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Synthesis and Characterization of Water-Soluble Conjugated Glycopolymer for Fluorescent Sensing of Concanavalin A

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Abstract: A neutral polyfluorene derivative that contains 20 mol% 2,1,3-benzothiadiazole (BT) is synthesized by Suzuki cross-coupling polymerization. A cationic conjugated polymer **A** and an α-mannose-bearing polymer **B** are subsequently obtained through different post-polymerization methods. As a result of the charged pendant groups or sugar-bearing groups attached to the polymer side chains, both **A** and **B** show good water-solubility. The titration of Concanavalin A (Con A) into polymer aqueous solution leads to different fluorescent responses for polymers **A** and **B**. Polymer **A** does not show any obvious fluorescence change upon interaction with Con A, whereas polymer **B** shows fluorescence increase in BT emission intensity when Con A is added. This is because of the specific interaction between α-mannose and Con A, which induces polymer aggregation, and then facilitates energy transfer from the phenylene–fluorene segments to the BT units. A practical calibration curve ranging from 1 nM to 250 nM is obtained by correlating the changes in BT emission intensity with Con A concentration. The advantage of polymer **B**-based Con A macromolecular probe is that it shows signal increase upon Con A recognition, which is significantly different from other conjugated polymer-based fluorescence quenching assays.

Keywords: benzothiadiazole · biosensors · click chemistry · FRET · polymers

Introduction

Concanavalin A (Con A), a lectin obtained from jack beans (*Canavalia ensiformis*),[1] is often used as a model protein to study sugar–protein interaction because it is less toxic than other lectins such as ricin, *Boulium* toxin and *Escherichia coli* (*E. Coli*) toxin. Because of its specific binding to both glucose and mannose-containing receptors, Con A plays a key role in cell adhesion and recognition of pathogens, such as viruses, bacteria, fungi, and protozoa.[2] It is also one of the most useful probes in studying cell carbohydrates and characterization of glycoproteins.[3] Individual carbohydrate–protein interaction is generally weak, which could be compensated by multiple cooperative interactions between the carbohydrate and proteins. One example is to attach many carbohydrate units to the polymer side chains and the resulting multivalent interactions between the carbohydrates and proteins have been demonstrated to be much stronger than the corresponding monovalent interaction.[4]

Conjugated polymers, decorated with receptors, are among the best candidates for biosensor applications because of their intrinsic fluorescence and high sensitivity to minor external stimuli.[5] A number of fluorescent assays have been reported for lectin detection in the past few years.[6] Sugar-substituted conjugated polymers with backbones of poly(*p*-phenyleneethynylene) (PPE),[6a,b,e,h,i] poly(*p*-phenylene)s (PPP),[6d] polythiophenes (PT),[6f] poly(2,7-fluorenylene-*alt*-1,4-phenylene) (PFP),[6j] poly(phenylene vinylene)s (PPV),[6c] and poly(phenylacetylene)s (PPA)[6g] have been developed for the detection of Con A or *E. Coli.* All these assays are based on polymer aggregation and fluorescence quenching induced by sugar–Con A interactions.

One strategy to develop fluorescence turn-on assays is to design polymers that contain low-energy traps.[7] Cationic conjugated polymers (CCPs), which contain a fractional substitution of fluorene fragments with 2,1,3-benzothiadiazole (BT) units, provide a multicolor platform for biological detection.[7a,8] The working hypothesis is that interchain fluorescence resonance energy transfer (FRET) is more efficient than intrachain FRET because of stronger electronic coupling and increased transfer dimensionality.[8a,9] Moreover,

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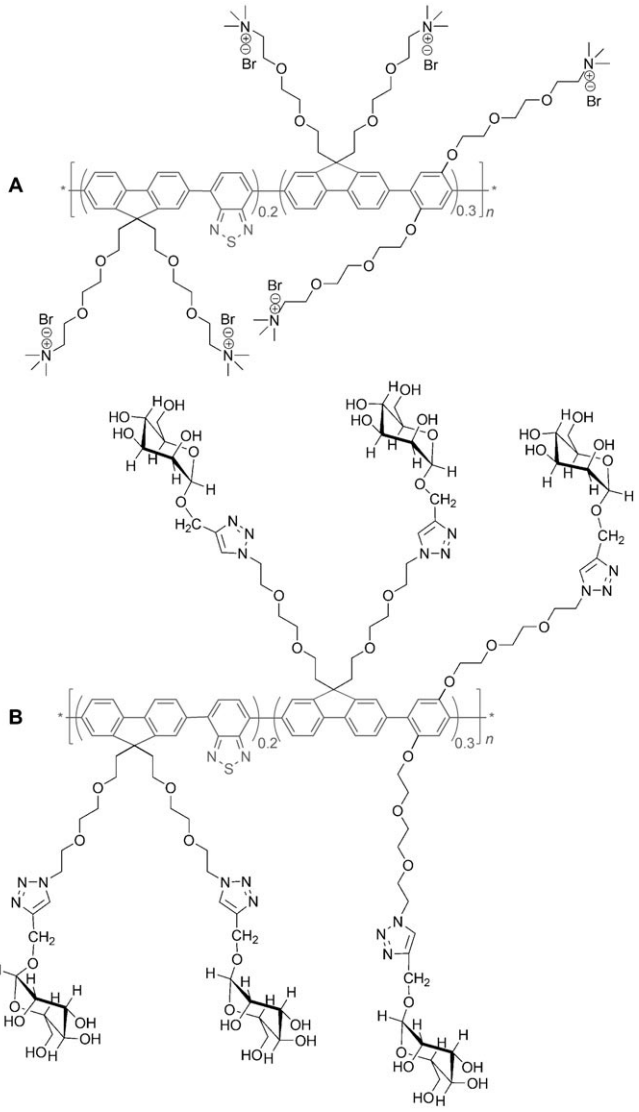
aggregation induced by complexation between the CCPs and the oppositely-charged analytes favor interchain FRET from donor (fluorene segments) to acceptor (BT units), which gives rise to fluorescence changes.[7a,8] Most of these assays are based on multivalent electrostatic interaction, which do not have specificity for analyte detection in biological media.[7,8,10] As a consequence, it is important to develop neutral water-soluble conjugated polymers with specific recognition groups for biosensor applications.

To attach mannose to the conjugated polymers, both pre-polymerization and post-polymerization approaches have been reported in the literature.[11] For pre-polymerization, well-defined carbohydrate-carrying monomers must be prepared by tedious product isolation. Post-polymerization, however, is generally more advantageous because it provides a versatile approach to rapidly attach a variety of carbohydrates to conjugated polymers, which allows easy control of the functionalization degree of carbohydrates along the same polymer backbone.[12] However, the yield of the functional group conversion is a major concern of post-polymerization. This problem could be solved by "click" chemistry, which has the advantages of nearly quantitative yield, mild reaction condition, and broad tolerance towards the functional groups.[13] In addition, the click reaction has been widely utilized to synthesize bioconjugates because both the reactants (acetylenes and azides) and the products (triazoles) are biocompatible.[14]

In this contribution, we report the design and synthesis of a cationic polymer **A** and an α-mannose-bearing polymer **B** (Scheme 1) by post-polymerization of a polyfluorene derivative that contains 20 mol% BT in the backbone. The optical properties of both the water-soluble polymers are studied to show the effect of mannose terminal groups on polymer fluorescence. Specific response of **B** to Con A has been demonstrated and the corresponding photoluminescence (PL) changes in the BT emission intensity with Con A concentration have been quantified to obtain the calibration for Con A quantification.

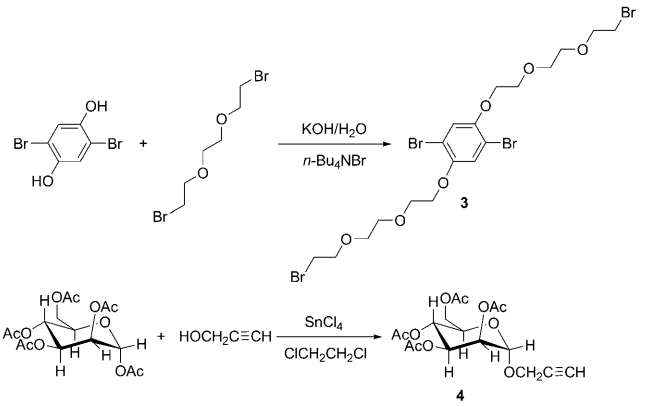


Scheme 1. Chemical structures of cationic polymer **A** and α-mannose-bearing polymer **B**.

Results and Discussion

Synthesis and Characterization

To obtain polymers of high solubility in water, monomers with ethylene glycol side chains were chosen for polymerization because of their hydrophilic nature. 2-(9,9-Bis(2-(2-(2-methoxyethoxy)ethoxy)ethyl)-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)fluorenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**1**) and 4,7-dibromo-2,1,3-benzothiadiazole (**2**) were prepared according to the literature methods.[15] The monomer 2,5-dibromo-1,4-bis[2-[2-(2-bromoethoxy)ethoxy] ethoxyl] benzene[16] (**3**) was synthesized in 58.9% yield by the deprotonation of 2,5-dibromohydroquinone with KOH and subsequent alkoxylation with 1,2-bis(2-bromoethoxy)-ethane in the presence of tetrabutylammonium bromide (Scheme 2). 2-Propynyl-2,3,4,6-tetra-O-acetyl-α-D-mannopyranose (**4**)[17] was synthesized by the reaction of 1,2,3,4,6-



Scheme 2. Synthetic route for monomers **3** and **4**.

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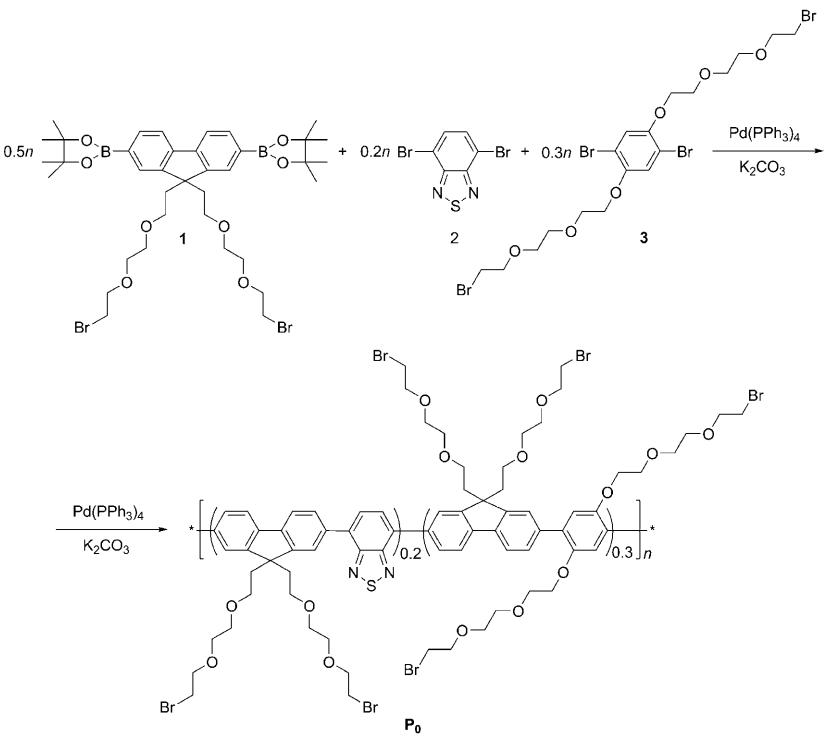


Scheme 3. Synthetic route for polymer $\mathbf{P_0}$.

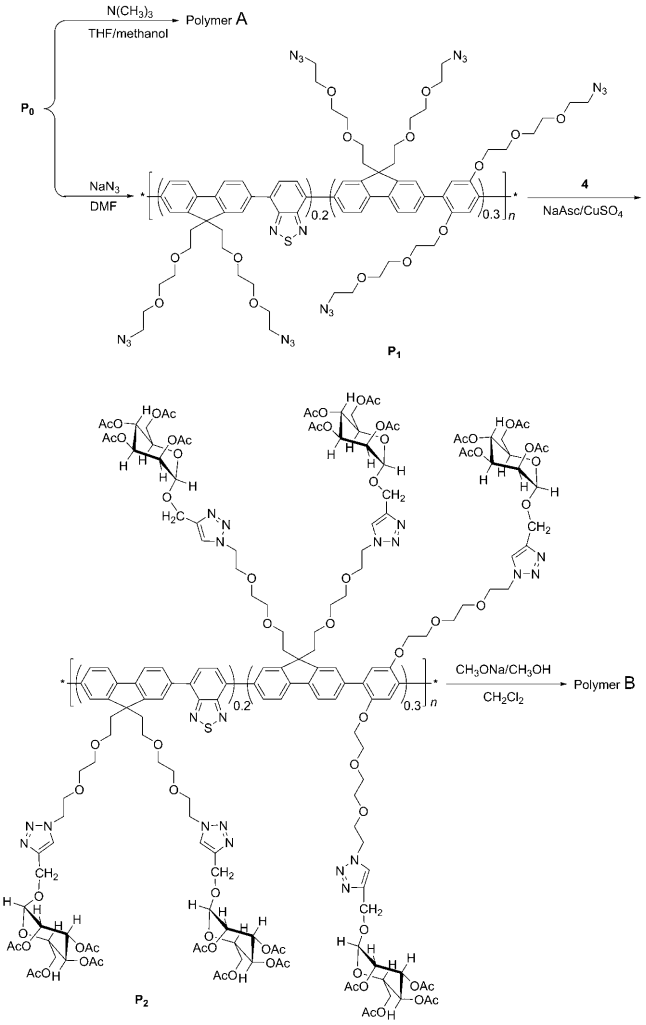
penta-O-acetyl-α-D-mannopyranose with 2-propynyl alcohol using $SnCl_4$ as the catalyst. The structure and purity of **3** and **4** were confirmed by NMR, MS, and elemental analysis.

The synthetic route to the polymers is shown in Schemes 3 and 4. The Suzuki cross-coupling copolymerization of **1**, **2**, and **3** at a feed ratio of 0.5:0.3:0.2 provided the neutral statistical random copolymer ($\mathbf{P_0}$). The chemical structure of $\mathbf{P_0}$ was determined by ^{1}H and ^{13}C NMR spectroscopies. The ratio of the integrated area of the aromatic protons to that of the alkoxy protons agrees well with the targeted structure. The number-average molecular weight and polydispersity of $\mathbf{P_0}$ determined by GPC using THF as the solvent and polystyrene as the standard, are 8500 and 2.14, respectively.

Treatment of $\mathbf{P_0}$ with trimethylamine in THF/methanol yielded the cationic conjugated polymer **A**. The structure of **A**, determined by ^{1}H NMR spectroscopy in deuterated methanol, was more than 90% quaternized. A mixture of $\mathbf{P_0}$ and sodium azide in DMF was stirred at room temperature for 24 h to yield polymer $\mathbf{P_1}$ with azide groups at the end of the side chains. The ^{1}H NMR spectra of $\mathbf{P_0}$, $\mathbf{P_1}$, $\mathbf{P_2}$, and **B** are shown in Figure 1. Upon treatment of $\mathbf{P_0}$ with NaN_3, the signal at 3.80 ppm for $\sim CH_2CH_2Br$ ($\mathbf{P_0}$) shifts to 3.53 ppm for $\sim CH_2CH_2N_3$ ($\mathbf{P_1}$), and the transformation from bromide to azide is nearly quantitative. The Cu^I-catalyzed click reaction between $\mathbf{P_1}$ and **4** was carried out in THF/H_2O at room temperature to afford the polymer $\mathbf{P_2}$ as a yellow fibrous solid. In the ^{1}H NMR spectrum of $\mathbf{P_2}$, the new signals at ~1.84–2.11 ppm correspond to acetyl protons, which indicate that the mannose groups have been successfully attached to the polymer side chains with ~98% functionalization degree. Deacetylation of $\mathbf{P_2}$ under Zemplém conditions in methanol and dichloromethane leads to polymer **B**, with almost no residual peaks for acetyl protons in the ^{1}H NMR spectrum. The successful removal of the acetate groups endows **B** with good water-solubility because of the presence of hydrophilic mannose groups.

Optical Properties

The normalized UV/Vis absorption and photoluminescence (PL) spectra of **A** and **B** in aqueous solution are shown in Figure 2. The polymer concentration based on repeat unit ([RU])[18] was 1.0×10^{-6} M. The absorption spectrum of **A** shows a maximum at 345 nm and a shoulder centered at 425 nm, which have been assigned to the π–π* transition of the fluorene–phenylene segments and the BT units, respectively.[19] The maximum absorption wavelength of **B** was at 350 nm and a shoulder peak was centered at 437 nm, both of which were slightly red-shifted compared to those for polymer **A**. Upon excitation at 350 nm, **A** had an emission maximum at 412 nm and there was almost no emission tail observed in the 500–650 nm region. Although polymer **B** also had an emission maximum at 412 nm, an obvious BT emission peak centered at ~555 nm was observed. This indicates that the structure of the side end groups significantly affect the optical properties of the water-soluble conjugated polymers. To understand the origin of BT emission for polymer **B**, laser light scattering (LLS) was used to study the polymer aggregation of both **A** and **B** in solution. There was no scattering signal observed for a solution of polymer **A** at different concentrations ([RU] = 1.0×10^{-5}, 3.0×10^{-5} and 5.0×10^{-5} M), which indicated that there was very limited interchain aggregation of polymer **A** in dilute solutions. When the same concentrations of polymer **B** were used for the study, obvious scattering signals were observed. Figure 3 shows the LLS results of **B** solution at [RU] = 3.0×10^{-5} M, based on the average of five measurements with ±10% errors. The effective diameter of the aggregates is about 218±4 nm, which is comparable to those at [RU] = 1.0×10^{-5} M (206±6 nm) and 5.0×10^{-5} M (230±7 nm), respectively. Because both polymers **A** and **B** have the same backbone structure, the aggregation of **B** most likely originated from hydrogen bonding interactions between the mannose groups.[6e]



Scheme 4. Synthetic route for polymers **A** and **B**.

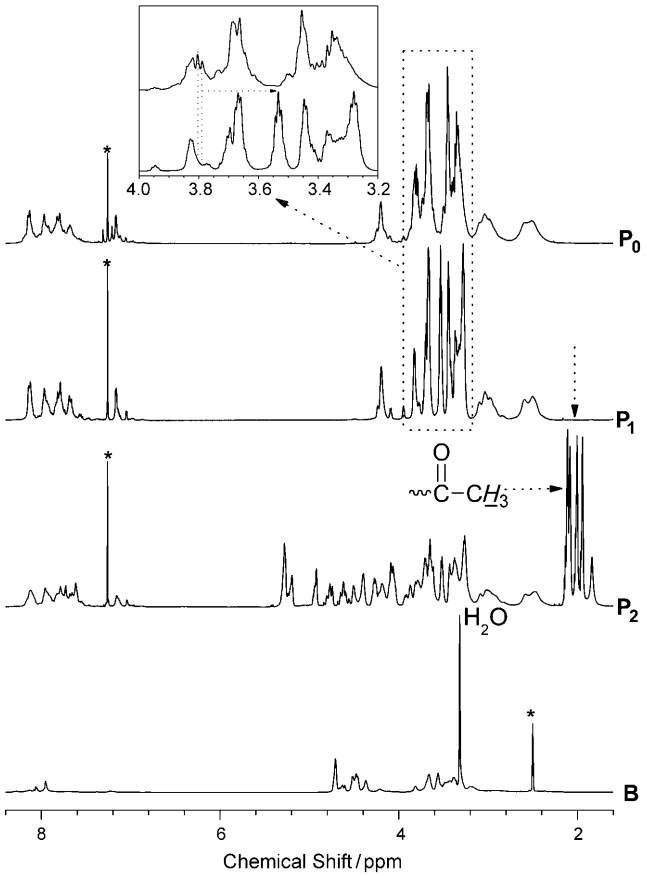


Figure 1. ^{1}H NMR spectra of $\mathbf{P_0}$, $\mathbf{P_1}$, $\mathbf{P_2}$, and **B**. The solvent peaks are marked with asterisks ($\mathbf{P_0}$, $\mathbf{P_1}$, and $\mathbf{P_2}$ in chloroform-*d*, **B** in [D$_6$]DMSO).

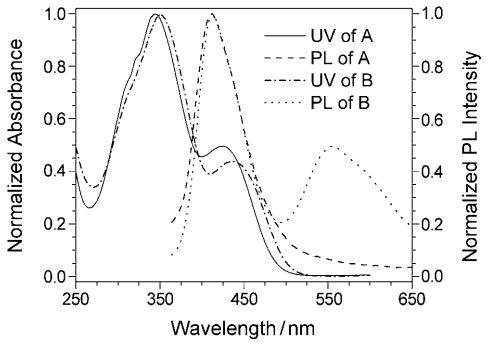


Figure 2. Normalized UV/Vis absorption and PL spectra of polymers **A** and **B** in H_2O. [RU] = 1.0×10^{-6} M. Excitation wavelength: 350 nm.

Fluorescence Response to Proteins

The proteins selected for this study were Con A (isoelectric point, pI = 4.5–5.5), bovine serum albumin (BSA, pI = 4.8), and lysozyme (pI = 11.0). Con A exists predominantly as a tetramer of four identical subunits in neutral or alkaline solutions. It has four binding sites and can bind to four α-mannose or α-glucose units in the presence of Ca^{II} and Mn^{II} ions.[20] Both BSA and lysozyme were used as disturbance proteins because BSA possesses net negative charges while lysozyme has net positive charges at pH 7.2. All titration experiments were carried out in phosphate buffer solution (pH 7.2, 25 mM) in the presence of 0.1 mM $CaCl_2$ and 0.1 mM $MnCl_2$ upon excitation at 350 nm. For the polymer **A** solution of [RU] = 1.0×10^{-6} M, the changes in PL spectra upon addition of BSA, lysozyme, or Con A at intervals of 1.0×10^{-8} M are shown in Figure 4. The PL spectra were normalized with the blue emission band. The BT emission intensity increases slightly for BSA and Con A, while there is almost no change observed for lysozyme. This indicates that polymer **A** has no selectivity for Con A. Polymer **B** was also used for protein titration under the same experimental conditions, and the normalized PL spectra obtained against the blue emission band are shown in Figure 5. Addition of BSA or lysozyme to the polymer **B** solution leads to very small changes in the BT emission band. However, the BT emission intensity increases when Con A is added to the polymer solution (Figure 5c). These results clearly indicate that the α-mannose-bearing polymer **B** can selectively respond to Con A. Because of the existence of four binding sites for one Con A molecule, cross-interactions between polymer **B** and

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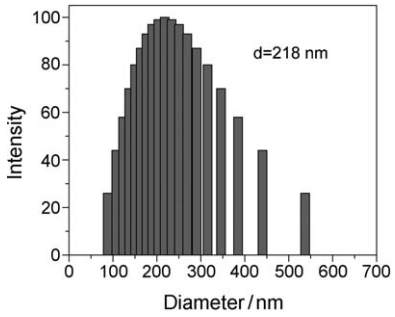


Figure 3. Effective diameters (EDs) determined by laser light scattering for polymer **B** ([RU] = 3.0×10^{-5} M) in H_2O.

Con A could lead to polymer interchain contact and aggregation, as shown in Scheme 5, which triggers FRET process from fluorene–benzene segments (donor) to BT units (acceptor) to yield increased BT emission signal. In addition, the aggregation-induced isolation of BT units from water molecules could also contribute to the increase in BT emission.[7b,16a]

Quantification of Con A

For Con A quantification, changes in the normalized PL spectra of polymer **B** upon Con A addition were correlated to Con A concentration. We define ϕ as [Eq. (1)]

$$\phi = (I - I_0)/I_0 \quad (1)$$

where I and I_0 are the relative intensities at 556 nm in the presence and absence of Con A, respectively. Figure 6 shows ϕ as a function of [Con A] along with its linear trend line. At [RU] = 2.0×10^{-6} M, a linear calibration curve was observed for Con A in the concentration range 0–250 nM. The slope of the curve is 0.028 nM^{-1} and the correlation coefficient is 0.999. This indicates that it is feasible to use polymer **B** as a probe for Con A quantification. To explore the low detection limit, further experiments were carried out at [RU] = 5.0×10^{-7} M. As shown in Figure 6b, an increase in [Con A] at the interval of 1 nM shows a progressive increase in emission intensity. The slope of the curve is 0.030 nM^{-1} and the correlation coefficient is 0.991. Thus the lowest experimentally measurable [Con A] is ~1 nM. As such, the calibration curve obtained at [RU] = 5.0×10^{-7} M is suitable for quantification of low Con A concentrations, while a wider calibration range could be obtained at [RU] = 2.0×10^{-6} M.

Conclusions

In summary, we have reported a post-polymerization approach to attach an α-mannose residue to a dual-emissive conjugated polymer by the "click" reaction. The specific interaction between Con A and α-mannose leads to polymer aggregation, which results in a FRET process from fluorene–phenylene segments to BT units and increased BT emission intensity. The linear growth of BT emission intensity upon addition of Con A allows Con A quantification in the concentration range from 1 nM to 250 nM. Because α-mannose residues also recognize other bacterial species, such as *Escherichia coli* and *Salmonella*,[21] this fluorescence detection assay may be easily adapted toward these analytes. In addition, the assay strategy could be used to develop other fluorescent sensors by selecting and attaching different recognition groups to the polymer side chains.

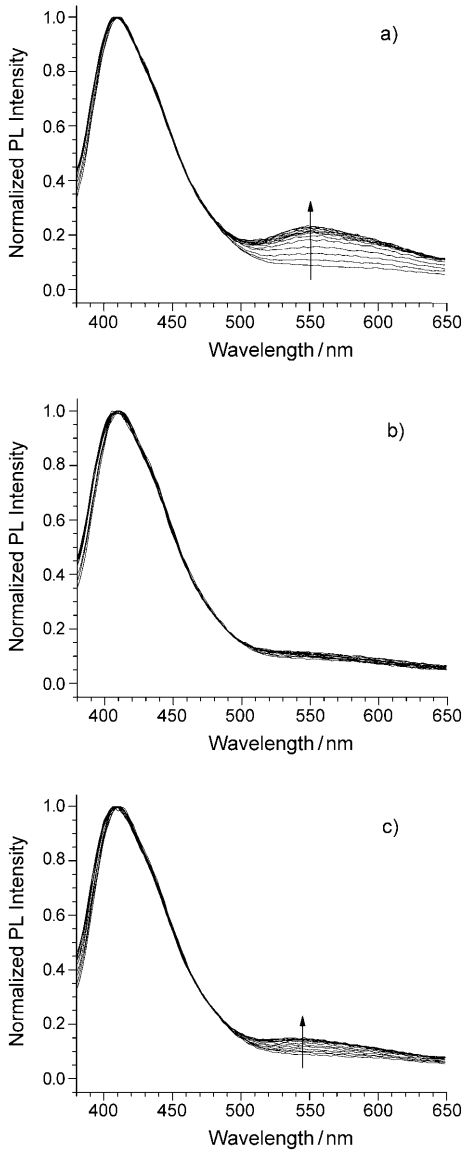


Figure 4. Normalized fluorescence spectra of polymer **A** in the absence and presence of a) BSA, b) lysozyme, and c) Con A in phosphate buffer (25 mM, pH 7.2) containing 0.1 mM $CaCl_2$ and 0.1 mM $MnCl_2$. Proteins are added at intervals of 1.0×10^{-8} M. [RU] = 1.0×10^{-6} M. Excitation wavelength: 350 nm.

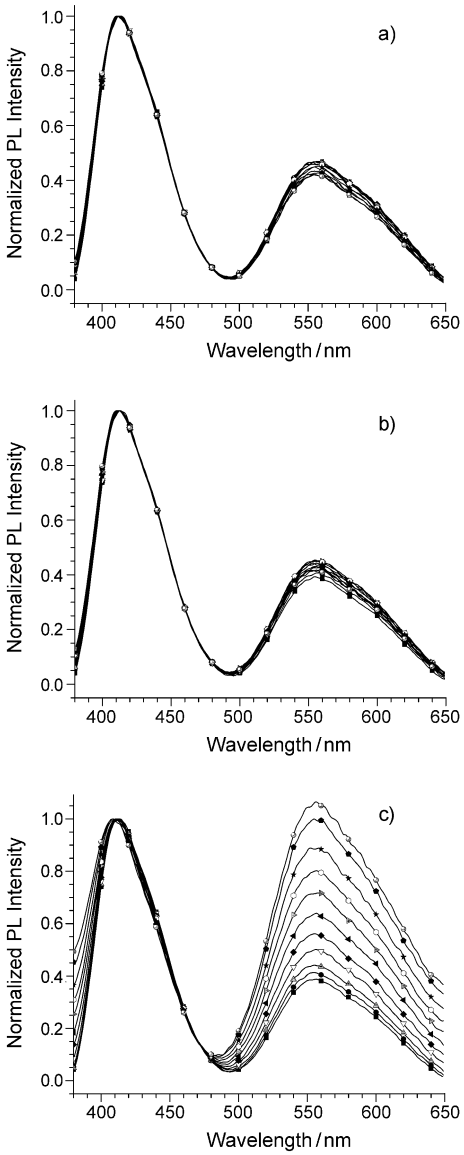
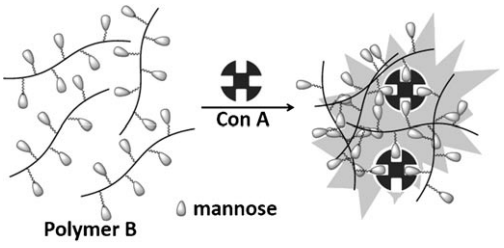


Figure 5. Normalized fluorescent spectra of polymer **B** in the absence and presence of a) BSA, b) lysozyme, and c) Con A in phosphate buffer (25 mM, pH 7.2) containing 0.1 mM $CaCl_2$ and 0.1 mM $MnCl_2$. Proteins are added at intervals of 1.0×10^{-8} M. $[RU]=1.0\times10^{-6}$ M. Excitation wavelength: 350 nm.



Scheme 5. Schematic representation of the change in polymer fluorescence upon interaction with Con A.

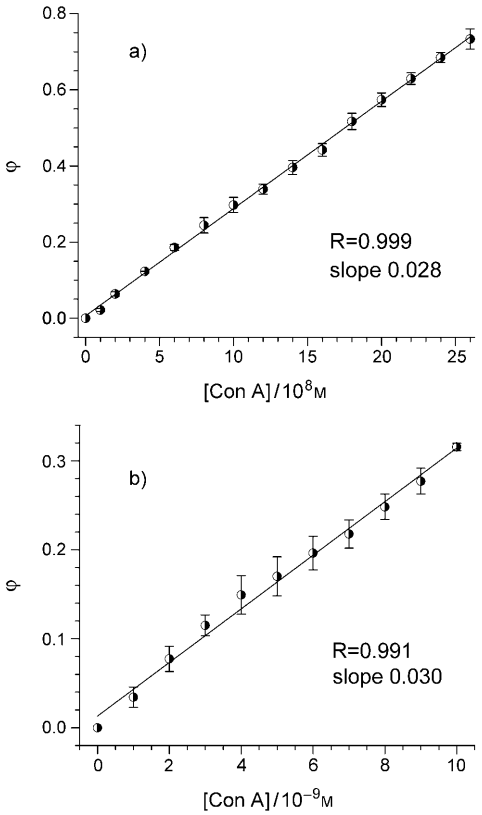


Figure 6. ϕ as a function of [Con A] at a) $[RU]=2.0\times10^{-6}$ M and b) $[RU]=5.0\times10^{-7}$ M in phosphate buffer (25 mM, pH 7.2) containing 0.1 mM $CaCl_2$ and 0.1 mM $MnCl_2$. The data are based on the average values of three independent experiments at excitation wavelength of 350 nm.

Experimental Section

Materials: 2-(9,9-Bis(2-(2-(2-methoxyethoxy)ethoxy)ethyl)-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)fluorenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**1**) and 4,7-dibromo-2,1,3-benzothiadiazole (**2**) were synthesized according to our previous reports.[15] 2,5-Dibromo-1,4-bis[2-[2-(2-bromoethoxy) ethoxy]ethoxyl] benzene(**3**)[16] and 2-propynyl-2,3,4,6-tetra-O-acetyl-α-D-mannopyranose (**4**)[17] were synthesized according to the literature. All other chemical reagents were purchased from Sigma–Aldrich and used as received.

Instrumentations: The UV/Vis spectra were recorded on a Shimadzu UV-1700 spectrometer. Fluorescence measurements were carried out on a Perkin–Elmer LS-55 equipped with a xenon lamp excitation source and a Hamamatsu (Japan) 928 PMT, using 90° angle detection for solution samples. The nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AMX-500 spectrometer using deuterated chloroform, dimethyl sulphoxide, or methanol as solvent. Gel-permeation chromatography (GPC) analysis was conducted with a Waters 2690 liquid-chromatography system equipped with a Waters 996 photodiode detector and a Waters 2420 evaporative light-scattering detector using three Phenogel GPC columns in one loop. The molecular weight and polydispersity were obtained using polystyrenes as the standard and THF as the eluent at a flow rate of 1.0 mL min^{-1} at 35 °C. Mass spectra were obtained using matrix assisted laser desorption ionization time-of-flight mass spectrometer (MALDI-TOF-MS) system, Autoflex II Bruker Daltronics from Bruker Daltronics, and performed by using 2,5-dihydroxybenzoic acid (DHB) as the matrix under the reflector mode for data acquisition. Elemental analysis was performed on Perkin–Elmer 2400 CHN/CHNS and Eurovector EA3000 elemental analyzers. Light scattering data were collected with laser light scattering (LLS) 90 Plus (Brookhaven Instrument

Corp.) at 25±1°C. Milli-Q water (18.2 MΩ) was used for all experiments.

Synthesis of 2,5-Dibromo-1,4-bis[2-[2-(2-bromoethoxy)ethoxy]ethoxyl]-benzene (3): 2,5-Dibromohydroquinone (1.3395 g, 5 mmol) was added to a mixture of aqueous potassium hydroxide (50 mL, 50 wt%), tetrabutylammonium bromide (0.3310 g, 1 mmol), and 1,2-bis(2-bromoethoxy)-ethane (8.2791 g, 30 mmol) at 75°C. After 1 h, the mixture was cooled to room temperature. After extraction with chloroform, the combined organic layers were washed successively with water, aqueous HCl (1 M), water, and brine. After drying over $MgSO_4$, the solvent was removed. The residue was purified by silica gel column chromatography using dichloromethane as the eluent to afford **3** as white crystals (1.9365 g, 58.9%). ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.13 (s, 2H), 4.11 (t, 4H), 3.85 (t, 4H), 3.80 (t, 4H), 3.73–3.76 (m, 4H), 3.66–3.69 (m, 4H), 3.45 ppm (t, 4H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=150.18, 119.03, 111.25, 71.12, 70.95, 70.50, 70.09, 69.53, 30.32 ppm; MS (MALDI-TOF): *m/z* (%) calcd for $C_{18}H_{26}Br_4O_6$: 657.84 [M^+]; found: 658.2; elemental analysis: calcd (%) for $C_{18}H_{26}Br_4O_6$: C 32.86, H 3.98, Br 48.57; found: C 32.83, H 3.92, Br 48.71.

Synthesis of 2-Propynyl-2,3,4,6-tetra-O-acetyl-α-D-mannopyranose (4): A solution of 1,2,3,4,6-Penta-O-acetyl-α-D-mannopyranose (1.5614 g, 4 mmol) in 1,2-dichloroethane was treated with $SnCl_4$ (0.46 mL, 4 mmol) for 20 min. 2-Propynyl alcohol (0.3364 g, 6 mmol) was then added and the mixture was stirred at room temperature for 5 h. The reaction mixture was quenched with 5% aqueous $NaHCO_3$ (20 mL). After extraction with chloroform, the combined organic layers were washed with water three times and dried over $MgSO_4$. After solvent removal, the residue was purified with neutral Al_2O_3 column chromatography using dichloromethane/hexane (1:1 *v/v*) as the eluent to afford **4** as white crystals (0.8921 g, 57.7%). ^{1}H NMR (300 MHz, $CDCl_3$): δ=5.26–5.31 (m, 3H), 5.02 (s, 1H), 4.25–4.31 (m, 3H), 4.01–4.12(m, 2H), 2.46 (t, 1H), 1.98–2.15 ppm (m, 12H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=170.57, 169.88, 169.78, 169.64, 96.23, 77.89, 75.54, 69.33, 68.97, 68.90, 66.02, 62.30, 54.92, 20.80, 20.68, 20.63, 20.60 ppm; MS (MALDI-TOF): *m/z* (%) calcd for $C_{17}H_{22}O_{10}$: 386.12 [M^+]; found: 386.3; elemental analysis: calcd (%) for $C_{17}H_{22}O_{10}$: C 52.85, H 5.74; found: C 52.85, H 5.29.

Synthesis of polymer P_0: Monomers **1** (202.06 mg, 0.25 mmol), **2** (29.40 mg, 0.1 mmol), **3** (98.70 mg, 0.15 mmol), [$Pd(PPh_3)_4$] (4.66 mg), and potassium carbonate (552.84 mg, 4 mmol) were placed in a 25 mL round-bottomed flask. A mixture of water (2 mL) and toluene (5 mL) was added to the flask, and the reaction vessel was degassed. The mixture was vigorously stirred at 95°C for 24 h and then precipitated into hexane. The polymer was filtered and washed with hexane and acetone and then dried under vacuum for 24 h to afford the polymer $\mathbf{P_0}$ as a yellow solid (203.87 mg, 89.9%). ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.68–8.15 (m, 3.4H), 7.05–7.31 (m, 0.6H), 2.52–4.20 ppm (m, 19.2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=154.34, 150.59, 150.07, 149.31, 149.05, 140.60, 140.34, 139.30, 139.07, 137.57, 137.21, 136.83, 136.47, 133.39, 131.25, 128.81, 128.04, 124.45, 124.25, 120.26, 119.81, 119.52, 116.98, 71.27, 71.12, 70.72, 70.65, 70.44, 69.99, 69.95, 69.43, 67.46, 51.85, 51.62, 51.39, 39.73, 30.36, 30.29, 30.22, 30.17 ppm.

Synthesis of polymer A: Condensed trimethylamine (2 mL) was added dropwise to a solution of $\mathbf{P_0}$ (50.0 mg) in THF (10 mL) at −78°C. The mixture was warmed to room temperature and stirred for 24 h. The precipitate formed was dissolved by the addition of methanol (10 mL). After the mixture was cooled to −78°C, additional trimethylamine (2 mL) was added. The mixture was stirred at room temperature for 24 h. After removal of the solvent, acetone was added to precipitate polymer **A** as an orange powder (57.3 mg, 94.8%). ^{1}H NMR (500 MHz, CD_3OD): δ= 7.70–8.39 (m, 3.4H), 6.95–7.44 (m, 0.6H), 3.15–4.35 ppm (m, 33.6H).

Synthesis of polymer P_1: Polymer $\mathbf{P_0}$ (50.4 mg) was dissolved in 10 mL of DMF, and NaN_3 (46.2 mg) was added to the solution. The mixture was stirred at room temperature for 24 h and filtered to remove NaN_3 and NaBr. The filtrate was added dropwise into hexane to obtain polymer $\mathbf{P_1}$ (41.3 mg, 94.6%). ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.56–8.14 (m, 3.4H), 7.04–7.16 (m, 0.6H), 2.50–4.20 ppm (m, 19.2H).

Synthesis of polymer P_2: Polymer $\mathbf{P_1}$ (55.0 mg, 0.14 mmol) and monomer **4** (131.4 mg, 0.34 mmol) were dissolved in THF. An aqueous solution of sodium ascorbate (6.7 mg, 0.034 mmol) and copper (II) sulphate (2.7 mg, 0.017 mmol) was then added. The mixture was stirred vigorously overnight at room temperature. The reaction mixture was washed with water and extracted with CH_2Cl_2 (3 times). The organic phase was separated, dried over $MgSO_4$, and concentrated to ~3 mL before the residue was added dropwise into hexane. The precipitate was filtered and dried in vacuum to give polymer $\mathbf{P_2}$ as a yellow solid (124.6 mg, 88.0%). ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.61–8.13 (m, 3.4H), 7.04–7.30 (m, 2.2H), 5.20–5.28 (m, 3.2H), 4.06–4.92 (m, 11.2H), 2.45–3.93 (m, 19.2H), 1.84–2.11 ppm (m, 19.2H).

Synthesis of polymer B: Polymer $\mathbf{P_2}$ (50.0 mg) was dissolved in methanol/dichloromethane (1:1, *v/v*), which was followed by the addition of CH_3ONa (1 M) in methanol (3 mL). The reaction mixture was stirred at room temperature for 3 h. After rotary evaporation of dichloromethane, the residue was dissolved in 20 mL of water. The solution was dialyzed in a cellulose dialysis bag (*Mw* cut off: 3000) against water for 2 days. Freeze drying of the polymer solution gave polymer **B** as a yellow solid (31.6 mg, 86.2%). ^{1}H NMR (500 MHz, [D_6]DMSO): δ=7.19–8.30 (m, 5.6H), 3.19–4.71 ppm (m, 40H).

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