Facile, Versatile Prepolymerization and Postpolymerization Functionalization Approaches for Well-Defined Fluorescent Conjugated Fluorene-Based Glycopolymers

Cuihua Xue, Venkat R. R. Donuru, and Haiying Liu*

Department of Chemistry, Michigan Technological University, Houghton, Michigan 49931

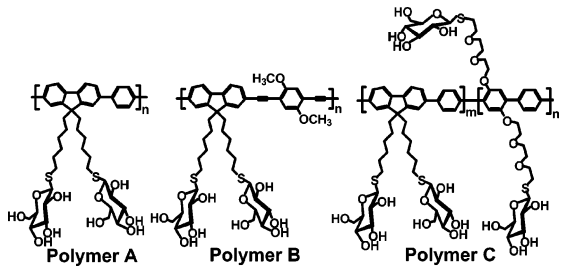
Received February 26, 2006; Revised Manuscript Received June 9, 2006

ABSTRACT: We have developed facile, versatile prepolymerization and postpolymerization functionalization approaches to prepare well-defined fluorescent conjugated fluorene-based glycopolymers (polymers **A** and **B**) through thioether formation. Well-defined glucose-carrying monomer (**5**) and conjugated polymer (polymer **2**) were prepared by coupling the key precursors, 2,7-diiodo-9,9-bis(6′-bromohexyl)fluorene (**2**) and poly[(9,9-bis-(6′-bromohexyl)-2,7-fluorenylene)-*alt*-1,4-phenylene] (polymer **1**) with an excess of 1-thio-*β*-D-glucose tetraacetate (**3**) in a basic condition, respectively. A well-defined poly(2,7-fluorenylene−ethynylene)-*alt*-1,4-(2,5-dimethyoxyphenylene−ethynylene) bearing peracetylated glucose residues (polymer **3**) was prepared by a palladium-catalyzed Sonogashira coupling polymerization of monomer **5** with a diethynylbenzene in the presence of 5% CuI, 5% $Pd(PPh_3)_2Cl_2$ and 10% PPh_3. Polymers **2** and **3** were deacetylated under Zemplén conditions in methanol and methylene chloride containing sodium methoxide, affording two fluorene-based conjugated glycopolymers **A** and **B**, respectively. Polymer **A** was also obtained by the reaction of polymer **1** with 1-thio-*β*-D-glucose sodium salt hydrate (**4**) in a basic condition. The composition and purity of the products were reliably analyzed by ^1H NMR. Water-soluble fluorene-based conjugated copolymer bearing glucose pendants with tri(ethylene glycol) tethers (polymer **C**) was prepared by the postpolymerization functionalization approach. This thioether formation reaction offers a very effective conjugation method to covalently attach protected or unprotected carbohydrate residues to fluorescent conjugated polymers.

Introduction

Protein−carbohydrate interactions are involved in a wide variety of cellular recognition processes including cell growth regulation, differentiation, adhesion, cancer cell metastasis, cellular trafficking, inflammation by bacteria and viruses, and the immune response.[1] However, since it is known that individual carbohydrate−protein interactions are generally weak,[2] multivalent forms of carbohydrate ligands, either glycopolymers or glycodendrimers, have been employed to demonstrate that inhibitory potencies of glycosides are enhanced through cooperative multiple interactions over their monovalent counterparts although the levels of enhancement vary.[3−5] Fluorescent conjugated glycopolymers combining scaffolding and reporting functions into one package are very attractive for study of protein−carbohydrate interactions due to their intrinsic fluorescence and their high sensitivity to minor external stimuli via amplification by a cooperative system response.[6−11] A few fluorescent conjugated glycopolymers such as poly(*p*-phenylene−ethynylene) (PPE) and polythiophene derivatives have been reported.[6−8,12] PPEs bearing α-mannose or *β*-glucose residues have been prepared by polymerizing well-defined carbohydrate-carrying monomers.[8,12] An alternative synthetic method has been used to prepare PPEs bearing α-mannose or α-galactose residues and polythiophene bearing α-mannose or sialic acid residues by postpolymerization functionalization of carboxylic acid-bearing PPE and polythiophene via an amidation reaction with amine-bearing carbohydrates.[6,7] The postpolymerization functionalization is generally advantageous because it provides a versatile approach to rapidly attach a variety of carbohydrates to conjugated polymers and to easily control a functionalization degree of carbohydrates along the polymer chain. However, incomplete reactions with the functional groups along the polymer chain may result in polymers with undefined structures.[8] Therefore, it is important to explore facile and versatile approaches to prepare fluorescent conjugated glycopolymers with well-defined structures.

Scheme 1. Chemical Structures of Conjugated Fluorene-Based Glycopolymers

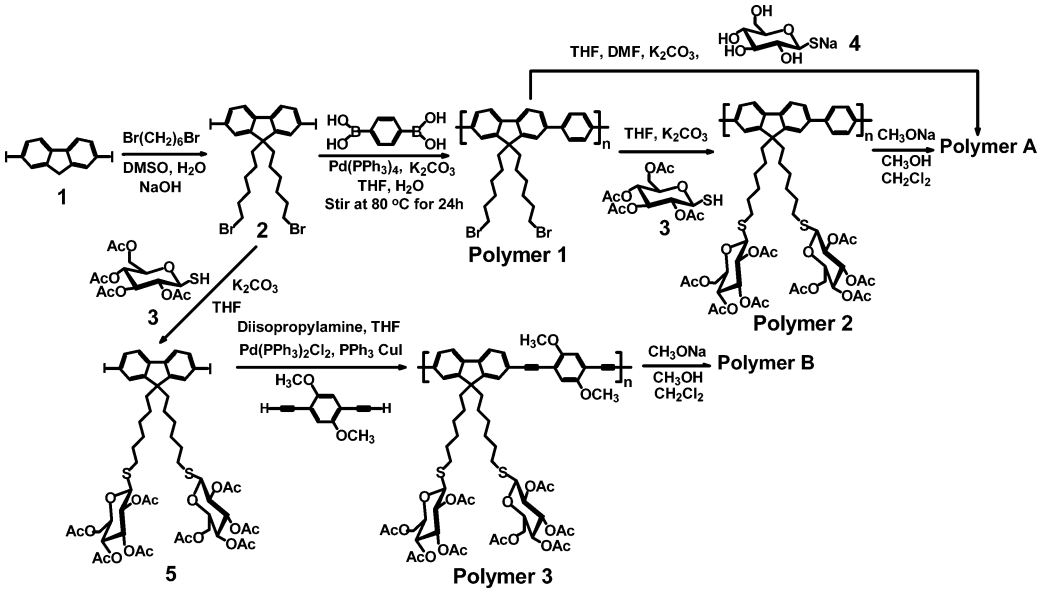


In this paper, we present facile, convenient, versatile prepolymerization and postpolymerization functionalization methods to prepare glucose-bearing conjugated fluorene copolymers with well-defined structures (Scheme 1). We choose fluorene-based conjugated polymers because of their high efficiencies both in photoluminescence and in electroluminescence.[13−15] The key precursors for the preparation of well-defined glucose-carrying monomer **5** and conjugated polymer **A** are 2,7-diiodo-9,9-bis-(6′-bromohexyl)fluorene and poly[(9,9-bis(6′-bromohexyl)-2,7-fluorenylene)-*alt*-1,4-phenylene] (polymer **1**) due to highly efficient reaction of their good leaving bromide groups with 1-thio-*β*-D-glucose tetraacetate (**3**) or 1-thio-*β*-D-glucose sodium salt (**4**) in a basic condition (Scheme 2). Polymer **1** was prepared through the Suzuki coupling polymerization of 2,7-diido-9,9-bis(6′-bromohexyl)fluorene and 1,4-phenyldiboronic acid with $Pd(PPh_3)_4$ as catalyst in a basic condition.[13,16] Well-defined poly-(2,7-fluorenylene−ethynylene)-*alt*-1,4-(2,5-dimethyoxyphe-

* Corresponding author. E-mail: hyliu@mtu.edu.

10.1021/ma060422e CCC: $33.50
Published on Web 07/28/2006

Scheme 2. Synthetic Route to Fluorene-Based Polymers Bearing Glucose Pendants



nylene−ethynylene) bearing peracetylated glucose residues (polymer **3**) was prepared by a palladium-catalyzed Sonogashira coupling polymerization of monomer **5** with a diethynylbenzene in the presence of 5% CuI, 5% $Pd(PPh_3)_2Cl_2$ and 10% PPh_3 in THF solution,[17−19] and sequentially deacetylated under Zemplén conditions,[20] resulting in glucose-bearing polymer **B** (Schemes 1 and 2). ^{1}H NMR analysis of the products proves that the functionalizations are 98% complete, and no side reactions occur. These prepolymerization and postpolymerization functionalization methodologies can be easily extended to the synthesis of well-defined fluorescent conjugated polymers bearing a variety of different carbohydrates. Water solubility of neutral conjugated glycopolymer (polymer **C**) can be significantly enhanced by using hydrophilic tri(ethylene glycol) as tethered spacers between the polymer backbone and glucose residues.

Experimental Section

Instrumentation. ^{1}H NMR and ^{13}C NMR spectra were taken on 400 MHz Varian Unity Inova spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$; chemical shifts (δ) are given in ppm relative to solvent peaks (^{1}H: δ 7.26; ^{13}C: δ 77.3) as internal standard. UV spectra were taken on a Hewlett-Packard 8452A diode array UV−vis spectrophotometer. Fluorescence spectra were recorded on a Spex Fluorolog 1681 0.22 m steady-state fluorometer. Fluorescence quantum yields of the polymers were measured in dilute chloroform, DMSO, or phosphate buffer solution, and calculated by using quinine sulfate in 0.1 N sulfuric acid as the reference absolute quantum efficiency (ϕ_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 and solvents were obtained from commercial suppliers (Aldrich, Sigma, Fluka, Acros Organics, Fisher Scientific, Lancaster) and were used without further purification. Air- and moisture-sensitive reactions were conducted in oven-dried glassware using standard Schlenk line or drybox techniques under an inert atmosphere of dry nitrogen. 1,4-(2,5-Dimethyoxyphenylene−ethynylene), 2,7-diiodofluorene (**1**), and 2,5-diiodo-1,4-bis{2-2-[(2-hydroxyethoxy)ethoxy]ethoxy}-ethoxy}benzene (**6**) were prepared and characterized according to reported procedures.[17,22,23]

2,7-Diiodo-9,9-bis(6′-bromohexyl)fluorene (2). To a mixture of 2,7-diiodofluorene (**1**) (5.0 g, 11.96 mmol) and catalyst amount of tetrabutylammonium iodide, 50 mL of DMSO (dimethyl sulfoxide) and 50 mL of 50% aqueous NaOH were added to a solution of 1,6-dibromohexane (17.51 g, 71.77 mmol) in 50 mL of DMSO. The reaction mixture was stirred for 24 h at room temperature and then poured into dilute hydrochloric acid. After the aqueous layer was extracted by using methylene chloride, the combined organic layer was washed with water and brine and then dried over $MgSO_4$. Removal of the solvent under vacuum followed by column chromatography using chloroform/hexane as a mobile phase produced a white solid (8.01 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66−7.64 (m, 4H), 7.41−7.39 (m, 2H), 3.28 (t, J = 6.8 MHz, 2H), 1.91−1.87 (m, 4H), 1.69−1.62 (m, 4H), 1.21−1.15 (m, 4H), 1.08−1.04 (m, 4H), 0.61−0.55 (m, 4H) ppm. ^{13}C NMR (400 MHz, $CDCl_3$): δ 152.35, 139.98, 136.44, 132.17, 121.87, 93.60, 55.64, 40.17, 34.19, 32.86, 29.18, 27.99, 23.71 ppm.

Polymer 1. 2,7-Diiodo-9,9-bis(6′-bromohexyl)fluorene (1.0 g, 1.34 mmol), 1,4-phenyldiboronic acid (1.25 g, 1.51 mmol), $Pd(PPh_3)_4$ (16 mg), and potassium carbonate (1.85 g, 13.4 mmol) were put in a 100 mL three-necked round-bottom flask under a nitrogen atmosphere. A degassed solution of water (10 mL) and THF (20 mL) was added to the flask, and the reaction mixture was stirred at 80 °C for 24 h. When the solvent was removed, the residue was dissolved in methylene chloride and washed with water (3 × 100 mL). The organic layer was collected, dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated and precipitated by addition to methanol. The resulting polymer was collected by filtration, further purified by extraction in a Soxhlet extractor with refluxing acetone for 2 days, and dried under a vacuum overnight to afford a light yellow solid (0.56 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81−7.45 (m, 10H), 3.25−3.10 (t, 4H), 2.09 (m, 4H), 1.66−1.63 (m, 4H), 1.24−1.12 (m, 8H), 0.78 (m, 4H) ppm. GPC (THF, polystyrene standard): M_n = 20 500 g/mol; polydispersity = 1.75. Polymer **1** displays absorption maxima at 364 nm and emission maxima at 408 nm with a vibronic shoulder peak at 428 nm in chloroform solution.

Polymer 2. Polymer **1** (0.2 g), 1-thiol-β-D-glucose tetraacetrate (**3**) (0.40 g, 1.09 mmol), and potassium carbonate (0.50 g, 3.62 mmol) were put in a 100 mL round-bottom flask under a nitrogen atmosphere. Degassed THF (50 mL) was added to the flask, and the reaction mixture was stirred at room temperature for 24 h. After

the solvent was removed under reduced pressure, methylene chloride (50 mL) was added to the residue. The organic phase was washed with water (3 × 100 mL) and dried over anhydrous $MgSO_4$. The filtrate was concentrated and poured into methanol to precipitate the polymer. The polymer was filtered, washed with methanol, and dried under vacuum for overnight to give a yellow solid (0.35 g, 91% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.8 (m, 5H), 7.7.62−7.67 (m, 5H), 5.16 (m, 2H), 4.95−5.02 (m, 4H), 4.38 (m, 2H), 4.16 (m, 2H), 4.06 (m, 2H), 3.61 (m, 2H), 2.52 (m, 4H), 1.97−2.06 (m, 24H), 1.61 (m, 4H), 1.40 (m, 4H), 1.12 (m, 8H), 0.73 (m, 4H) ppm. Polymer **2** displays absorption maxima at 364 nm and emission maxima at 408 nm with a vibronic shoulder peak at 428 nm in chloroform solution.

Polymer A. Polymer **2** (0.2 g) was added to a solution of dry CH_2Cl_2 (5 mL) and CH_3OH (10 mL) under a nitrogen atmosphere and followed by 0.5 M CH_3ONa solution (1 mL). The reaction mixture was stirred at room temperature for 24 h. After removal of the solvent under reduced pressure, 10 mL of water was added to the residue. The resulting solution was put in a cellulose dialysis tube (cutoff 12 000), dialyzed against water for 2 days (10 water changes), and lyophilized to give a yellow solid (0.13 g, 92.8%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.83−7.72 (m, 10H), 4.92−4.96 (m, 4H), 4.84 (m, 2H), 4.37 (m, 2H), 4.11−4.14 (m, 2H), 3.58 (m, 2H), 3.36−3.28 (m, 4H), 3.01−3.06 (m, 4H), 2.91 (m, 2H), 2.48 (m), 2.18 (m, 4H), 1.32 (m, 4H), 1.08 (m, 8H), 0.62 (m, 4H) ppm. Polymer **A** displays absorption maxima at 370 nm and emission maxima at 416 nm with a vibronic shoulder peak at 438 nm in DMSO solution (Figure 5) and an absorption maximum peak at 380 nm. Polymer **A** was also prepared by postpolymerization functionalization of polymer **1** with 1-thiol-β-D-glucose salt hydrate (**4**) as follows: polymer **1** (0.2 g), 1-thiol-β-D-glucose salt hydrate (**4**) (0.50 g), and potassium carbonate (0.65 g) were put in a 100 mL round-bottom flask. A degassed solution of THF (10 mL) and DMF (60 mL) was added to the flask, and the reaction mixture was stirred at room temperature for 3 days. The resulting mixture was put in a cellulose dialysis tube (cutoff 12 000), dialyzed against water for 3 days to remove THF, DMF, and free 1-thiol-β-D-glucose, and lyophilized to give polymer **A**. ^{1}H NMR spectral data of polymer **A** obtained by functionalization of polymer **1** with 1-thiol-β-D-glucose salt hydrate (**4**) are the exact same as those obtained by functionalization of polymer **1** with 1-thiol-β-D-glucose tetraacetrate (**3**) in a basic condition and subsequent deacetylation under Zemplén conditions.

Monomer 5. 2,7-Diiodo-9,9-bis(6′-bromohexyl)fluorene (1.0 g, 1.34 mmol), 1-thiol-β-D-glucose tetraacetrate (**3**) (1.50 g, 4.12 mmol), and potassium carbonate (5.0 g, 36.2 mmol) were put in a 100 mL round-bottom flask in a nitrogen atmosphere. Degassed THF (50 mL) was added to the reaction flask, and the resulting mixture was stirred at room temperature for 24 h. After removal of the solvent, the residue was dissolved in methylene chloride (50 mL), washed with water (3 × 100 mL), and dried over anhydrous $MgSO_4$. After the solvent was removed under a reduced pressure, the crude product was purified by column chromatography on silica gel with hexane/EtOAc (10:1) to give the target compound (1.61 g, 91.5% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.59−7.64 (m, 4H), 7.38 (m, 2H), 5.16 (m, 2H), 4.93−5.05 (m, 4H), 4.39 (m, 2H), 4.17 (m, 2H), 4.08 (m, 2H), 3.62 (m, 2H), 2.51−2.54 (m, 4H), 1.96−2.01 (m, 24H), 1.84−1.88 (m, 4H), 1.38 (m, 4H), 1.03−1.10 (m, 8H), 0.52 (m, 4H) ppm. ^{13}C NMR (400 MHz, $CDCl_3$): δ 170.79, 170.36, 169.59, 169.53, 152.36, 139.94, 136.38, 132.10, 121.81, 93.38, 83.77, 76.01, 74.08, 70.06, 68.51, 62.34, 55.61, 40.25, 30.05, 29.61, 29.53, 28.55, 23.75, 20.94, 20.91, 20.81, 20.78 ppm.

Polymer 3. Monomer **5** (0.6 g, 0.46 mmol), 1,4-(2,5-dimethyoxyphenylene−ethynylene) (0.09 g, 0.48 mmol), $Pd(PPh_3)_2Cl_2$ (0.02 g, 0.03 mmol), CuI (0.01 g, 0.05 mmol), and PPh_3 (0.03 g, 0.11 mmol) were put in round-bottom flask in a nitrogen atmosphere. A degassed solution of diisopropylamine (10 mL) and THF (20 mL) was added to the flask, and the reaction mixture was stirred at 45 °C for 24 h. After removal of the solvent, the residue was dissolved in CH_2Cl_2, washed with water (3 × 100 mL), and dried over anhydrous $MgSO_4$. The resulting filtrate was concentrated and poured into methanol to precipitate the polymer. The solid was filtered, washed with methanol, and dried under vacuum for 24 h to give the polymer **3** as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.67 (m, 2H), 7.51−7.55 (m,2H), 7.35−7.37 (m, 2H), 7.10 (m, 2H), 5.16 (m, 2H), 4.93−5.00 (m, 4H), 4.38−4.40 (m, 2H), 4.17 (m, 2H), 4.05−4.08 (m, 2H), 3.96 (s, 3H), 3.91 (s, 3H), 3.63 (m, 2H), 2.53 (m, 4H), 1.97−2.02 (m, 24H), 1.54 (m, 4H), 1.40 (m, 4H), 1.09 (m, 8H), 0.58 ppm (m, 4H). GPC (THF, polystyrene standard): M_n = 28 600 g/mol; polydispersity = 2.14. Polymer **3** displays absorption maxima at 430 nm and emission maxima at 457 nm in chloroform solution.

Polymer B. Polymer **3** (0.2 g) was added to a solution of dry CH_2Cl_2 (10 mL) and CH_3OH (10 mL) and followed by 0.5 M CH_3ONa solution (1 mL). The reaction mixture was stirred at room temperature for 24 h. After removal of the solvent under a reduced pressure, 5 mL of water was added to the residue. The resulting solution was dialyzed against water for 2 days and lyophilized to give a yellow solid (0.13 g, 90%). Polymer **B** is slightly soluble in DMSO and DMF, and insoluble in solvents such as $CHCl_3$ and H_2O, so we were not able to obtain satisfactory NMR data. It exhibits an absorption maximum peak at 450 nm and an emission maximum peak at 508 nm with a vibronic shoulder peak at 512 nm in DMSO solution.

Monomer 7. Bromine (1.02 g, 6.37 mmol) was added slowly to CH_3CN solution (15 mL) containing triphenylphosphine (1.67 g, 6.37 mmol) at 0 °C under a nitrogen atmosphere. Compound **6** (2.0 g, 3.19 mmol) dissolved in CH_3CN (15 mL) was added dropwise to the mixed solution. After the reaction mixture was stirred for 48 h at room temperature, the solvent was removed, and the residue was dissolved in ethyl acetate (EtOAc) and washed with saline solution. The organic layer was collected and dried over anhydrous $MgSO_4$. After removal of the solvent, the residue was purified by column chromatography on silica (EtOAc/hexane = 5/1) to give the target compound as a white solid (1.7 g, 71%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.22 (s, 2H), 4.09 (t, J = 4.8 Hz, 4H), 3.86 (t, J = 4.8 Hz, 4H), 3.79 (t, J = 6.4 Hz, 4H), 3.76 (t, J = 2.4 Hz, 4H), 3.68 (t, J = 2.4 Hz, 4H), 3.45 (t, J = 6.4 Hz, 4H) ppm. ^{13}C NMR (400 MHz, $CDCl_3$): δ 153.33, 123.70, 86.65, 71.49, 71.37, 70.88, 70.53, 69.91, 30.63 ppm.

Polymer 4. 2,7-Diiodo-9,9-bis(6′-bromohexyl)fluorene (0.279 g, 0.375 mmol), 1,4-phenyldiboronic acid (0.125 g, 0.75 mmol), compound **7** (0.282 g, 0.375 mmol), potassium carbonate (1.25 g, 9.06 mmol), and $Pd(PPh_3)_4$ (8 mg) were added to a 100 mL flask under a nitrogen atmosphere. A degassed solution of water (10 mL) and THF (20 mL) was added to the flask, and the reaction mixture was stirred at 80 °C for 24 h. After the solvent was removed in a reduced pressure, the residue was dissolved in methylene chloride, washed with water, and dried over anhydrous $MgSO_4$. The filtrate was concentrated and poured into methanol to precipitate the polymer. The polymer was collected by filtration, further purified by washing with refluxing ethanol in a Soxhlet extractor for 2 days, and dried under vacuum overnight to afford a light yellow solid (0.41 g). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80−7.39 (m, 14H), 7.12 (s, 2H), 4.17−4.09 (m, 4H), 3.82−3.76 (m, 8H), 3.64−3.59 (m, 8H), 3.41 (t, 4H), 3.28−3.26 (t, 4H), 2.08 (m, 4H), 1.64 (m, 4H), 1.24−1.22 (m, 8H), 0.76 ppm (m, 4H). GPC (THF, polystyrene standard): M_n = 24 600 g/mol; polydispersity = 1.86. Polymer **4** displays absorption maxima at 360 nm and emission maxima at 408 nm with a vibronic shoulder peak at 432 nm in chloroform solution.

Polymer C. Polymer **4** (0.1 g), 1-thio-β-D-glucose sodium salt hydrate (0.30 g), and K_2CO_3 (1.0 g) were added to a 50 mL round-bottom flask under a nitrogen atmosphere. A degassed solution of THF (6 mL) and DMF (30 mL) was added to the flask, and the reaction mixture was stirred at room temperature for 48 h. The resulting solution was put in a cellulose dialysis tube (cutoff 12 000), dialyzed against water for 3 days, and lyophilized to give polymer **C** (0.12 g, 85.7%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.86−7.38 (m, 14H), 7.18 (s, 2H), 4.96−4.83 (m, 12H), 4.43−4.37 (m, 4H), 4.24−4.12 (m, 8H), 3.76−3.56 (m, 20H), 3.36

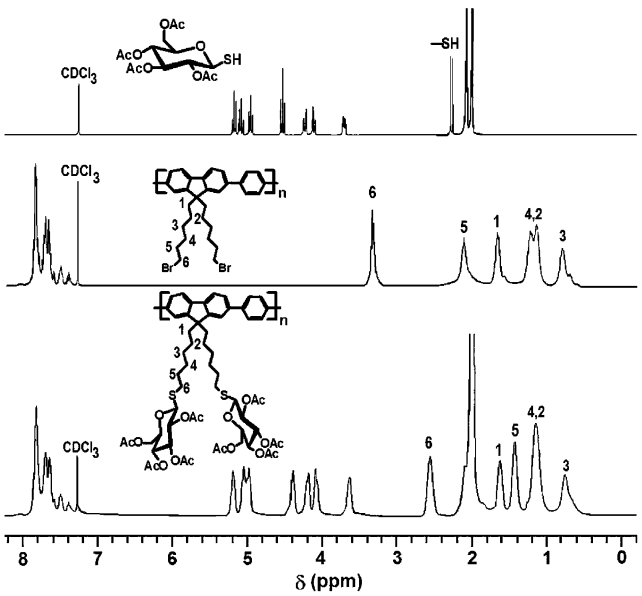


Figure 1. ^{1}H NMR spectra of 1-thio-β-D-glucose tetraacetate (**3**) and polymers **1** and **2** in $CDCl_3$ solution.

(m, 4H), 3.28 (m, 4H), 3.07−3.01 (m, 8H), 2.91 (m, 4H), 2.48 (m), 2.10 (m, 4H), 1.29 (m, 4H), 1.17−1.05 (m, 8H), 0.59 ppm (m, 4H). Polymer **C** displays absorption maxima at 360 nm and emission maxima at 414 nm in phosphate buffer solution (pH 7.2).

Results and Discussion

We explore prepolymerization and postpolymerization functionalization approaches to prepare well-defined fluorescent conjugated fluorene-based polymers bearing glucose residues. A copolymer (polymer **A**) is based on the alternating fluorene and phenylene backbone (Scheme 1), which represents an example of neutral conjugated fluorene copolymer bearing glucose pendants and exhibits efficient blue light emission. We use a synthetic strategy of postpolymerization functionalization since the polymerization for poly(2,7-fluorenylene-*alt*-1,4-phenylene) is usually achieved at high temperature.[13,24] Monomer **2** was obtained by alkylation of 2,7-didofluorene (**1**) with 6 equiv of dibromohexane in a mixed solution of DMSO and water containing 50% NaOH at room temperature for 24 h. An alternating copolymer, poly[(9,9′-bis(6′-bromohexyl)-2,7-fluorenylene)-*alt*-1,4-phenylene] (polymer **1**), was synthesized by the palladium-catalyzed Suzuki coupling reaction of monomer **2** with 1,4-phenylenediboronic acid in excellent yield and with a high degree of polymerization, according to gel permeation chromatography (yield 85%, M_n = 20 500 g/mol; polydispersity = 1.75 by gel permeation chromatography).[13,16] Its derivative bearing peracetylated glucose residues (polymer **2**) was obtained through a postpolymerization treatment on the terminal bromide groups with 1-thio-β-D-glucose tetraacetate in a basic condition at room temperature. ^{1}H NMR spectra of polymer **1** show that methylene groups adjacent to bromide atoms in polymer **1** display the signal peaks around 3.25−3.10 ppm (Figure 1). Treatment of polymer **1** with thio-β-D-glucose tetraacetate caused the signal peaks corresponding to these methylene groups shift to the higher field region around 2.50 ppm (Figure 1), indicating 98% complete formation of thioether linkage in polymer **2**. This postpolymerization functionalization method can be used to quantitatively introduce carbohydrate residues to fluorescent conjugated polymers. Polymer **A** was prepared by sequentially deacetylating polymer **2** under Zemplén conditions in methanol and methylene chloride containing sodium methoxide at room temperature (Scheme 2). Polymer **A** was also easily obtained by the reaction of polymer **1** with 1-thio-β-D-glucose sodium salt hydrate in mixed solution of THF and DMF in the presence of K_2CO_3. ^{1}H NMR spectra of polymer **A** obtained by both methods are the same (Figure 2). This thioether forming reaction offers a very effective conjugation method to functionalize fluorescent conjugated polymers with protected or unprotected carbohydrate residues (Scheme 2).

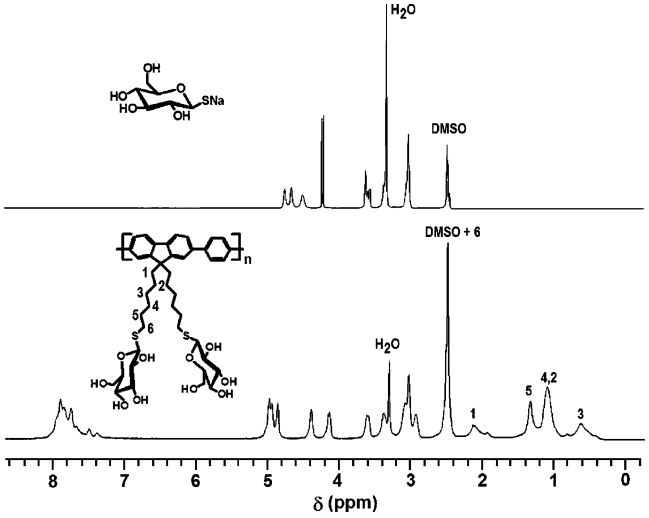


Figure 2. ^{1}H NMR spectra of polymer **A** and 1-thio-β-D-glucose sodium salt hydrate (**4**) in DMSO-d_6 solution.

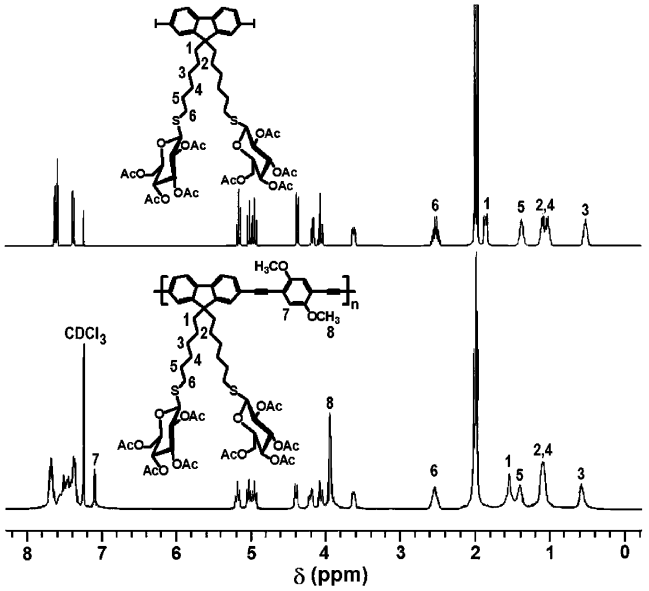


Figure 3. ^{1}H NMR spectra of monomer **5** and polymer **3** in $CDCl_3$ solution.

To conjugate glucose groups to the backbone of poly(2,7-fluorenylene−ethynylene)-*alt*-(1,4-phenylene−ethynylene), we developed a synthetic strategy based on the use of the intermediate 2,7-diido-9,9-bis(6′-bromohexyl)fluorene (**2**). 2,7-Diiodofluorene derivative bearing peracetylated glucose pendants (**5**) was obtained by a reaction of 2,7-diido-9,9-bis(6′-bromohexyl)fluorene (**2**) with 1-thio-β-D-glucose tetraacetate (**3**) in the presence of K_2CO_3 at room temperature for 24 h (Scheme 2). Well-defined poly(2,7-fluorenylene−ethynylene)-*alt*-1,4-(2,5-dimethyoxyphenylene−ethynylene) bearing peracetylated glucose residues (polymer **3**) was prepared by a palladium-catalyzed Sonogashira coupling polymerization of a well-defined glucose-bearing monomer (**5**) with a diethynylbenzene in the presence of 5% CuI, 5% $Pd(PPh_3)_2Cl_2$ and 10% PPh_3.[10,17,18,25−27] Figure 3 shows ^{1}H NMR spectra of monomer **5** and polymer **3**

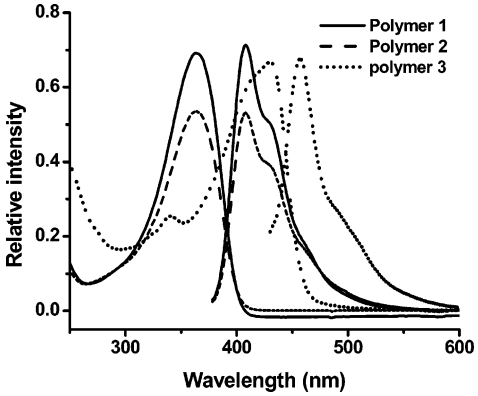


Figure 4. UV and fluorescence spectra of polymers **1**−**3** in chloroform solution. Excitation wavelengths are 364 nm for polymers **1** and **2** and 430 nm for polymer **3**.

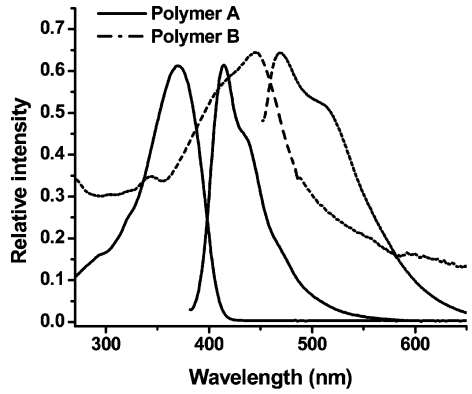


Figure 5. UV and fluorescence spectra of polymers **A** and **B** in DMSO solution.

in $CDCl_3$ solution. These data clearly indicate that well-defined fluorescent conjugated glycopolymer can also be prepared by polymerizing well-defined carbohydrate-bearing monomers. Polymer **B** was obtained by sequentially deacetylating polymer **3** under Zemplén conditions in methanol and methylene chloride containing sodium methoxide (Scheme 2).

The solubility of the glycopolymers is different from its precursor. The precursor polymer **1** is readily soluble in common solvents such as THF, chloroform, and methylene chloride, and moderately soluble in DMF and DMSO, but insoluble in ethanol, methanol, acetone, and water. Polymer **2** is readily soluble in common solvents such as THF, chloroform, methylene chloride, toluene, DMF, and DMSO but insoluble in water. Polymer **3** displays the similar solubility to polymer **1**. Polymer **A** is readily soluble in DMF and DMSO and slightly soluble in water. Polymer **B** is slightly soluble in DMF and DMSO but insoluble in water. The insolubility of polymer **B** in water is similar to that of poly(*p*-phenylene−ethynylene)s bearing glucose pendants,[12] which may be due to strong $\pi-\pi$ stacking interactions between the polymer backbone.

The precursor polymer **1** displays absorption maximum peak at 364 nm and emission maximum peak at 408 nm with a vibronic shoulder peak at 428 nm in chloroform solution, which were ascribed to the $\pi-\pi^*$ transition of the conjugated polymer backbone (Figure 4). Polymer **2** bearing peracetylated glucose residues shows the similar absorption and emission maximum peaks to the precursor polymer **1** in chloroform solution (Figure 4). Polymer **3** displays significant red shifts in absorption and emission compared with polymer **2** since it shows an absorption maximum peak at 430 nm and an emission maximum peak at 457 nm in chloroform. The red shifts are due to enhanced conjugation of poly(2,7-fluorenylene−ethynylene)-*alt*-1,4-(2,5-dimethyoxyphenylene−ethynylene) backbone and electron-donating effect from the dimethyoxy groups.[26, 27]

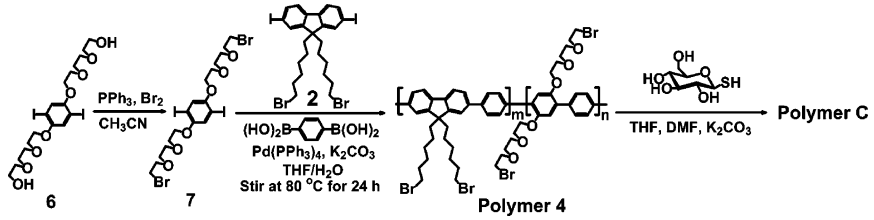
Polymer **A** exhibits an absorption maximum peak at 370 nm and an emission maximum peak at 416 nm with a vibronic shoulder peak at 438 nm in DMSO solution (Figure 5). The red shift in both absorption and emission spectra for polymer **A** with increased polarity can be due to enhanced planar conformation or aggregation of polymer main chain in the polar solvent. Polymer **B** exhibits an absorption maximum peak at 450 nm and an emission maximum peak at 508 nm with a vibronic shoulder peak at 512 nm in DMSO solution (Figure 5). Polymer **B** shows significant red shifts in absorption and emission compared with polymer **3** in DMSO solution, which arises from aggregation of polymer **B**, resulting in low fluorescent quantum yield of 12% (Table 1).

Fluorescent conjugated glycopolymers should be soluble in water for potential biosensing applications. Conventional strategies for making conjugated polymers water-soluble consist of introducing hydrophilic ionic side chains to the polymers to overcome $\pi-\pi$ stacking interactions between the hydrophobic polymer backbones via electrostatic repulsion and enhance enthalpic interactions with water. Ionic groups such as carboxylic, sulfonic, ammonium, or phosphonate have been employed on side chains to conjugated polymers.[11,13] Neutral poly(*p*-phenylene−ethynylene)s (PPEs) bearing carbohydrate pendants reported display low water solubility,[12] which might be due to strong $\pi-\pi$ stacking interactions of hydrophobic PPE backbones. Introduction of anionic groups such as carboxylic acid to PPE significantly enhanced water solubility of carbohydrate-bearing PPE.[28] However, the presence of ionic groups in conjugated polymers might cause interfering responses due to nonspecific electrostatic interactions in complicated biological samples.[28] To prevent potential nonspecific electrostatic interactions in biosensing applications, we prepare neutral water-soluble fluorescent conjugated glycopolymers by using hydrophilic

Table 1. Fluorescent Quantum Yield of Fluorene-Based Conjugated Polymers

polymer	**1**	**2**	**3**	**A**	**B**	**4**	**C**
fluorescent quantum yield (%)	47	50	48	47	12	62	40
solvent	$CHCl_3$	$CHCl_3$	$CHCl_3$	DMSO	DMSO	$CHCl_3$	phosphate buffer (pH 7.0)

Scheme 3. Synthetic Route to Water-Soluble Fluorene-Based Conjugated Glycopolymer



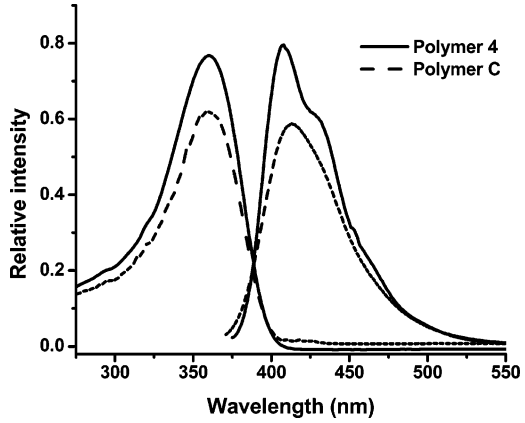


Figure 6. UV and fluorescence spectra of polymer **4** in chloroform and polymer **C** in phosphate buffer (pH 7.0) at an excitation wavelength of 350 nm.

oligo(ethylene glycol) as tethering spacers between polymer backbone and carbohydrates. To covalently attach glucose to conjugated polymer backbone through tri(ethylene glycol) tether, we developed a synthetic strategy based on the use of monomer **7**, which was obtained by bromination of 2,5-diiodo-1,4-bis-{2-2-[(2-hydroxyethoxy)ethoxy]ethoxy}ethoxy}benzene (**6**) in acetonitrile solution containing bromine and triphenylphosphine. Bromide-bearing fluorene-based copolymer (polymer **4**) was synthesized by the palladium-catalyzed Suzuki coupling of *p*-phenylenediboronic acid with monomers **7** and **2**. β-Glucose was conjugated to polymer **4** through the postpolymerization functionalization approach in a basic condition, affording water-soluble fluorene-based conjugated glycopolymer (polymer **C**) (Scheme 3). This approach not only renders conjugated glycopolymers soluble in water but also allows for control of orientation and density of carbohydrates along the conjugated polymers for study of multivalent effect on carbohydrate−protein interactions.

Polymer **4** shows absorption maxima at 360 nm and emission maxima at 408 nm with a vibronic shoulder peak at 432 nm in chloroform solution (Figure 6). It is highly fluorescent with fluorescent quantum yield of 62% in chloroform solution. Polymer **C** displays absorption maxima at 360 nm and emission maxima at 414 nm in phosphate buffer solution (pH 7.2) with high fluorescent quantum yield of 40% (Figure 6 and Table 1).

Conclusion

In conclusion, we have described facile, versatile prepolymerization and postpolymerization functionalization strategies to attach glucose residues to fluorene-based conjugated copolymers. These strategies use the reaction of thiols with bromide groups in the monomer and copolymers to form thiother bridges. This thioether formation reaction offers a very effective method to conjugate protected or unprotected glucose to fluorescent conjugated polymers. We believe this method could be of great utility in the preparation of conjugated glycopolymers bearing a variety of different carbohydrate pendants, as it should work equally well with any monosaccharide thiol substrate and oligosaccharides.

Acknowledgment. The authors greatly acknowledge Research Excellence Fund of Michigan Technological University for support of this work and United States Department of Agriculture for partial support of the work through National Research Initiative Competitive Grants Program.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra of intermediates and monomers. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Mammen, M.; Choi, S. K.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2755−2794.
(2) Lis, H.; Sharon, N. *Chem. Rev.* **1998**, *98*, 637−674.
(3) Roy, R. *Trends Glycosci. Glycotechnol.* **2003**, *15*, 291−310.
(4) Cloninger, M. J. *Curr. Opin. Chem. Biol.* **2002**, *6*, 742−748.
(5) Rockendorf, N.; Lindhorst, T. K. *Dendrimers IV* **2001**, *217*, 201−238.
(6) Baek, M. G.; Stevens, R. C.; Charych, D. H. *Bioconjugate Chem.* **2000**, *11*, 777−788.
(7) Disney, M. D.; Zheng, J.; Swager, T. M.; Seeberger, P. H. *J. Am. Chem. Soc.* **2004**, *126*, 13343−13346.
(8) Kim, I. B.; Wilson, J. N.; Bunz, U. H. F. *Chem. Commun.* **2005**, 1273−1275.
(9) McQuade, D. T.; Pullen, A. E.; Swager, T. M. *Chem. Rev.* **2000**, *100*, 2537−2574.
(10) Bunz, U. H. F. *Chem. Rev.* **2000**, *100*, 1605−1644.
(11) Pinto, M. R.; Schanze, K. S. *Synthesis-Stuttgart* **2002**, 1293−1309.
(12) Kim, I. B.; Erdogan, B.; Wilson, J. N.; Bunz, U. H. F. *Chem.—Eur. J.* **2004**, *10*, 6247−6254.
(13) Liu, B.; Bazan, G. C. *Chem. Mater.* **2004**, *16*, 4467−4476.
(14) Xu, Q. H.; Gaylord, B. S.; Wang, S.; Bazan, G. C.; Moses, D.; Heeger, A. J. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 11634−11639.
(15) Wang, S.; Gaylord, B. S.; Bazan, G. C. *J. Am. Chem. Soc.* **2004**, *126*, 5446−5451.
(16) Liu, M.; Kaur, P.; Waldeck, D. H.; Xue, C. H.; Liu, H. Y. *Langmuir* **2005**, *21*, 1687−1690.
(17) Xue, C. H.; Chen, Z.; Luo, F. T.; Palaniappan, K.; Chesney, D. J.; Liu, J.; Chen, J.; Liu, H. Y. *Biomacromolecules* **2005**, *6*, 1810−1815.
(18) Chen, Z.; Xue, C. H.; Shi, W.; Luo, F. T.; Green, S.; Chen, J.; Liu, H. Y. *Anal. Chem.* **2004**, *76*, 6513−6518.
(19) Ellison, S. T.; Gies, A. P.; Nonidez, W. K.; Xue, C. H.; Liu, H. Y. *Int. J. Polym. Anal. Charact.* **2005**, *10*, 245−258.
(20) Smith, E. A.; Thomas, W. D.; Kiessling, L. L.; Corn, R. M. *J. Am. Chem. Soc.* **2003**, *125*, 6140−6148.
(21) Demasa, J. N.; Crosby, G. A. *J. Phys. Chem.* **1971**, *75*, 991−1024.
(22) Khan, M. S.; Al-Mandhary, M. R. A.; Al-Suti, M. K.; Corcoran, T. C.; Al-Mahrooqi, Y.; Attfield, P. J.; Feeder, N.; David, W. I. F.; Shankland, K.; Friend, R. H.; Kohler, A. *New J. Chem.* **2003**, *27*, 140−149.
(23) Lee, S. H.; Nakamura, T.; Tsutsui, T. *Org. Lett.* **2001**, *3*, 2005−2007.
(24) Gaylord, B. S.; Heeger, A. J.; Bazan, G. C. *J. Am. Chem. Soc.* **2003**, *125*, 896−900.
(25) Xue, C. H.; Chen, Z.; Wen, Y.; Luo, F. T.; Chen, J.; Liu, H. Y. *Langmuir* **2005**, *21*, 7860−7865.
(26) Pschirer, N. G.; Byrd, K.; Bunz, U. H. F. *Macromolecules* **2001**, *34*, 8590−8592.
(27) Pschirer, N. G.; Bunz, U. H. F. *Macromolecules* **2000**, *33*, 3961−3963.
(28) Kim, I.-B.; Dunkhorst, A.; Bunz, U. H. F. *Langmuir* **2005**, *21*, 7985−7989.

MA060422E