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Dai Geun Kim [a], Minjung Lee [b] & Taek Seung Lee [c]

[a] Organic and Optoelectronic Materials Laboratory, Department of Nanotechnology, Chungnam National University, Daejeon, Korea

[b] Department of Oriental Medicine and Food Biotechnology, Joongbu University, Chubu-myon, Kumsan-gun, Chungnam, Korea

[c] Organic and Optoelectronic Materials Laboratory, Department of Advanced Organic Materials and Textile System Engineering and Graduate School of Analytical Science and Technology (GRAST), Chungnam National University, Daejeon, Korea

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DAI GEUN KIM,[1] MINJUNG LEE,[2] AND TAEK SEUNG LEE[3]

[1]Organic and Optoelectronic Materials Laboratory, Department of Nanotechnology, Chungnam National University, Daejeon, Korea
[2]Department of Oriental Medicine and Food Biotechnology, Joongbu University, Chubu-myon, Kumsan-gun, Chungnam, Korea
[3]Organic and Optoelectronic Materials Laboratory, Department of Advanced Organic Materials and Textile System Engineering and Graduate School of Analytical Science and Technology (GRAST), Chungnam National University, Daejeon, Korea

We have synthesized water-soluble hyperbranched poly(p-phenylene)s having boronic acid groups capable of reacting with diol-containing saccharides. The polymerization was carried out by palladium-catalyzed Suzuki cross-coupling reaction using tribromo monomer for the preparation of hyperbranched type structure. The water-soluble hyperbranched polymer ***HP*** *exhibited much higher sensitivity toward glucose compared with the linear polymer* ***LP*** *presumably due to the difference in the number of the boronic acids which are present in the end groups of the polymers.*

Keywords Fluorescence; glucose sensor; water-soluble hyperbranched polymer

Introduction

In the past decade, one of the important challenges in the management of diabetes is the monitoring the glucose concentrations [1]. The most commonly used technology for blood glucose concentration determination is an enzyme-based method, which requires frequent collection of blood samples [2]. However, use of enzyme shows some practical limitations in the development of implantable sensors for continuous glucose monitoring in blood or in interstitial tissue.

The boronic acids have been known for a few decades for their ability to interact with diol-containing compounds such as carbohydrates [3,4]. They have been used for the development of receptor and fluorescent probes for sugars [5,6]. The main advantage of using boronic acid as ligand group for sugars are the fast and reversible

Address correspondence to Taek Seung Lee, Organic and Optoelectronic Materials Laboratory, Department of Advanced Organic Materials and Textile System Engineering, Chungnam National University, Daejeon 305-764, Korea. E-mail: tslee@cnu.kr

interaction with sugars [7,8]. Besides, many substituted phenylboronic acids are commercially available allowing the development of a variety of synthetic fluorescent probes for sugars with slight modification. Different mechanisms have been employed to induce spectral change following the interaction of the boronic acid and the sugars.

Hyperbranched polymers have also been employed to reduce intermolecular interaction because of their highly branched and globular molecular structures [9]. Hyperbranched structure is of greater advantage over its linear counterpart in high solubility, easiness in processing, and minimization of unfavorable intermolecular interactions and crystallization [10]. Furthermore, by controlling the nature of the peripheral groups in hyperbranched polymers, it is possible to endow them with functions such as enhanced adhesion and energy harvesting as well as biomolecular sensing characteristics.

Recently water-soluble conjugated polymers have received a great deal of attention due to their unique optoelectronic properties, which may serve as a basis for a new generation of optoelectronic devices and biochemical detection [11–13]. Their special characteristics stems from the combination of optoelectronic properties of conventional polymers and water solubility induced by ionic nature of polyelectrolytes [14,15].

In this work, we are reporting the first water-soluble hyperbranched poly(p-phenylene) containing boronic acid that showed high selectivity and the significant fluorescence intensity changes within the range of physiological glucose concentration. The number of glucose-binding ligand, boronic acid was maximized through the synthesis of the hyperbranched polymer structure. The structure-sensing property of the hyperbranched polymer was investigated in detail by comparison with linear counterpart. The results demonstrated that the hyperbranched structure is effective in enhancing sensitivity and selectivity toward glucose.

Experimental

Reagents and Instrumentation

1,4-Butane sultone, benzene-1,4-diboronic acid, 4-bromobenzaldehyde, tris(4-bromophenyl)amine, and tetrakis(triphenylphosphine)palladium(0) were purchased from Aldrich. All the chemicals were used without further purification. ^{1}H NMR spectra were obtained on a Bruker DRX-300 spectrometer (Korea Basic Science Institute). The elemental analysis was determined with a CE Instruments EA-1110 elemental analyzer. FT-IR spectra were obtained from Mattson Genesis II spectrometer. UV-vis absorption spectra were recorded on a PerkinElmer Lambda 35 spectrometer. Photoluminescence spectra were taken from a Varian Cary Eclipse equipped with a xenon lamp excitation source.

Synthesis of 2,5-Dibromohydroquinone (1)

To a 500 ml round-bottomed flask were added 10 g (90.8 mmol) of hydroquinone and 100 ml of acetic acid. 9.32 g (181.6 mmol) of Br_2 in 10 ml of acetic acid was added dropwise to the hydroquinone mixture. The mixture was stirred at room temperature for 2 h and then water was added. Precipitate was isolated by filtration and crystallized from water. Yield 8.71 g (35.8%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.84 (s, 2H), 7.04 (s, 2H) ppm.

Synthesis of 1,4-Dibromobenzene-2,5-bis-4-butoxysulfonic acid (2)

To a 250 ml round-bottomed flask were added 1.96 g (49.09 mmol) of NaOH, 3.3 g (12.27 mmol) of **1** and 150 ml of ethanol. 5 ml (49.09 mmol) of 1,4-butane sultone was added dropwise. The mixture was refluxed for 20 h. After the reaction, the crude product was filtered and washed with ethanol. The white solid was dried in vacuum. To a 500 ml beaker were added 3 g of the white product and 90 ml of water. 35% HCl was added dropwise to the mixture. The precipitate was formed and isolated by filtration. The product was dried in vacuum. Yield 6.9 g (96%). ^{1}H NMR (300 MHz, D_2O): δ 7.43 (s, 2H), 4.18-4.14 (t, 4H), 3.08-3.03 (t, 4H), 2.00-1.96 (m, 8H) ppm. FT-IR (cm^{-1}): 719 (S–O), 1018 (aryl C–Br), 1204, 1358 (S=O), 1489 (C=C), 2936 (C–H), 3351 (OH). Anal. Calcd. For $C_{14}H_{20}Br_2O_8S_2$: C, 31.12%; H, 3.73%; S, 11.87%. Found: C, 30.38%; H, 3.86%; S, 11.35%.

Synthesis of HP

To a 100 ml round-bottomed flask were added 1 g (1.85 mmol) of **2**, 0.38 g (2.31 mmol) of benzene-1,4-diboronic acid, 0.148 g (0.308 mmol) of tris(4-bromophenyl)amine, 18 ml of 2 M aqueous Na_2CO_3 solution, and 30 ml of dry DMF under nitrogen. After addition of tetrakis(triphenylphosphine)palladium(0) (5 mol %) as a catalyst the mixture was heated up to 85°C. The mixture was stirred for 40 h. The reaction mixture was poured into acetone. Precipitate was redissolved in deionized water and the solution was dialyzed using a membrane (3500 cutoff) for 3 days. The polymer was obtained after drying in freeze drier. Yield 0.41 g (40.2%). ^{1}H NMR (300 MHz, D_2O): δ 7.7-6.8 (m, 8H), 4.2-3.7 (m, 6H), 3.2-2.8 (t, 6H), 2.1-1.6 (d, 12H) ppm. FT-IR (cm^{-1}): 727 (S–O), 1155 (aryl C–N), 1379 (S=O), 1611 (C=C), 2944 (C–H), 3428 (OH). Anal. Found. For $C_{14}H_{20}Br_2O_8S_2$: C, 39.72%; H, 4.17%; S, 10.66%.

Synthesis of LP

To a 100 ml round-bottomed flask were added 0.5 g (0.825 mmol) of **2**, 0.183 g (1.11 mmol) of benzene-1,4-diboronic acid, 18 ml of 2 M aqueous Na_2CO_3 solution, and 30 mL of dry THF under nitrogen. After addition of 5 mol% of Pd(0) catalyst the mixture was heated up to 85°C. Then the mixture was stirred for 48 h. The reaction mixture was poured into acetone. Precipitate was redissolved in deionized water and the solution was dialyzed using a membrane (3500 cutoff) for 3 days. The polymer was obtained after drying in freeze drier. Yield 0.203 g (39.84%). ^{1}H NMR (300 MHz, D_2O): δ 7.6-6.7 (m, 6H), 4.1-3.5 (m, 4H), 3.2-2.8 (t, 4H), 2.1-1.6 (d, 8H) ppm. FT-IR (cm^{-1}): 725 (S–O), 1381 (S=O), 1615 (C=C), 2930 (C–H), 3430 (OH). Anal. Calcd.: C, 50.18%; H, 5.18%; S, 10.31%; Found: C, 49.15%; H, 5.48%; S, 10.48%.

Glucose Sensing Test

Monosaccharide sensing studies were carried out in a 10 mm quartz cuvette. The fluorescence spectral changes in **HP** and **LP** (8×10^{-7} M) in the presence of each monosaccharide were recorded in their 6 mM sodium phosphate buffer solutions (pH 7.4).

Results and Discussion

The synthetic routes for monomer and polymers are depicted in Figure 1. Monomers **1** and **2** for the preparation of the polymers were synthesized according to the literature procedures [16,17]. Poly(p-phenylene)s are typically prepared by palladium-catalyzed Suzuki cross-coupling of diboronic acid and dibromo compound (plus tribromo monomer for hyperbranched polymer, **HP**). For the preparation of control **LP, 2** and slight excess of 1,4-phenylenediboronic acid in the presence of catalytic amount of Pd(0) were reacted in THF with aqueous sodium carbonate solution.

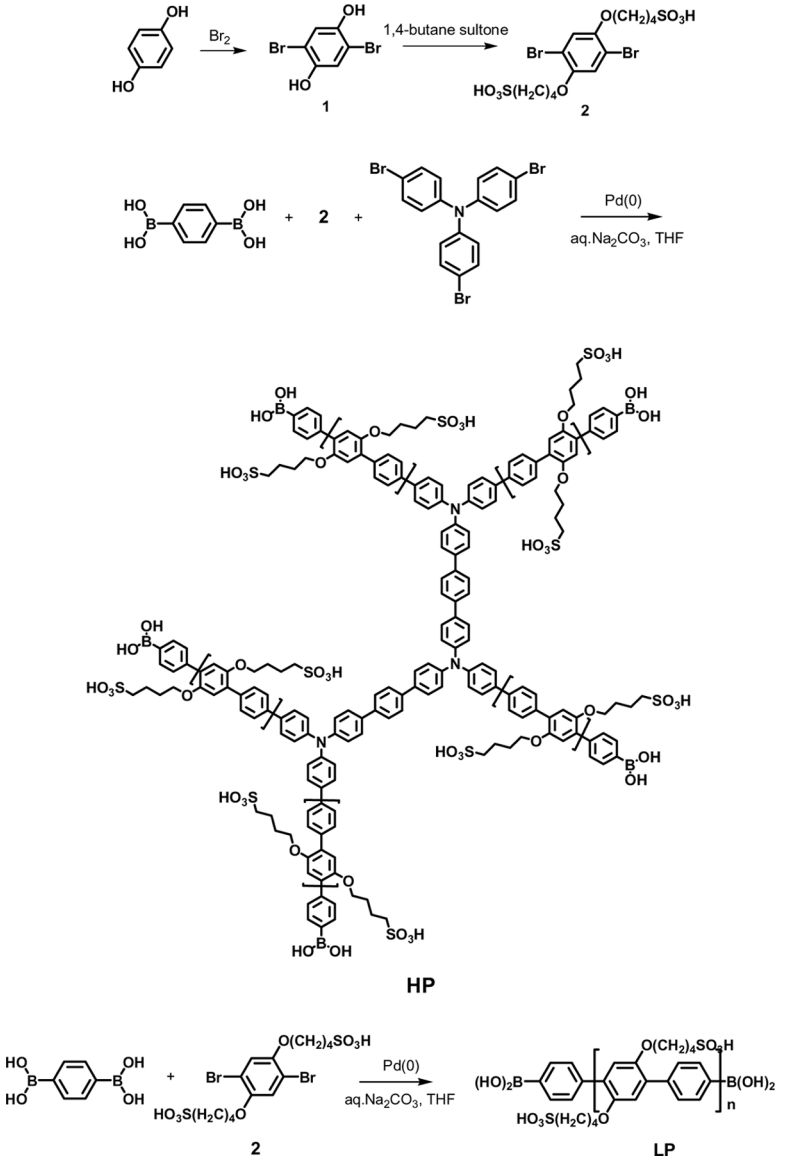

Figure 1. Synthetic routes for **HP** and **LP**.

In the case of **HP**, the polymerization time was selected experimentally before the appearance of precipitates under the same polymerization condition. **HP** obtained before precipitation was still soluble in water. However, the product precipitated was not soluble in water mainly due to its crosslinked structure. Thus precise control of the reaction time and the content of tribromo monomer, tris(4-bromophenyl)amine is essential to obtain water-soluble hyperbranched polymer. **LP** showed good solubility in water even for the prolonged reaction time. **HP** and **LP** were characterized by ^{1}H NMR, IR, and elemental analysis. ^{1}H NMR spectra of **HP** and **LP** exhibited chemical shifts of phenylene group and alkylene group at 7.7-6.7 and 4.2-1.6 ppm, respectively, thus, which the polymers were successfully synthesized.

The optical properties of the polymers were investigated with a spectrophotometer and spectrofluorometer in 6 mM sodium phosphate buffer solution at physiological pH of 7.4. Due to the presence of water-soluble sulfonic acid group at the side chain of **HP** and **LP**, they showed good solubility in the buffer solution. Their absorption and fluorescence spectra in the buffer solutions are shown in Figure 2. The linear **LP** shows absorption band at 307 nm with a shoulder band around 330 nm. However, the shoulder band was strengthened in the case of **HP**, which exhibited λ_{max} at 333 nm. This corresponds to the intensified push-pull structure of HP, which had electron-donating triphenylamine group.

On the while, the fluorescence bands of the HP and LP are concentrated at 418 nm, indicating that the conjugation lengths of the emitting species are almost identical. This should be attributed to effective excitation energy transfer from the short conjugated species to the larger ones which are the main emitting species [18].

Fluorescence experiments were performed to evaluate the affinity of the polymer for glucose. Specifically, 2 ml of the polymer aqueous solution (8×10^{-7} M, 6 mM sodium phosphate buffer, pH 7.4) was mixed with aqueous saccharide solution in the same buffer at various concentrations, and then the fluorescence intensity was recorded (Fig. 3). Fluorescence intensity decreased upon exposure to the saccharides.

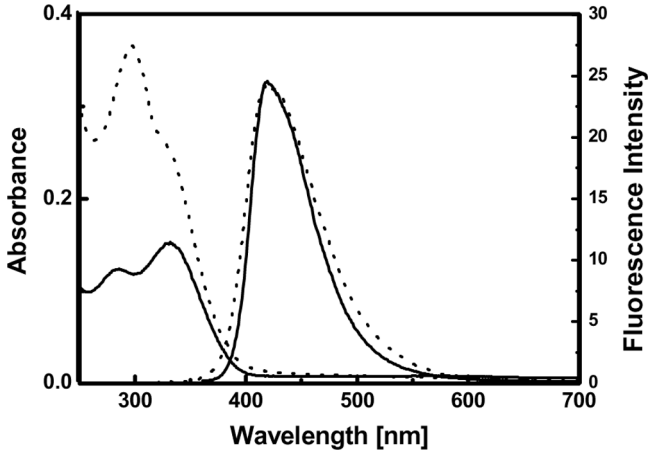

Figure 2. Absorption and emission spectra of **LP** (dotted, excited at 307 nm) and **HP** (solid, excited at 333 nm) in aqueous solution (polymer concentration: 10^{-5} M for absorption spectra).

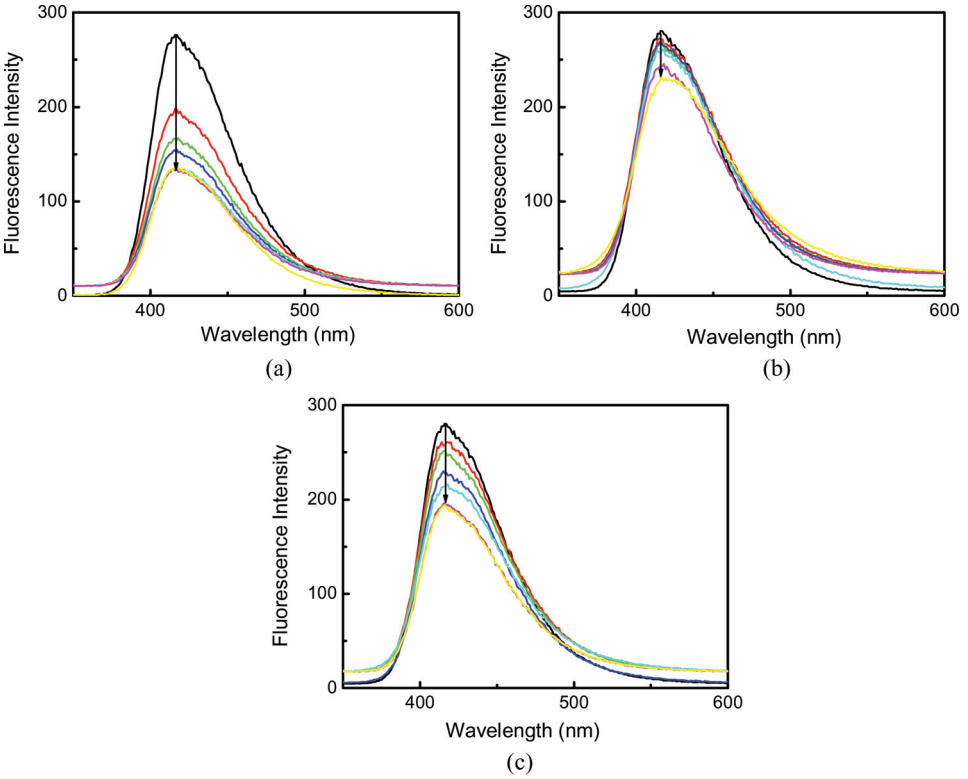

Figure 3. Fluorescence spectra of **HP** (8×10^{-7} M) upon addition of (a) glucose; (b) galactose; (c) fructose (0, 6, 12, 25, 50, 100, and 200 mM) in 6 mM aqueous sodium phosphate buffer at pH 7.4; $\lambda_{ex} = 333$ nm.

Among the saccharides investigated, **HP** showed highest selectivity for glucose over fructose and galactose in terms of the fluorescence intensity changes. It needs to be noted that the boronic acid has more affinity to fructose over glucose [3,19]. Sensory HP represents an improvement of sensitivity for glucose over fructose compared with fluorescence quenching.

Based on the complex formation between boronic acid and diol groups in glucose, the hyperbranched **HP** offers a great advantage over a linear counterpart **LP**, because a number of end groups (boronic acid) can be incorporated in the backbone. Figure 4 illustrates the fluorescence change was saturated at the concentration of 6 mM of glucose (even in fructose) due to the limited number of diol-binding sites, which are existing only at two end groups per polymer chain.

It should be noted that HP showed the most sensitive fluorescence intensity changes to glucose in mM region (Fig. 5(a)). Compared with the case of **LP** shown in Figure 5(b), consecutive fluorescence quenching can be observed in **HP** according to the concentration of glucose, while all the three saccharides can quench the fluorescence intensity of **LP** at the similar level. The results suggests that a number of boronic acid functional group on **HP** provide a potential binding with various concentration of glucose, whereas **LP** has low tendency for the complex formation between boronic acid and glucose exhibiting saturated detection limit of ~6 mM.

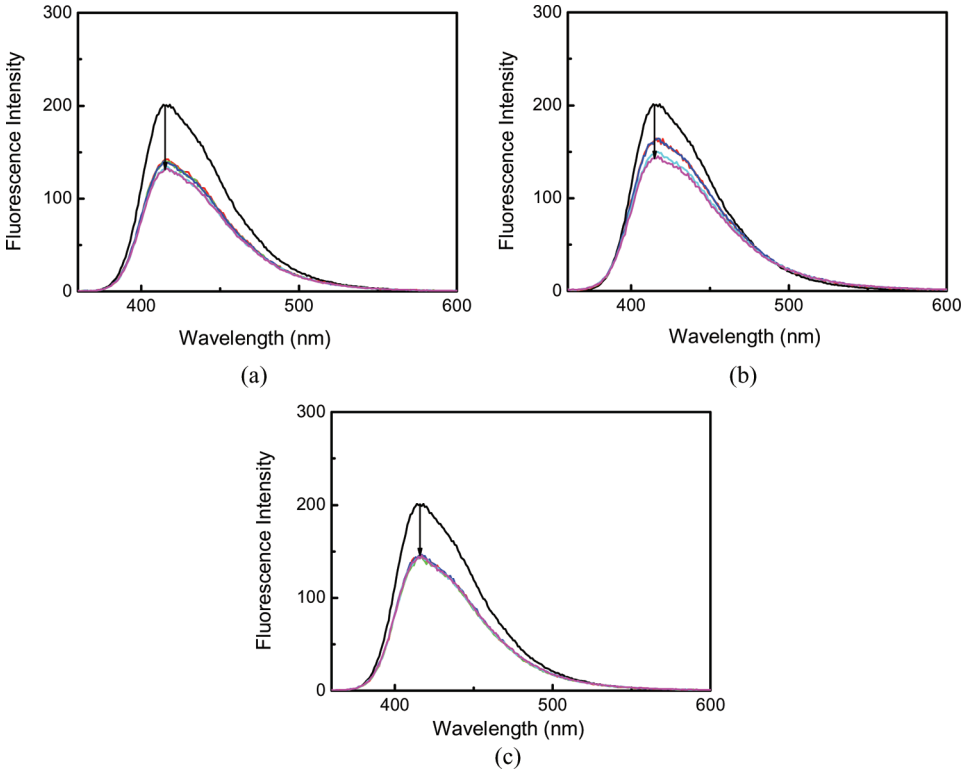

Figure 4. Fluorescence spectra of **LP** (8×10^{-7} M) upon addition of (a) glucose; (b) galactose; (c) fructose (0, 6, 12, 25, 50, 100, and 200 mM) in 6 mM aqueous sodium phosphate buffer at pH 7.4; $\lambda_{ex} = 307$ nm.

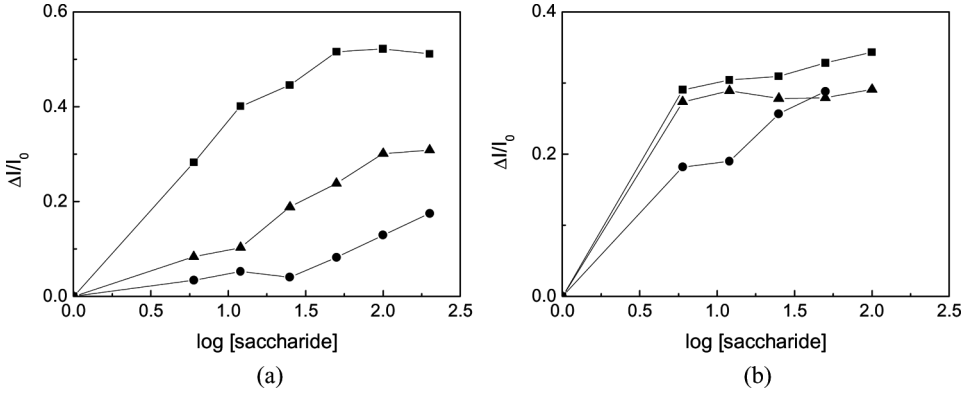

Figure 5. Emission intensity changes ($\Delta I/I_0$) of (a) **HP** and (b) **LP** as a function of the saccharide concentration; 8×10^{-7} M in 6 mM aqueous sodium phosphate buffer at pH 7.4; $\lambda_{ex} = 333$ nm and $\lambda_{em} = 418$ nm for **HP**; $\lambda_{ex} = 307$ nm and $\lambda_{em} = 418$ nm for **LP**; ■ glucose; ● galactose; ▲ fructose.

Conclusion

We synthesized a blue emitting hyperbranched water-soluble conjugated poly(p-phenylene) with a number of boronic acid functional groups in its ends via Suzuki coupling polymerization. As a control, linear water-soluble conjugated poly(p-phenylene) with boronic acid only at both chain ends. The polymers showed similar absorption and emission bands in their aqueous solutions. According to the investigations on their sensing properties toward saccharides, hyperbranched type polymer exhibited more selective and sensitive toward glucose than its linear type counterpart.

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