CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.200701376

Ultrasensitive Fluorescent Responses of Water-Soluble, Zwitterionic, Boronic Acid-Bearing, Regioregular Head-to-Tail Polythiophene to Biological Species

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Abstract: Water-soluble regioregular head-to-tail zwitterionic fluorescent conjugated boronic acid-bearing polythiophene (polymer **2**) was prepared through a postpolymerization quaternization of a pyridine group of 3-pyridineboronic acid with bromide groups of regioregular head-to-tail poly(3-bromohexylthiophene) (polymer **1**). Titration of monosaccharides, lactose, ascorbic acid, or dopamine with 0.1 M phosphate buffer (pH 7.4), containing 4.0 μM of polymer **2**, results in significant concentration-dependent quenching of the polymer fluorescence. The polymer displays an optimum response to the biological species at pH 7.0. The binding constants of polymer **2** with mannose, fructose, glucose, galactose, vitamin C, dopamine, and lactose are 3.33×10^4, 1.13×10^5, 1.23×10^5, 1.69×10^5, 3.17×10^5, 3.27×10^5, and 4.60×10^5, respectively.

Keywords: boronic acid · carbohydrates · fluorescence · polythiophene · Sensors

Introduction

Conjugated polymers have become attractive sensing materials because they display higher sensitivities to analytes than devices which use small molecules.[1–6] The sensitivities arise from collective optical or conducting properties of the conjugated polymers that are extremely sensitive to minor external structural perturbations or to electron-density changes within the polymer backbone in the presence of analytes.[1–5] In particular, water-soluble conjugated polymers show promising biosensing applications for DNA,[4] enzymes,[7] proteins,[8–10] bacteria,[8,11] and other biologically important species.[12] Conjugated polymers have been made water-soluble by introducing highly branched hydroxyls,[13] carbohydrates,[8,9,11,14,15] or hydrophilic ionic groups, such as sulfonic,[16,17] carboxylic,[18,19] ammonium,[4] or phosphonate groups,[20] to the polymer side chains to overcome π–π stacking interactions among the hydrophobic polymer backbones and enhance enthalpic interactions with water. Boronic acids are well known to bind diol species with high affinities via reversible boronate formation in aqueous solution.[21–25] Their common interactions are with *cis*-1,2 or 1,3-diols of saccharides to form five- or six-membered rings via two covalent bonds.[25] As a result, boronic acids have been widely incorporated into small molecules as artificial receptors in circular dichroism and fluorescent detection of saccharides.[21–25] Poly(aniline boronic acid) has been prepared by electrochemistry for potentiometric detection of saccharide.[26] Incorporation of boronic acid as side chain residues is expected to offer a highly sensitive fluorescent sensing material for carbohydrates by amplification through cooperative multivalent interactions among carbohydrates and boronic acid residues. In this article, we first report synthesis of a water-soluble zwitterionic boronic acid-bearing regioregular head-to-tail conjugated polythiophene and investigate its fluorescent responses to monosaccharides, lactose, ascorbic acid, and dopamine. The polymer displays ultrasensitive responses to these biological species through multivalent interactions, and possesses a binding constant of up to 4.60×10^5 with lactose.

Results and Discussion

The synthetic route to regioregular head-to-tail polythiophene bearing boronic acid residues is shown in Scheme 1. We used a synthetic strategy of post-polymerization functionalization of regioregular head-to-tail conjugated poly(3-bromohexylthiophene) (polymer **1**) with 3-pyridineboronic acid through a quaternization reaction between a pyridine

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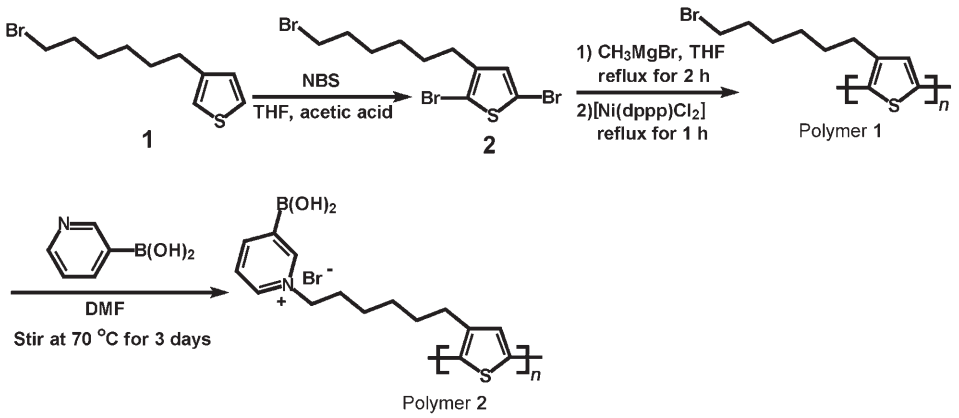


Scheme 1. Synthetic route to water-soluble zwitterionic boronic acid-bearing regioregular head-to-tail polythiophenes. NBS: *N*-bromosuccinimide, dppp: 1,2-bis(diphenylphosphino)propane.

group of 3-pyridineboronic acid with the polymer bromide groups for water-soluble zwitterionic regioregular head-to-tail glycopolythiophene bearing boronic acid residues (polymer **2**), as the polymerization for regioregular head-to-tail polythiophenes is typically achieved by the Grignard metathesis method initially reported by McCullough et al.[27–31] Regioregular head-to-tail poly(3-bromohexylthiophene) (polymer **1**) was synthesized by means of condensation polymerization by using the Grignard metathesis method in excellent yield and with a high degree of polymerization (Scheme 1),[29] according to gel-permeation chromatography (yield: 90%, M_n: 25,900 g mol^{-1}; polydispersity: 1.75).

The solubility of zwitterionic boronic acid-bearing regioregular head-to-tail polythiophenes is different from its precursor polymer. The precursor polymer **1** is readily soluble in common solvents, such as THF, chloroform, methylene chloride, and moderately soluble in DMF and DMSO, but insoluble in ethanol, methanol, acetone, and water. The zwitterionic polymer **2** is insoluble in ethanol, acetone, acetonitrile, THF, chloroform, methylene chloride, and toluene, but readily soluble in water. Water solubility of polymer **2** arises from the hydrophilic feature of its ionic pyridinium groups which overcomes the π–π stacking interactions among the hydrophobic polymer backbones and enhances enthalpic interactions with water

The precursor polymer **1** exhibits an UV/Vis absorption maximum peak at $\lambda_{max}=400$ nm, and emission maximum peak at $\lambda_{max}=558$ nm in chloroform solution, which were ascribed to the π–π* transition of the conjugated polymer backbone (Figure 1a). Its fluorescent quantum yield in chloroform solution was 2.0% by using quinine sulfate in 0.1 N sulfuric acid as the reference for absolute quantum efficiency ($\varphi_n=55\%$).[32] Polymer **2** displays a red shift relative to polymer **1** as it exhibits maximum absorption and mission peaks at $\lambda_{max}=410$ and 560 nm in aqueous solution (Figure 1b). Introduction of boronic acid residues to polythiophene does not quench the polymer fluorescence as polymer **2** possesses a little higher fluorescent quantum yield (with fluorescent quantum efficiency of 2.1% in 0.1 M phosphate buffer (pH 7.4)) than that of polymer **1**.

The UV/Vis absorption and fluorescent spectra of polymer **2** were measured in 0.1 M phosphate buffer solution (pH 7.4) in the absence and presence of monosaccharides, lactose, vitamin C, and dopamine. Figure 2a–d shows typical fluorescent spectra of polymer **2** in the absence and presence of glucose, lactose, dopamine, and ascorbic acid, respectively. Titration of these biological species to a polymer phosphate buffer resulted in a significant concentration-dependent quenching of the polymer fluorescence. The quenching of the polymer fluorescence might be due to the polymer confirmation changes caused by multivalent interactions among boronic acid residues and carbohydrates. In addition, titration of lactose, vitamin C, or dop-

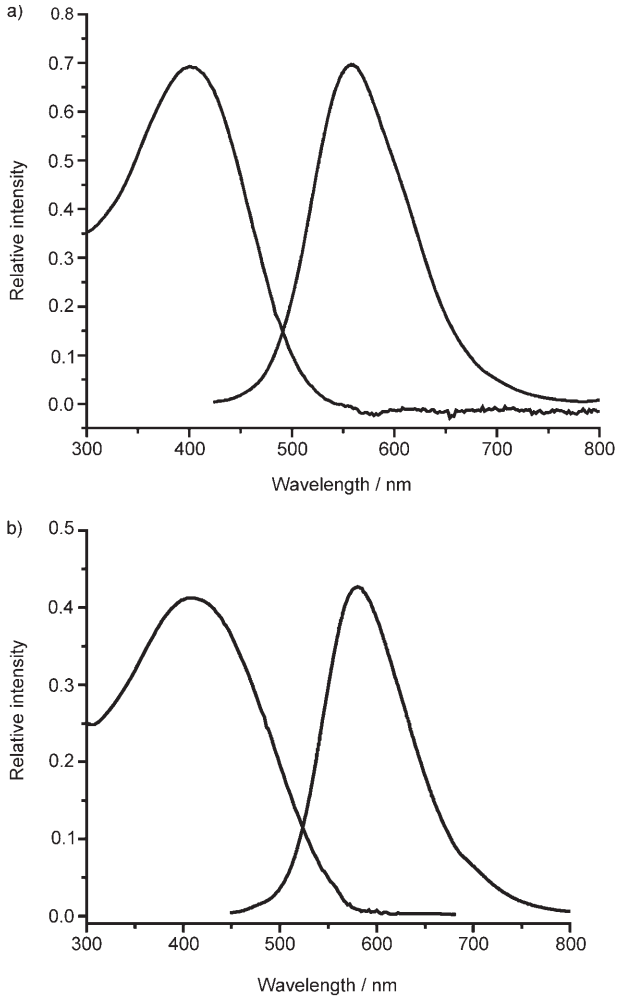
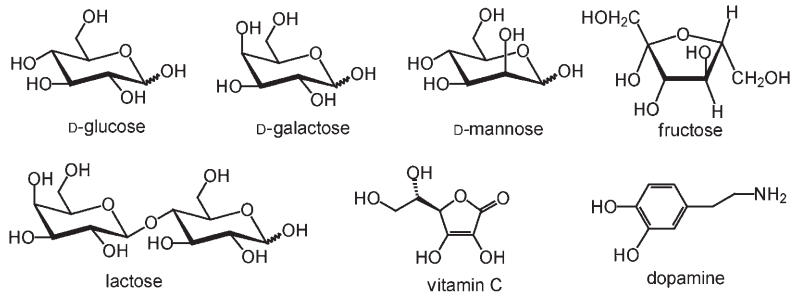


Figure 1. UV/Vis absorption and fluorescent spectra of a) polymer **1** in chloroform solution and b) polymer **2** in aqueous solution.



amine to a phosphate buffer solution of polymer **2** caused a slight red shift of the polymer fluorescence (Figure 2b–d). However, titration of these biological species to the same polymer phosphate buffer only caused slight decreases of UV/Vis absorbance of the polymer without significant Stokes shift (please see the Supporting Information).

Boronic acid readily binds to diols of these saccharides to form boronate anion species in phosphate buffer (pH 7.4). Monosaccharides usually bearing five OH groups tend to form 1+2 monosaccharide-phenylboronic acid complexes as one phenylboronic acid reacts with two OH groups (1,2- and 1,3-diols) of monosaccharides to form five- or six-membered rings via two covalent bonds, respectively.[33,34] The observed selectivity order of these complexes with one phenylboronic acid is always the same, which is governed by the inherent structures of monosaccharides. Among the monosaccharides, fructose possesses the highest association constant whereas glucose has the lowest association constant as a five-membered ring with *cis*-1,2-diols of fructose being usually the most stable.[33,34] In contrast, the boronic acid-grafted polymer is expected to display a different stability order through multivalent interactions compared with one phenylboronic acid as it can react with four of the five OH groups of monosaccharides, and with up to eight groups of disaccharides. As a result, polymer **2** exhibits the most sensitive response to lactose through multivalent interactions between lactose and boronic acid groups.

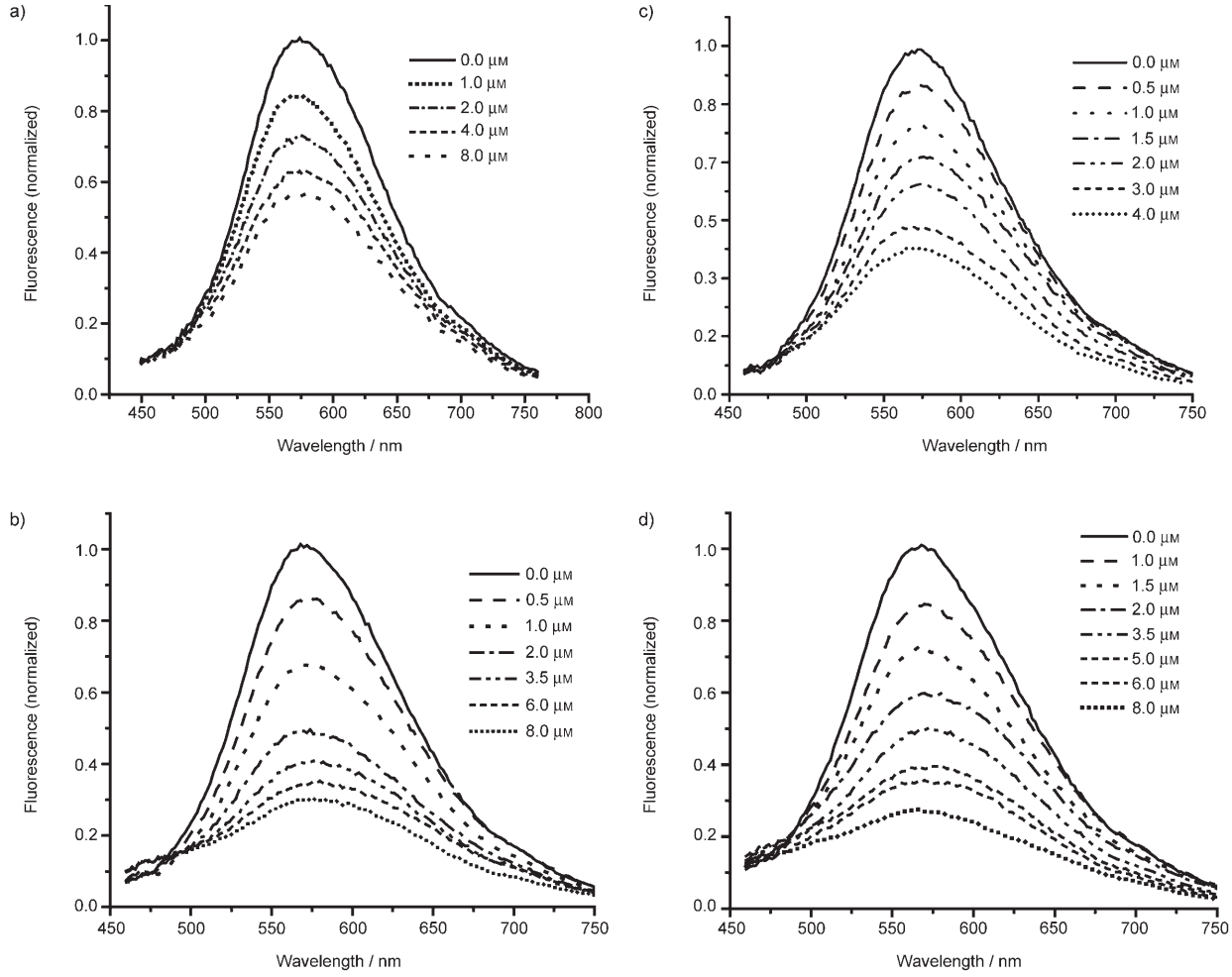


Figure 2. Fluorescent spectra of polymer **2** (4.0 μM) in 0.1 M phosphate buffer (pH 7.4) in the absence and presence of different concentrations of a) glucose, b) lactose, c) dopamine, and d) vitamin C.

Conjugated polymers feature short emissive lifetimes on the order of 0.2–0.5 ns, unless they contain organometallic fragments.[17,35,36] As a result, only static quenching is predominant.[17,35,36] In static quenching, the quencher forms a ground-state complex with the fluorophore which is then quenched after excitation: quenching constant K_{SV} in static quenching equals the apparent complex formation constant of quencher to fluorophore. The use of the Stern–Volmer relationship offers a simple way to determine binding constants (Figure 3).[37] A quantitative measure of the fluorescent quenching can be achieved by determining the well-known Stern–Volmer constant, K_{SV}:[37]

$$I_0/I = 1 + K_{SV}[Q] \quad (1)$$

in which [Q] is the concentration of quencher, I_0 stands for the intensity of fluorescence in the absence of the quencher, and I is the fluorescence intensity as a function of quencher concentration [Q]. The equation reveals that I_0/I increases in direct proportion to the concentration of the quencher,

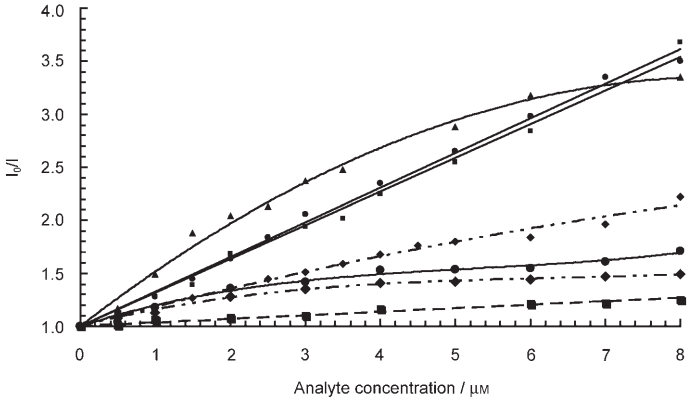
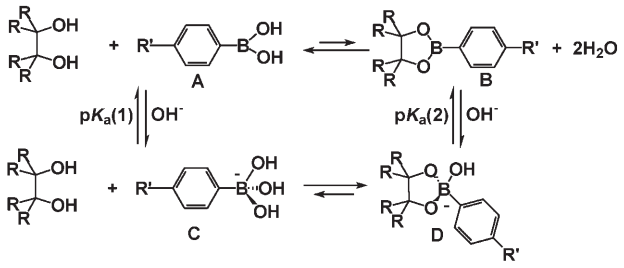


Figure 3. The Stern–Volmer curves of polymer **2** with different concentrations of biological species in 0.1 M phosphate buffer (pH 7.4; ▲ lactose, ● dopamine, ■ vitamin C, ◆ galactose, ● glucose, ◆ fructose, ■ mannose).

and K_{SV}, slope of the plots, is the Stern–Volmer constant, defining the efficiency of fluorescent quenching. When all other variables are held constant, the higher the value of K_{SV}, the lower the concentration of quencher required to quench the fluorescence.[37] The Stern–Volmer quenching constants of polymer **2** by different carbohydrates, dopamine and vitamin C were calculated and are listed in Table 1.

Ester formation between boronic acids and diols in aqueous solution strongly depends on pH according to the equilibria in Scheme 2. To obtain a favorable binding, the formation of the hydroxyboronate (**D**), which contains a tetrahedral boron atom, is desirable. Because pK_a(2) is 1–2 units



Scheme 2. Equilibria between boronic acids and diols.

lower than pK_a(1),[38] the complex **D** will only exist in fair amounts at neutral pH if the boronic acid has a p$K_a \leq 7.0$. As the aromatic boronic acid derivatives have pK_a values ranging from 8 to 10,[39] it requires more alkaline conditions to form strong complexes (Scheme 2). Strong electron-withdrawing substituents on the aromatic moiety are required to lower the pK_a values of boronic acid derivatives. For example, 4-carboxy-3-nitrophenylbronic acid has a pK_a of 7.0.[40] Our approach for measuring carbohydrates at neutral pH is to take advantage of a zwitterionic pyridinium hydroxyboronate with a very low pK_a value of 4.0,[41] which was obtained through post-polymerization functionalization of bromide-bearing polythiophene with 3-pyridineboronic acid. Alkylation of the nitrogen in 3-pyridineboronic acid would give a pyridinium salt, which not only functions as an electron-withdrawing group but also makes the polymer soluble in water. We investigated the pH effect on fluorescent responses of polymer **2** to carbohydrates (Figure 4). As we expected, the polymer displayed an optimum response to fructose or lactose at pH 7.0 in 0.1 M phosphate buffer.

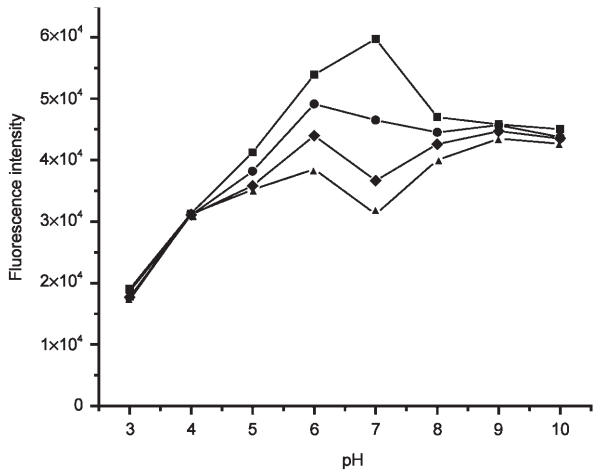


Figure 4. Fluorescent responses of polymer to fructose at different pH values. Concentration of lactose: ■ 0.0 μM, ● 1.0 μM, ◆ 2.0 μM, ▲ 4.0 μM.

Table 1. The Stern–Volmer quenching constants of polymer **2** by biological species.

Species	Fructose	Glucose	Galactose	Mannose	Vitamin C	Dopamine	Lactose
quenching constant	1.13×10^5	1.23×10^5	1.69×10^5	3.33×10^4	3.17×10^5	3.27×10^5	4.60×10^5

Conclusion

A water-soluble zwitterionic boronic acid-bearing regioregular head-to-tail polythiophene has been synthesized and its fluorescent responses to carbohydrates, dopamine, and ascorbic acid have been studied. The polymer exhibits ultrasensitive responses to carbohydrates through multivalent cooperative interactions at pH 7.4 in 0.1 M phosphate buffer.

Experimental Section

Instrumentation: ^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz Varian Unity Inova spectrophotometer. UV absorption spectra were taken on a Hewlett–Packard 8452 A Diode Array UV/Vis spectrophotometer. Fluorescence spectra were obtained on a steady-state Spex Fluorolog 1681 fluorometer. All concentrations of polymers reported in the paper are prepared in terms of a polymer repeat unit. Analytical gel permeation chromatography (GPC) was obtained from a Waters 6000 A equipped with a Waters Model 440 ultraviolet absorbance detector at a wavelength of $\lambda = 254$ nm and a Waters Model 2410 refractive index detector. 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. THF was used as an eluent and the molecular weight was measured relative to polystyrene standards.

Materials synthesis: Unless otherwise indicated, all reagents 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 drybox techniques under an inert atmosphere of dry argon. 3-Bromohexylthiophene and 2,5-dibromo-3-bromohexylthiophene were prepared according to a reported procedure.[29]

Preparation of poly(3-bromohexylthiophene) (polymer 1): Regioregular head-to-tail conjugated poly(3-bromohexylthiophene) was prepared according to a reported procedure.[29] 3-Bromohexyl-2,5-dibromothiophene (**2**) (2.02 g, 5.0 mmol) was added to dry THF (30 mL) containing CH_3MgBr (1.7 mL, 5 mmol). [$NiCl_2$(dppp)] (14 mg, 0.03 mmol) was added after the mixture had been stirred at 75 °C for 2 h. After further stirring at 75 °C for 1 h, the resulting solution was cooled to room temperature and poured into methanol (200 mL) to precipitate the polymer. The solid polymer was filtered, further purified by extraction in a Soxhlet extractor with refluxing methanol for 24 h, and dried under vacuum to give 0.9 g of 98% regioregular head-to-tail coupled polymer **1**. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.97$ (s, 1H), 3.41 (t, 2H), 2.81 (m, 2H), 1.87 (m, 2H), 1.71 (m, 2H), 1.48 ppm (m. 4H); GPC (THF, polystyrene standard): M_n: 25,900 g mol^{-1}, polydispersity: 1.75; displays UV/Vis absorption maxima at $\lambda_{max} = 400$ nm and emission maxima at $\lambda_{max} = 558$ nm in chloroform solution.

Preparation of polythiophene bearing boronic acid residues (polymer 2): 3-Pyridineboronic acid (0.3 g) was added to a solution of polymer **1** (0.2 g) dissolved in a mixture of DMF (30 mL) and THF (20 mL). The flask was degassed and refilled with N_2. The mixture was stirred at 70 °C under a N_2 atmosphere for 48 h. After removing most of the solvent, the residue was precipitated in THF (60 mL). The precipitate was collected by filtration, washed with THF (100 mL) several times, and dried under vacuum at room temperature to obtain polymer **2** as a dark-red solid. ^{1}H NMR (400 MHz, D_2O): $\delta = 8.49$ (m, 4H), 7.74 (m, 2H), 7.50 (s, 1H), 4.38 (m, 2H), 3.38 (m, 2H), 1.84 (m, 4H), 1.30 ppm (m, 4H); we failed to collect a nice ^{13}C NMR spectrum as the polymer concentration in water is not high enough for the experiment; displays UV/Vis absorption maxima at $\lambda_{max} = 410$ nm and emission maxima at $\lambda_{max} = 570$ nm in aqueous solution; IR (KBr): $\tilde{\nu} = 3339$, 2920, 1630, 1376, 1055, 793, 678 cm^{-1}.

Acknowledgements

We acknowledge the Research Excellence Fund of Michigan Tech, 21st Century Jobs Fund of Michigan and the United States Department of Agriculture (contract number: 2007-35603-17740) for support of this work through the National Research Initiative Competitive Grants Program.

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Received: August 31, 2007
Revised: October 9, 2007
Published online: November 28, 2007

