few fluorescent, conjugated glycopolymers, such as glycopoly(*p*-phenylene ethynylene)s (PPEs), glycopolythiophenes, and glycopoly(*p*-phenylene)s have been prepared for biosensing applications for lectins and bacteria through pre- and post-polymerization functionalization approaches.[10–14] However, some neutral, conjugated glycopolymers display low water solubility,[15–17] probably due to strong π–π stacking interactions among hydrophobic polymer backbones. Introduction of anionic groups such as carboxylic acid to conjugated polymers significantly enhances water solubility of the conjugated glycopolymers by preventing the π–π stacking interactions of polymer backbones through charge repulsion.[18] However, the presence of ionic groups in conjugated polymers could cause potential interfering responses due to non-specific electrostatic interactions in complicated biological samples.[18] Therefore, it is important to explore new approaches to prepare a variety of highly water-soluble, neutral, fluorescent, conjugated glycopolymers for bacterial and viral biosensing applications.

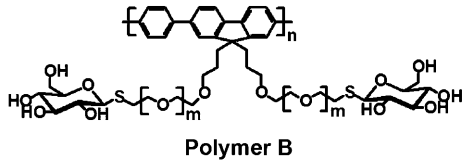
In this article, we report the synthesis of highly water-soluble, fluorene-based, conjugated glycopolymers by using

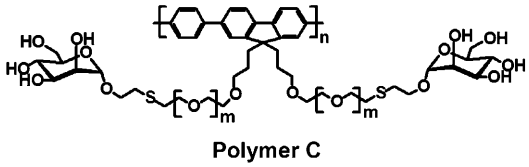
[a] C. Xue, S. Velayudham, S. Johnson, A. Smith, W. Brewer, P. Murthy, H. Liu
Department of Chemistry
Michigan Technological University
1400 Townsend Drive, Houghton, MI 49931 (USA)
E-mail: hyliu@mtu.edu

[b] R. Saha, S. T. Bagley
Department of Biological Science
Michigan Technological University
1400 Townsend Drive, Houghton, MI 49931 (USA)
Fax: (+1) 906-4872062

poly(ethylene glycol) as tethered spacers between the polymer backbone and carbohydrate residues. The glycopolymers were prepared by our unique post-polymerization functionalization approach, which covalently attaches thiol-functionalized carbohydrates to bromide-bearing, fluorene-based, conjugated polymers through thioether bridges under basic conditions. Use of long, flexible poly(ethylene glycol) as the tethered spacers not only makes the glycopolymers highly soluble in water, but also helps reduce steric hindrance for cellular recognition and prevents non-specific interactions. Strong cooperative multivalent interactions between the polymeric carbohydrates and pili of *E. coli* result in significant red shifts of UV/Vis absorption and fluorescent spectra. In contrast, fluorene-based, conjugated glycopolymer (**polymer A**), with a short oligo(ethylene glycol) as the tethered spacer, displays poor solubility in aqueous solution due to strong hydrophobic feature of fluorene-based, polymer backbone.



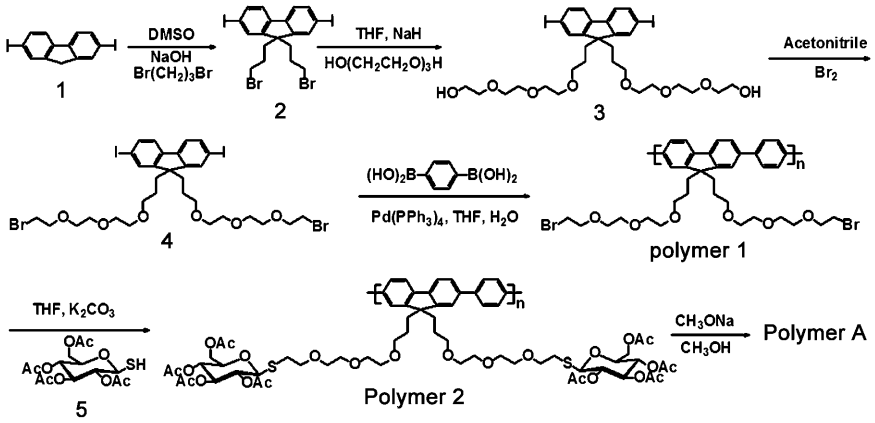




Results and Discussion

We chose fluorene-based, conjugated polymers to demonstrate the feasibility of our strategy to use poly(ethylene glycol) as tethered spacers between the polymer backbone and carbohydrate residues to enhance water-solubility of the glycopolymers, because the fluorene ring is rigid and highly hydrophobic. For a control experiment, we used a shorter oligo(ethylene glycol) as the tethered spacer between the polymer backbone and glucose residues. A bromide-bearing, fluorene-based, polymer was prepared (from compounds **1**–**4**) by the Suzuki coupling polymerization of a bromide-functionalized, fluorene monomer with an oligo(ethylene glycol) tether (**4**), which was obtained by reacting one equivalent of 2,7-diiodo-9,9-bis(3′-bromo-propanyl)-fluorene (**2**) with six equivalents of tri(ethylene glycol) in dry THF in the presence of NaH to give compound **3**, followed by bromination in acetonitrile in the presence of bromine and triphenylphosphine (Scheme 1). Glucose was covalently attached to the polymer through thioether bridges by our unique post-polymerization functionalization of the bromide-bearing, polymer precursor with 1-thiol-β-D-glucose tetraacetrate (**5**) under basic conditions, affording a glycopolymer bearing peracetylated glucose residues (polymer **2**) (Scheme 2). Two well-separated peaks at $\delta = 3.37$ and 3.14 ppm in the ^{1}H NMR spectrum, corresponding to methylene protons at the 3- and 4-positions in monomer **4**, become around $\delta =$ 3.22 ppm after polymerization. The ^{1}H NMR spectrum of the precursor **polymer 1** shows that the signal peak at $\delta =$ 3.23 ppm, corresponding to methylene protons (at the 4-position) adjacent to bromide group, shifts to high field and splits into two peaks at $\delta = 2.86$ and 2.72 ppm after formation of thioether bond; this behavior was also observed in regioregular head-to-tail conjugated glycopolythiophene (Figure 1).[13] The signal peaks at $\delta = 1.07$, 2.16 and 3.23 ppm corresponding to methylene protons at the 1-, 2-, and 3-positions, respectively, in **polymer 1** are almost unchanged relative to those in **polymer 2** as they are far from the thioether group (Figure 1). **Polymer 2** was de-acetylated under Zemplén conditions in methanol and methylene chloride containing sodium methoxide, affording fluorene-based, fluorescent, conjugated **polymer A** (Figure 2). As we expected, polymer **A** with shorter oligo(ethylene glycol)-tethered spacers displays poor solubility in aqueous solution due to strong hydrophobic feature of the fluorene-based polymer backbone.

A fluorene-based, conjugated polymer bearing bromide groups with poly(ethylene glycol) tethers (**polymer 3**) was prepared (from compounds **2**, **6**, and **7**) by the Suzuki cou-



Scheme 1. Synthetic route to **polymer A**.

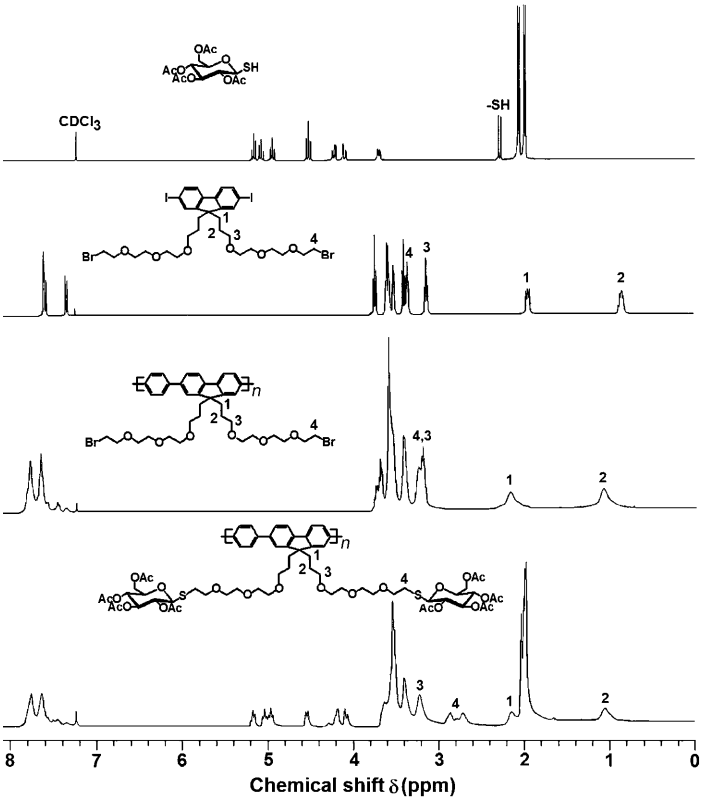


Figure 1. ^{1}H NMR spectra of 1-thiol-β-D-glucose tetraacetrate, monomer **4**, **polymer 1** and **polymer 2** in $CDCl_3$.

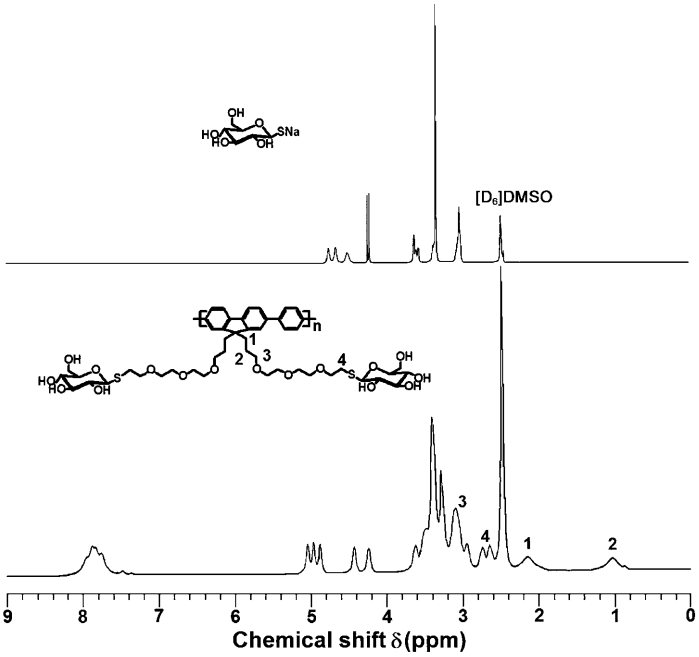
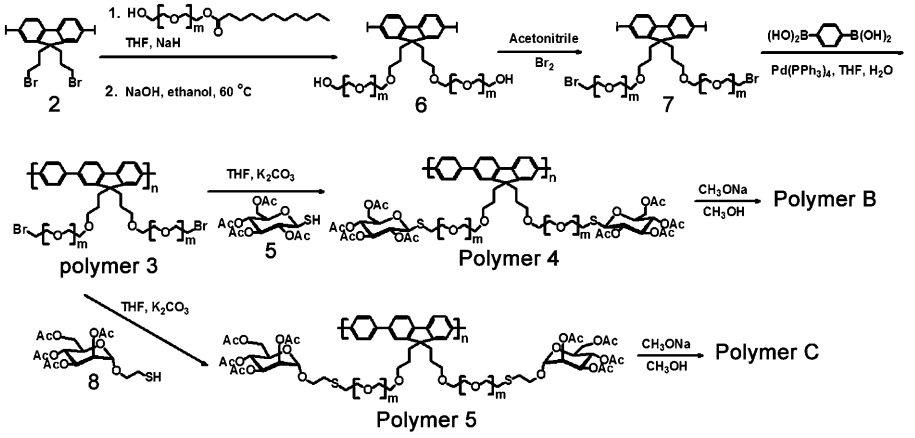


Figure 2. ^{1}H NMR spectra of **polymer A** in [D_6]DMSO.

pling polymerization of phenyldiboronic acid and bromide-bearing monomer **7** bearing poly(ethylene glycol) tethers with $Pd(PPh_3)_4$ as catalyst under basic conditions (Scheme 2). Bromide-bearing monomer **7** was prepared by reacting compound **2** with poly(ethylene glycol) monolaurate in a dry THF in the presence of NaH, and hydrolyzing the reaction product to give compound **6**, and followed by bromination in acetonitrile in the presence of bromine and triphenylphosphine (Scheme 2). β-Glucose- and α-mannose-bearing conjugated glycopolymers with poly(ethylene glycol) tethers were prepared by post-polymerization functionalization of bromide-bearing **polymer 3** with 1-thiol-β-D-glucose tetraacetrate (**5**) and 1-thiolethyl-α-D-mannose tetraacetrate (**8**) under basic conditions, affording **polymers 4** and **5**, respectively; de-acetylation of **polymers 4** and **5** under Zemplén conditions in methanol and methylene chloride containing sodium methoxide. lead to the formation of **polymers B** and **C**. Two well-separated peaks in the ^{1}H NMR spectrum at $\delta = 3.36$ and 3.14 ppm for monomer **7**, become a broad peak between $\delta =$ 3.20 and 3.14 ppm after the polymerization; such behavior was also observed in **polymer 1** (Figure 3). Formation of thioether bonds of bromide groups with compound **8** causes the peak at $\delta = 3.20$ ppm adjacent to bromide groups in **polymer 3** shift to high field around $\delta = 2.75$ ppm. Peaks at 3.20 and 1.02 ppm corresponding to methylene protons at the 3- and 2-positions remain almost unchanged after polymerization, since they are far away from the thioether bonds.

The precursor **polymer 1** displays absorption spectral maximum peak at $\delta = 376$ nm, and emission spectral maximum peak at 416 nm with a vibronic shoulder peak at 438 nm in DMF solution, which were ascribed to the π–π* transition of the conjugated polymer backbone. **Polymers 2**,



Scheme 2. Synthetic route to **polymers B** and **C**.

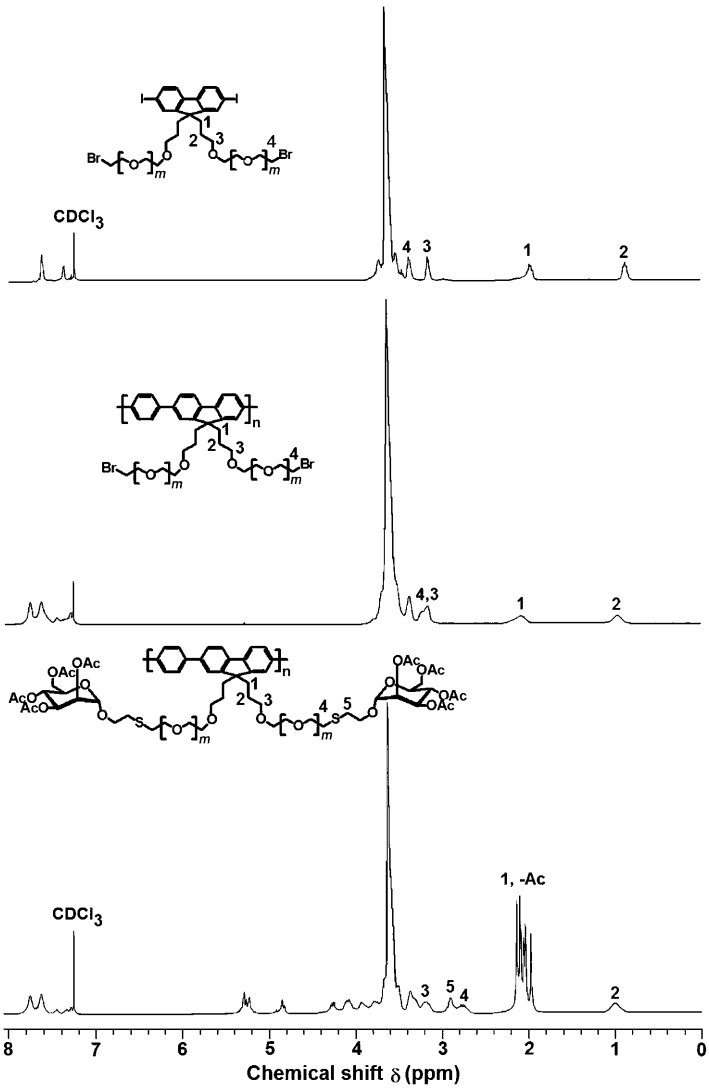


Figure 3. ^{1}H NMR spectra of monomer **7** and **polymers 3** and **5** in $CDCl_3$.

3, **4**, **5** and **A** in DMF exhibit similar optical properties to those of **polymer 1** except their fluorescence quantum yields as polymers with poly(ethylene glycol) tethers are a little higher than those of their counterparts with oligo(ethylene glycol)-tethered spacers (Table 1). **Polymers B** and **C** with poly(ethylene glycol) tethers are highly soluble and fluorescent with fluorescent quantum yield of about 44% in phosphate buffer, and exhibit absorption maximum peak at 380 nm, and emission maximum peak at 426 nm with a vibronic shoulder peak at 440 nm in 0.1 M phosphate buffer (pH 7.4) (Table 1).

Table 1. Fluorescent quantum yield (ϕ) of fluorene-based conjugated polymers.

	ϕ [%]	Solvent
polymer 1	42	DMF
polymer 2	43	DMF
polymer A	39	DMF
polymer 3	48	DMF
polymer 4	52	DMF
polymer 5	53	DMF
polymer B	44	buffer[a]
polymer C	45	buffer[a]

[a] 0.1 M phosphate buffer (pH 7.4).

Type 1 pili of *E. coli*, encoded by the *fim* gene cluster (*fimA–fimH*), possess a short tip fibrillum containing the FimH adhesion, which is joined to the distal end of the FimA pilus rod.[19] The FimH adhesion binds specifically to mannosylated glycoproteins present in the bladder epithelium.[19] We chose two strains ORN178 and ORN208 of *E. coli* for testing and control experiments to investigate the specific binding of the α-mannose-bearing **polymer C** to the bacterial pili.[20] The ORN178 strain expresses the wild-type type 1 pili that specifically bind to mannose, whereas the ORN208 strain is deficient of the *fimH* gene and expresses abnormal type 1 pili that lack the ability to mediate α-mannose-specific binding. Phosphate buffer (1.0 mL) containing different numbers of the bacterial cells were individually incubated with 10–50 μg of **polymer C** for one hour with gentle shaking, centrifuged at 10,000 g, and were washed five times with PBS buffer. The final cell pellet was re-suspended in PBS buffer. Incubation of **polymer C** with ORN178 strain resulted in the formation of fluorescently stained bacterial clusters from which the polymer was not removed by rinsing and centrifugation. These bacterial clusters were visualized under fluorescent microscope (Figure 4, left and middle). Strong multivalent cooperative interactions between the polymeric carbohydrates and the bacterial pili cross-link the bacteria, forming highly fluorescent bacterial clusters.

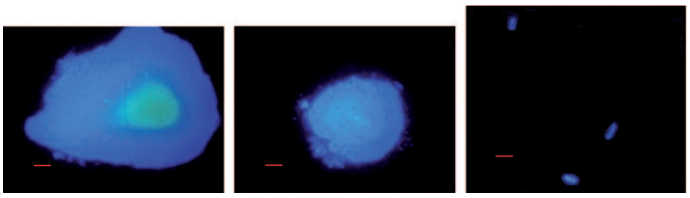


Figure 4. Fluorescent microscopy images of fluorescent glycopolymer-stained *E. coli* bacteria clusters with 10^9 and 10^6 bacterial cells (left and middle), respectively, and fluorescent glycopolymer-stained *E. coli* bacterial cells of the ORN178 strain (right). The scale bar is 10 μm.

We further confirmed multivalent interactions between bacterial pili and the polymeric α-mannose by measuring optical properties of the glycopolymers in the absence and presence of *E. coli*. Formation of fluorescently stained clusters of the ORN178 strain with mannose-bearing **polymer C** causes significant red shifts in UV/Vis absorption and fluorescent spectra of the polymer (Figure 5). These red shifts might arise from enhanced π conjugation of the conjugated glycopolymers through multivalent interactions with the bacterial pili as the glycopolymers might lie on the bacterial surface (Figure 4, right). The red shift in fluorescent spectra of the conjugated glycopolymer in the presence of a large number of bacteria results in a green region in image of fluorescently stained bacterial cluster (Figure 4, left).

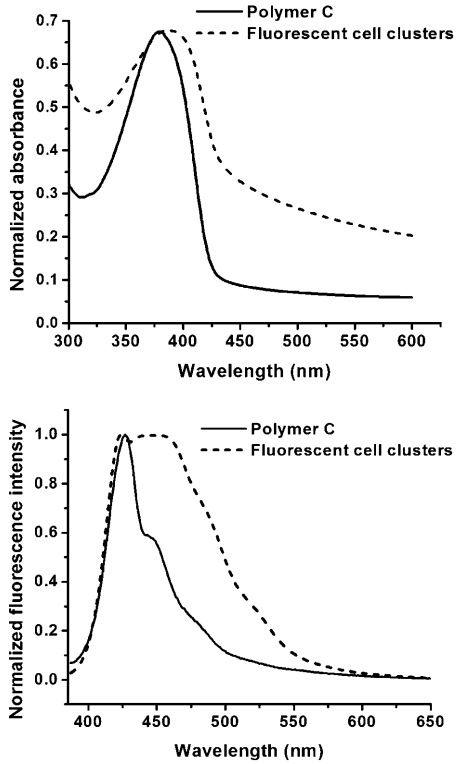


Figure 5. UV/Vis absorption (top) and fluorescent (bottom) spectra of polymer **C** in the absence and presence of *E. coli* bacteria (1×10^8 cells). Excitation wavelength is 370 nm.

The presence of the ORN208 strain does not cause any significant changes in the optical properties of the polymer, indicating that **polymer C** does not bind ORN208 strain. The experimental results indicate that **polymer C** selectively binds the pili of the ORN178 strain, but not those of the mutant ORN208 strain, demonstrating specific binding of polymer **C** to the bacterial FimH proteins.

Conclusions

Combining the post-polymerization functionalization approach with the use of poly(ethylene glycol) as glycoside-tethered spacers will provide a facile and versatile way to prepare a variety of highly water-soluble, neutral, conjugated glycopolymers and with significantly reduced steric binding hindrance and non-specific interactions for promising biosensing applications for cells and viruses.

Experimental Section

Instrumentation: ^{1}H NMR and ^{13}C NMR spectra were taken on 400 MHz Varian Unity Inova spectrophotometer. UV spectra were taken on a Hewlett Packard 8452A Diode Array UV/Vis spectrophotometer. Fluorescence spectra were obtained on a Spex Fluorolog 1681 0.22 m steady-state fluorometer. Fluorescence quantum yields of the polymers were measured in DMF, or phosphate buffer solution, and calculated by using quinine sulfate in 0.1 N sulfuric acid as the reference absolute quantum efficiency ($\phi_n=55\,\%$).[21] Molecular weights of the polymers were determined by gel permeation chromatography (GPC) by using a Waters Associates Model 6000A liquid chromatograph. Three American Polymer Standards Corp. Ultrastyragel columns in series with porosity indices of 10^3, 10^4, and 10^5 Å were used and housed in an oven thermostated at 30 °C. Mobile phase was HPLC grade THF which was filtered and degassed by vacuum filtration through a 0.5 μm Fluoropore filter prior to use. The polymers were detected by a Waters Model 440 ultraviolet absorbance detector at a wavelength of 254 nm and a Waters Model 2410 refractive index detector. Molecular weight was measured relative to polystyrene standards.

Materials: Unless otherwise indicated, all reagents, poly(ethylene glycol) monolaurate ($M_n=600$, average repeated unit of poly(ethylene glycol) = 9), and solvents were obtained from commercial suppliers (Aldrich, Fluka, Acros, Lancaster), and were used without further purification. Air- and moisture-sensitive reactions were conducted in oven-dried glassware by using standard Schlenk line or dry-box techniques under an inert atmosphere of dry nitrogen. 1-Thiolethyl-α-D-mannose tetraacetate and 1-thiolethyl-α-D-mannose were prepared and characterized according to the literature.[22–24]

Cell growth and incubation of glycopolymers with cells: Two different *E. coli* bacterial strains of ORN178, a mannose-binding strain and ORN208, a mutant strain that does not bind mannose, were used for this study. ORN178 and ORN208 are generous gifts from Professor Paul Orndorff, North Carolina State University. Cell growth was conducted according to a reported procedure.[14] An aliquot of the glycopolymer (10–40 μg) was added to an aliquot of the bacterial cells (1 mL) in PBS buffer with $CaCl_2$ (1.0 mM) and $MnCl_2$ (1.0 mM). The suspension was incubated for 1 h at room temperature with gentle shaking, and then centrifuged to form cell pellets. The pellets were re-suspended in the same buffer and centrifuged. Cells were imaged by using a Zeiss Axioplan 2 fluorescence microscope with 375 nm of excitation wavelength.

2,7-Diido-9,9-bis-(3′-bromo-propyl)-fluorene (2): 2,7-Diiodofluorene (**1**; 2.0 g, 4.8 mmol) was added to a mixture of aqueous potassium hydroxide (100 mL, 50 %), tetrabutylammonium bromide (0.33 g, 1.0 mml), and 1,3-dibromopropane (10.0 g, 49.5 mmol) at 75 °C. After the mixture was stirred for 30 min, it was cooled to room temperature, and then extracted with CH_2Cl_2. The combined organic layers were washed with water, aqueous HCl (1 M) and brine, and then dried over $MgSO_4$. The solvent was removed and the residue was purified by silica gel column chromatography (hexane/CH_2Cl_2, 8:1) to give compound **2** (2.2 g, 71 %) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.69$–7.66 (m, 4H), 7.41–7.39 (d, $J=8$ Hz, 2H), 3.12–3.09 (t, $J=6.4$ Hz, 4H), 2.12–2.08 (m, 4H), 1.13–1.09 ppm (m, 4H); ^{13}C NMR (400 MHz, $CDCl_3$): $\delta=150.83$, 139.87, 137.05, 132.23, 122.02, 93.80, 54.53, 38.64, 33.89, 27.20 ppm; MS: *m/z* calcd for $C_{19}H_{18}Br_2I_2$ $[M]^+$: 657.8; found: 657.7.

Compound 3: Compound **2** (1.6 g, 2.42 mmol) was added to a mixture of triethylene glycol (7.2 g, 48 mmol) and NaH (1.0 g, 25 mmol) in THF at room temperature. After stirring for 24 h, the mixture was extracted with CH_2Cl_2. The combined organic layers were washed with brine and then dried over $MgSO_4$. After removal of the solvent, the residue was purified by silica gel column chromatography (EtOAc) to give the compound **3** (1.3 g, 66 %) as a pasty oil. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.64$–7.59 (m, 4H), 7.36–7.34 (d, $J=8$ Hz, 2H), 3.67–3.66 (m, 4H), 3.65–3.63 (m, 4H), 3.59–3.52 (m, 12H), 3.38–3.35 (t, $J=5.2$ Hz, 4H), 3.16–3.13 (t, $J=6.8$ Hz, 4H), 2.0–1.94 (m, 4H), 0.87–0.85 ppm (m, 4H); ^{13}C NMR (400 MHz, $CDCl_3$): $\delta=151.88$, 140.01, 136.54, 132.33, 121.82, 93.51, 72.69, 71.25, 70.82, 70.71, 70.54, 70.05, 61.93, 55.08, 36.36, 24.19 ppm; MS: *m/z* calcd for $C_{31}H_{44}I_2NaO_8$ $[M+Na]^+$: 821.1; found: 821.3.

Compound 4: Br_2 (0.44 g, 2.75 mmol) was added dropwise to the suspension of triphenylphosphine (0.72 g, 2.75 mmol) in CH_3CN under N_2 at 0 °C , and a solution of compound **3** (1.1 g, 1.38 mmol) in CH_3CN was then added dropwise. The resulting mixture was stirred for 48 h at room temperature. After removal of the solvent, the residue was dissolved in EtOAc and washed with solution of NaCl. The organic layer was collected and dried over anhydrous $MgSO_4$. After removing the solvent, the residue was purified by column chromatography on silica (EtOAc/hexane 5:1) to give the target compound as a white solid (1.04 g, 82 %). ^{1}H NMR

(400 MHz, $CDCl_3$): $\delta = 7.61$–7.59 (m, 4H), 7.36–7.34 (d, $J = 8$ Hz, 2H), 3.75–3.72 (m, 4H), 3.62–3.57 (m, 8H), 3.53–3.51 (m, 4H), 3.41–3.34 (m, 8H), 3.15–3.12 (t, $J = 6.8$ Hz, 4H), 1.96–1.92 (m, 4H), 0.87–0.83 ppm (m, 4H); ^{13}C NMR (400 MHz, $CDCl_3$): $\delta = 151.83$, 140.01, 136.56, 132.26, 121.88, 93.59, 71.38, 71.23, 70.81, 70.73, 70.14, 55.06, 36.38, 30.64, 24.28 ppm; MS: m/z calcd for $C_{31}H_{42}Br_2I_2NaO_6$ $[M+Na]^+$: 944.9; found: 944.8.

Polymer 1: Compound **4** (1.25 g, 1.35 mmol), 1,4-phenyldiboronic acid (0.23 g, 1.36 mmol), and potassium carbonate (1.87 g, 13.6 mmol) were placed in a 100 mL round-bottomed flask. $[Pd(PPh_3)_4]$ (12 mg) was added to the flask under N_2 atmosphere in glove box. The reaction mixture was heated to reflux at 80 °C under N_2 atmosphere for 36 h, after which a mixed degassed solution of water (10 mL) and THF (20 mL) was added to the flask. After removal of the solvent, the residue was dissolved in methylene chloride, washed with water and dried over anhydrous $MgSO_4$. When the solvent was reduced (to 3 mL) by evaporation, the polymer was precipitated by methanol, collected by filtration, washed with methanol, and dried under vacuum for 24 h at room temperature to afford the **polymer 1** (0.62 g, 62%). ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.78$ (m, 4H), 7.65 (m, 4H), 7.41 (m, 2H), 3.74–3.67 (m, 4H), 3.58–3.53 (m, 12H), 3.41–3.39 (m, 4H), 3.24–3.17 (m, 8H), 2.16 (m, 4H), 1.07 ppm (m, 4H); ^{13}C NMR (400 MHz, $CDCl_3$): $\delta = 151.12$, 140.58, 140.08, 132.28, 129.02, 127.82, 127.44, 126.56, 121.62, 120.55, 72.14, 71.73, 71.38, 70.81, 70.41, 70.12, 54.93, 36.85, 30.60, 24.59 ppm; GPC (THF, polystyrene standard), M_n: 26700 g mol^{-1}; polydispersity (M_w/M_n): 1.70; UV/Vis and fluorescence (DMF): $\lambda_{max} = 376$ (abs), 416 (em), 438 nm (sh).

Polymer 2: Compound **5** (0.4 g, 1.1 mmol) and potassium carbonate (1.5 g, 10.8 mmol) were added to the solution of **polymer 1** (0.4 g) in THF (30 mL). The mixture was stirred at room temperature for 48 h. After removal of the solvent, the residue was dissolved in methylene chloride, washed with water and dried over anhydrous $MgSO_4$. After removing most of the solvent, the residue was poured into methanol, and polymer was filtered, washed with methanol and dried under vacuum for 24 h to give the **polymer 2** (0.5 g, 68% yield) as a yellow solid. ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.76$ (m, 4H), 7.64 (m, 4H), 7.43 (m, 2H), 5.17 (m, 2H), 5.04 (m, 2H), 4.97 (m, 2H), 4.53 (m, 2H), 4.18 (m, 2H), 4.10 (m, 2H), 3.64–3.54 (m, 18H), 3.40 (m, 4H), 3.23 (m, 4H), 2.86–2.72 (m, 4H), 2.16 (m, 4H), 2.03–1.98 (m, 24H), 1.06 ppm (m, 4H). UV/Vis and fluorescence (DMF): $\lambda_{max} = 376$ (abs), 415 (em), 436 nm (sh).

Polymer A: **Polymer 2** (0.5 g) was dissolved in a mixed solution of dry CH_2Cl_2 (10 mL) and CH_3OH (20 mL), and a solution of CH_3ONa in CH_3OH (1 mL) was added. The reaction mixture was stirred at room temperature for 30 h. After the solvent was removed, water (10 mL) was added to the residue, and the mixture was then put in a cellulose dialysis tube (cutoff 12000) for dialysis against water for 2 days (10 water changes). The light yellow solid was precipitated from the water, collected, and dried under vacuum for 24 h to give **polymer A** (0.34 g, 92% yield). ^1H NMR (400 MHz, DMSO): $\delta = 7.87$–7.76 (m), 5.04–4.87 (m), 4.43 (m), 4.23 (m), 3.62 (m), 3.50–3.28 (m), 3.09 (m), 2.94 (m), 2.74 (m), 2.64 (m), 2.18 (m), 0.83 ppm (m); polymer **A** shows poor water solubility; UV/Vis and fluorescence (DMF): $\lambda_{max} = 376$ (abs), 417 (em), 438 nm (sh).

Compound 6: Compound **2** (1.0 g, 1.52 mmol) was added to a solution of polyethylene glycol monolaurate (5.0 g) and NaH (1.0 g, 25 mmol) in THF (50 mL) at room temperature. After the resulting solution was stirred for 48 h, the solvent was evaporated. The residue was dissolved in methylene chloride and washed with distilled water five times. After the solvent was removed, NaOH (1.0 g) and ethanol (25 mL) were added to the residue. The mixture was stirred under a nitrogen atmosphere at 60 °C for 24 h before the solvents were evaporated by rotary evaporator. The residue was extracted with CH_2Cl_2, and the combined organic layer was washed with brine, and dried over $MgSO_4$. After removal of the solvent, the residue was treated with a mixed solution of EtOAc and hexane (1:10), and dried to give the compound **6** (1.0 g) as a light yellow pasty oil. ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.62$ (m, 4H), 7.36 (m, 2H), 3.70–3.67 (m, 4H), 3.64–3.58 (m), 3.54–3.50 (m, 4H), 3.38–3.35 (m, 4H), 3.17–3.14 (t, 4H), 2.64 (m, 2H), 1.97–1.93 (m, 4H), 0.89–0.86 ppm (m, 4H).

Compound 7: Br_2 (0.2 g, 1.25 mmol) was added dropwise to the suspension of triphenylphosphine (0.32 g, 1.22 mmol) in CH_3CN (30 mL) under N_2 at 0 °C. Compound **3** (1.0 g) in CH_3CN (30 mL) was added dropwise to the mixture and the resulting mixture was stirred for 48 h at room temperature. After removal of the solvent, the residue was dissolved in CH_2Cl_2 and washed with saturated NaCl solution, and dried over anhydrous $MgSO_4$. After removing the solvent, the residue was treated with a mixed solution of EtOAc and hexane (1:10), and dried under a vacuum oven to give compound **7** (0.75 g) as a light yellow sticky oil. ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.62$ (m, 4H), 7.36 (m, 2H), 3.69–3.50 (m), 3.36 (m, 4H), 3.15–3.13 (m, 4H), 1.97–1.95 (m, 4H), 0.88–0.86 ppm (m, 4H).

Polymer 3: Compound **7** (0.75 g) and 1,4-phenyldiboronic acid (0.07 g, 0.42 mmol), and potassium carbonate (0.6 g, 4.3 mmol) were placed in 100 mL round-bottom flask. $Pd(PPh_3)_4$ (8 mg) was added to the flask under a N_2 atmosphere in a glove box. When a degassed mixed solution of water (10 mL) and THF (10 mL) was added to the flask, the mixture was stirred at 80 °C under N_2 atmosphere for 36 h. After removal of the solvent, the residue was dissolved in methylene chloride and washed with water. After the solvent was removed, ethanol (10 mL) was added to the residue, and the solution was put in a cellulose dialysis tube (cutoff 12000) for dialysis against water containing 20% ethanol for 2 days (10 changes of the solution). When the solution was extracted with CH_2Cl_2, the organic layer was collected, dried over $MgSO_4$, and filtered. The filtrate was collected and the solvent was evaporated to give **polymer 3** (0.4 g) as a brown sticky oil. ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.74$ (m, 4H), 7.62 (m, 4H), 7.41 (m, 2H), 3.69–3.50 (m), 3.36 (m, 4H), 3.20–3.14 (m, 8H), 2.04 (m, 4H), 1.02 ppm (m, 4H); GPC (THF, polystyrene standard), M_n: 45,900 g mol^{-1}; polydispersity (M_w/M_n): 2.15; UV/Vis and fluorescence (DMF): $\lambda_{max} = 375$ (abs), 416 (em), 438 nm (sh).

Polymer 4: Compound **5** (0.2 g, 0.55 mmol) and potassium carbonate (0.4 g, 3.6 mmol) were added to a solution of **polymer 3** (0.2 g) in THF (20 mL). After the mixture was stirred at room temperature for 48 h, the polymer was purified by means of the same procedure used for **polymer 3** to give the **polymer 4** (0.23 g) as a brown sticky oil. ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.72$ (m, 4H), 7.60 (m, 4H), 7.43 (m, 2H), 5.20–5.14 (m), 5.07–4.98 (m), 4.39–4.07 (m), 3.73–3.34 (m), 3.14–3.12 (m), 2.89–2.73 (m), 2.08–1.83 (m), 1.01 ppm (m, 4H); UV/Vis and fluorescence (DMF): $\lambda_{max} = 375$ (abs), 416 (em), 438 nm (sh).

Polymer B: **Polymer 4** (0.20 g) was dissolved in a mixture of dry CH_2Cl_2 (5 mL) and CH_3OH (20 mL), and then solution of CH_3ONa (0.5 M) in CH_3OH (1 mL) was added. The reaction mixture was stirred at room temperature for 30 h. After the solvent was removed, water (10 mL) was added to the residue. The mixture was put in a cellulose dialysis tube (cutoff 12000), dialyzed against water for 2 days (10 water changes), and lyophilized to give **polymer B** (0.14 g) as a brown sticky oil. ^1H NMR (400 MHz, D_2O): δ 7.44–6.89 (m), 5.0–4.01 (m, D_2O + glucose), 4.0–3.19 (m), 2.86 (m), 1.98 (m), 1.2–0.9 ppm (m); **polymer B** is highly soluble in water; UV/Vis and fluorescence (0.1 M phosphate buffer with pH 7.4): $\lambda_{max} = 380$ (abs), 426 (em), 440 nm (sh); $\phi = 44\%$ in 0.1 M phosphate buffer (pH 7.4).

Polymer 5: Compound **8** (0.5 g) and potassium carbonate (0.4 g, 3.6 mmol) were added to **polymer 3** (0.2 g) in THF (20 mL). The mixture was stirred at room temperature for 48 h. The polymer was purified by using the same procedure described for **polymer 3** to give the **polymer 5** (0.24 g) as a brown sticky oil. ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.75$ (m, 4H), 7.64–7.61 (m, 4H), 7.42 (m, 2H), 5.28–5.21 (m), 4.84 (m), 4.26–4.23 (m), 4.12–4.07 (m), 3.93–3.90 (m), 3.78–3.72 (m), 3.61–3.49 (m), 3.36–3.31 (m), 3.18–3.14 (m), 2.92–2.88 (m), 2.78–2.73 (m), 2.12–1.96 (m), 0.98 ppm (m, 4H); UV/Vis and fluorescence (DMF): $\lambda_{max} = 375$ (abs), 416 (em), 438 nm (sh).

Polymer C: **Polymer 5** (0.21 g) was dissolved in a mixture of dry CH_2Cl_2 (5 mL) and CH_3OH (20 mL), and then solution of CH_3ONa (0.5 M) in CH_3OH (1 mL) was added. The reaction mixture was stirred at room temperature for 30 h. The polymer was purified by using the same procedure described for **polymer B** to give **polymer C** (0.14 g) as a brown sticky oil. ^1H NMR (400 MHz, D_2O): $\delta = 7.45$–6.8 (m), 4.74–4.58 (m, D_2O + mannose), 4.0–3.11 (m), 2.68 (m), 1.98 (m), 1.12–0.9 ppm (m); **polymer C** is highly soluble in water; UV/Vis and fluorescence (0.1 M phos-

phate buffer with pH 7.4): $\lambda_{max}=380$ (abs), 426 (em), 440 nm (sh); $\phi=45\,\%$ in 0.1 M phosphate buffer (pH 7.4).

Acknowledgements

We would like to graciously thank Professor P. E. Orndorff for his kind donation of the ORN 178 and ORN 208 strains. The authors greatly acknowledge Research Excellence Fund of Michigan Technological University and 21st Century Jobs Fund of Michigan (contract number: 06-1-P1-0283) for support of the work. This project was also partially supported by National Research Initiative Grant no. 2007-35603-17740 from the USDA Cooperative State Research, Education, and Extension Service—Nanoscale Science and Engineering for Agriculture and Food Systems.

[1] H. Lis, N. Sharon, *Chem. Rev.* **1998**, *98*, 637–674.
[2] M. Mammen, S. K. Choi, G. M. Whitesides, *Angew. Chem.* **1998**, *110*, 2908–2953; *Angew. Chem. Int. Ed.* **1998**, *37*, 2754–2794.
[3] R. Jelinek, S. Kolusheva, *Chem. Rev.* **2004**, *104*, 5987–6015.
[4] Z. Juan, T. M. Swager in *Poly(arylene ethynylene)s in Chemosensing and Biosensing, Vol. 177*, Springer, Berlin, **2005**, pp. 151–179.
[5] U. H. F. Bunz, *Chem. Rev.* **2000**, *100*, 1605–1644.
[6] D. T. McQuade, A. E. Pullen, T. M. Swager, *Chem. Rev.* **2000**, *100*, 2537–2574.
[7] B. Liu, G. C. Bazan, *Chem. Mater.* **2004**, *16*, 4467–4476.
[8] M. R. Pinto, K. S. Schanze, *Synthesis* **2002**, 1293–1309.
[9] S. W. Thomas, G. D. Joly, T. M. Swager, *Chem. Rev.* **2007**, *107*, 1339–1386.
[10] I. B. Kim, J. N. Wilson, U. H. F. Bunz, *Chem. Commun.* **2005**, 1273–1275.
[11] M. D. Disney, J. Zheng, T. M. Swager, P. H. Seeberger, *J. Am. Chem. Soc.* **2004**, *126*, 13343–13346.
[12] M. G. Baek, R. C. Stevens, D. H. Charych, *Bioconjugate Chem.* **2000**, *11*, 777–788.
[13] C. Xue, F. T. Luo, H. Y. Liu, *Macromolecules* **2007**, *40*, 6863–6870.
[14] C. H. Xue, S. P. Jog, P. Murthy, H. Y. Liu, *Biomacromolecules* **2006**, *7*, 2470–2474.
[15] I. B. Kim, B. Erdogan, J. N. Wilson, U. H. F. Bunz, *Chem. Eur. J.* **2004**, *10*, 6247–6254.
[16] C. H. Xue, V. R. R. Donuru, H. Y. Liu, *Macromolecules* **2006**, *39*, 5747–5752.
[17] A. Takasu, K. Iso, T. Dohmae, T. Hirabayashi, *Biomacromolecules* **2006**, *7*, 411–414.
[18] I.-B. Kim, A. Dunkhorst, U. H. F. Bunz, *Langmuir* **2005**, *21*, 7985–7989.
[19] H. Connell, W. Agace, P. Klemm, M. Schembri, S. Marild, C. Svanborg, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 9827–9832.
[20] J. Bouckaert, J. Mackenzie, J. L. de Paz, B. Chipwaza, D. Choudhury, A. Zavialov, K. Mannerstedt, J. Anderson, D. Pierard, L. Wyns, P. H. Seeberger, S. Oscarson, H. De Greve, S. D. Knight, *Mol. Microbiol.* **2006**, *61*, 1556–1568.
[21] J. N. Demasa, G. A. Crosby, *J. Phys. Chem.* **1971**, *75*, 991–1024.
[22] C. H. Xue, Z. Chen, F. T. Luo, K. Palaniappan, D. J. Chesney, J. Liu, J. Chen, H. Y. Liu, *Biomacromolecules* **2005**, *6*, 1810–1815.
[23] G. B. Africa, G. L. Juan, I. G. Joaquin. , O. C. Fernando, U. Clara, V. B. Antonio, S. G. Francisco, *Synthesis* **2001**, 1057–1064.
[24] C. Grandjean, C. Rommens, H. Gras-Masse, O. Melnyk, *Tetrahedron Lett.* **1999**, *40*, 7235–7238.

Received: September 11, 2008
Published online: January 13, 2009

