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Polymeric Monosaccharide Receptors Responsive at Neutral pH

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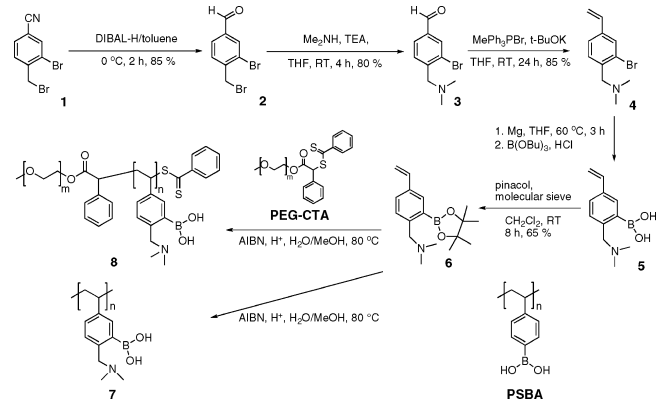
Binding of saccharides to organoboronic acids via the formation of reversible covalent bonds has been studied extensively due to its practical importance in the development of sensors and drug releasing platforms for glucose-related human disorders such as diabetes.[1] In particular, polymers and block copolymers containing boronic acid moieties are attracting recent interest as stimuli-responsive materials that switch their solubility in water in correspondence to the glucose concentration in the medium.[2−4] However, the sugar-responsive behavior of these materials requires aqueous media of relatively high pH (>9) because the hydrolysis of boronate esters is favored at neutral pH conditions.[5] This high pH medium requirement for stimuli-responsiveness is a disadvantage for the use of boronic acid containing polymers under physiological conditions.

Wulff ingeniously showed that aromatic boronic acids having *o*-dialkylaminomethyl groups (Wulff-type boronic acids) exhibit a lower pK_a value due to the intramolecular interaction between B and N atoms, thus stabilizing boronate esters at neutral pH.[6] Shinkai and co-workers utilized this concept to develop fluorescent sensors detecting glucose in a methanol/water mixture at neutral pH.[7] The incorporation of these Wulff-type boronic acids into polymers would be a highly attractive method to create sugar-responsive materials, which could operate under physiological conditions. However, until now, these materials have hardly been studied, presumably due to the unavailability of procedures for the synthesis of monomers that can be polymerized in a controlled manner.[8] Here we present the first detailed report of the synthesis of Wulff-type styrenic monomers and their polymerization by radical addition−fragmentation chain transfer (RAFT) methods. The resulting polymers and block copolymers exhibit sugar-responsive solubilization in aqueous buffer solutions (pH = 7.4−7.8) in the presence of monosaccharides such as D-fructose and D-glucose.

Wulff-type styrenic boronic acid **5** was synthesized from 3-bromo-4-bromomethylbenzonitrile (**1**) in five steps (Scheme 1, Supporting Information (SI)). To facilitate purification and characterization, boronic acid **5** was reacted with pinacol to yield the pinacol boronate ester **6**. Compound **6** was obtained as a white crystalline solid in 65% yield from **5**. X-ray crystallographic studies on **6** (Figure 1A) revealed the presence of a N−B dative bond (1.735 Å) and a strained geometry of the B center (tetrahedral character 58.3%).[9]

Compound **6** could not be polymerized by any free radical or controlled radical polymerization method. We presumed that the steric hindrance imposed by the bulky pinacol boronate on the *m*-position with respect to the vinyl group prevented any further propagation after initiation. Therefore, we tried a free radical polymerization of **6** in an acidic water/methanol mixture (1:1 by volume, pH ≈ 3). Under these conditions, pinacol boronate was hydrolyzed *in situ*, yielding **5**, which could be consequently polymerized. The polymerization was performed at 80 °C for 8 h. After raising the pH of the reaction mixture to ca. 9 by adding aqueous 1 M NaOH, the solution was dialyzed against pure water

Scheme 1. Synthesis and Polymerization of Wulff-Type Boronic Acid Monomer, Its Pinacol Boronate Ester



for 24 h. Precipitation of the aqueous solution into dioxane gave **7** as a white powder. Polymer **7** was insoluble in most common organic solvents except in methanol. Protection of **7** with pinacol only gave partially protected polymers because of the steric reason. Molecular weight determination of these materials by GPC ($CHCl_3$) was unsuccessful due to the high affinity of the polymer to the GPC column material. However, size exclusion chromatography in 70% formic acid (pH = 1) showed a single peak indicating that the polymerization was successful (Figure 1B).

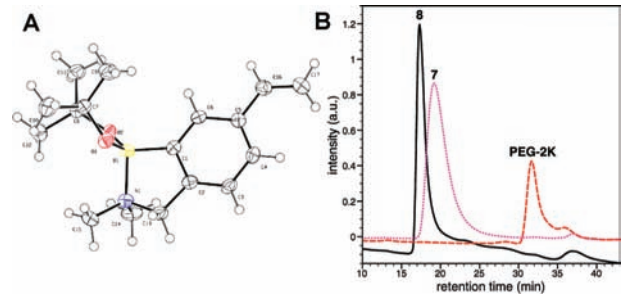


Figure 1. (A) X-ray crystal structure of **6** (ellipsoids are shown at 50% probability). Selected bond lengths (Å) and angles (deg): B_1-N_1 1.735 (2), C_1-B_1 1.607(2), B_1-O_2 1.432(2); $C_1-B_1-N_1$ 95.57(11), $O_2-B_1-O_1$ 107.67(13), $O_2-B_1-N_1$ 112.49(12), $O_2-B_1-C_1$ 117.38(13), $C_{15}-N_1-C_{14}$ 109.09(14). (B) SEC traces of **7** and **8** (70% formic acid, pH = 1) compared to PEG (M_n = 2000 g/mol).

To investigate the possibility of applying this monomer in the synthesis of stimulus responsive block copolymers, we performed a RAFT polymerization of **6** by using a poly(ethylene glycol)-chain transfer agent (PEG-CTA, M_n = 2000 g/mol)[10] (Scheme 1). For polymerization to occur, compound **6** was again hydrolyzed in situ in an acidic water/methanol mixture (pH ≈ 3). The polymerization was conducted for 24 h at 80 °C ([M]:[CTA]:[I] = 100:1:0.2, conversion >90% by ^{1}H NMR), and the resulting block copolymer was thoroughly purified by dialysis and washing with $CHCl_3$ to

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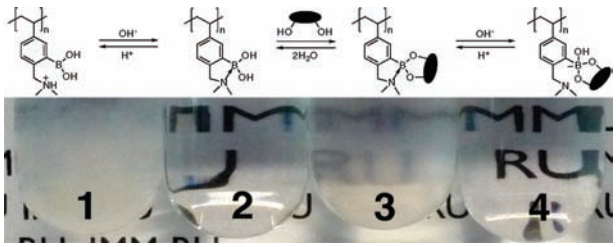

Figure 2. A schematic representation of the polymer **7** with monosaccharides in different pH environments. The photograph shows tubes containing the polymer **7** in different buffers after 18 h. Tube 1: **7** in PBS (pH = 7.4), 2: in PBS/D-fructose (50 mM), 3: in PBS/D-glucose (100 mM), 4: in TRIS/D-glucose (100 mM, pH = 7.8).

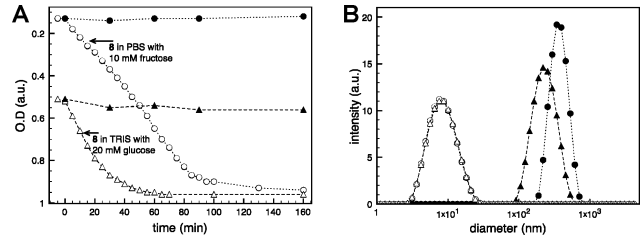

Figure 3. (A) Optical density measurement of the colloidal solution of **8** in PBS (●) and in TRIS (▲) buffers. Upon addition of fructose (10 mM, ○) or glucose (20 mM, △), optical density decreases. (B) DLS results of the colloidal solutions (●: **8** in PBS, D_{avg} = 335 nm; ▲: **8** in TRIS, D_{avg} = 194 nm) and dissociated **8** with monosaccharides in the same buffers (○, △: D_{avg} = 9.2 nm).

remove any residual impurities. The degree of polymerization of the purified block copolymer **8** was ~200 as determined by ^{1}H NMR integration (estimated M_n = 43 000 g/mol, Figure S1 in SI). The resulting block copolymer **8** was soluble in pure water and methanol but remained insoluble in neutral pH buffers. SEC of this block copolymer (Figure 1B) clearly indicated that **8** was successfully prepared by showing a sharp signal free from any residual PEG-CTA.

Boronic acid containing polymers increase their solubility in water upon binding of saccharides to the boronic acid moieties.[3,4] We first screened the solubility of polymer **7** in aqueous media at varying pH's.[11] The polymer was well soluble when the pH of the medium was in a weakly acidic (<6) or in a moderately basic (>9) region. Polymer **7** remained insoluble in a PBS buffer (pH = 7.4) until D-fructose (50 mM, 3.4 equiv to boronic acid) was added. Addition of D-glucose (100 mM, 6.8 equiv to boronic acid) did not solubilize polymer **7** in PBS but rendered **7** to be completely soluble in a TRIS/HCl buffer (pH = 7.8) (Figure 2). Dynamic light scattering results on the buffer solutions of **7** with monosaccharides showed average hydrodynamic diameters (D_{avg}) of 11.2 nm for the fructose/PBS solution and 12.6 nm for the glucose/TRIS solution, which indicates the presence of molecularly dissolved species (Figure S2 in SI). The solubilized **7** in the buffer/monosaccharide turned insoluble upon dialysis against buffers (MW cutoff: 13 000 g/mol, 24 h), indicating that the sugar responsiveness of **7** is reversible with respect to the concentration of monosaccharides. For comparison, we checked the solubility of poly(styrene-4-boronic acid) (PSBA, Scheme 1) in the same monosaccharide/buffer media. The polymer remained insoluble after a prolonged time (3 weeks).

When **8** was dispersed in PBS and TRIS/HCl buffers with the aid of THF (15% by volume) colloidal suspensions were formed.[12] As Figure 3 shows, these suspensions remained stable when no monosaccharides were added. Upon addition of monosaccharides (10−20 mM), the colloidal particles of **8** dissociated into smaller objects (D_{avg} = 9.2 nm), and the turbidity of the solution disappeared with time. The results therefore indicate block copolymer **8** exhibits similar sugar responsiveness as the homopolymer **7** in neutral pH buffers.

These results indicate that Wulff-type boronic acids in polymers **7** and **8** indeed bind with monosaccharides under neutral pH conditions due to the presence of the intramolecular B−N interaction, which lowers the pK_a of boronic acid. Given the low binding efficiency of D-glucose to Wulff-type boronic acids in pure water,[13] it is clear that numerous weak bindings of D-glucose to the boronic acids of **7** and **8** must contribute to the solubility change of the polymer. These results demonstrate that Wulff-type boronic acid containing polymers may be utilized as sugar-responsive materials under physiological conditions, which removes one of the main limitations of the currently applied materials. Following up on the synthetic procedures described here, we are currently working on the design of polymers with well-defined architectures to be used in glucose sensing and drug delivery.

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Supporting Information Available: X-ray crystallographic data of **6** (CIF) and synthetic details and characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) In all cases, the solid powder of **7** (3 mg) in the solvent (1 mL) was sonicated for 1 min and stored at room temperature up to 16 h. The solubility was decided visually after this time.
(12) **8** in THF/MeOH was dispersed in PBS or TRIS/HCl with a vigorous stirring. THF was evaporated from the suspension in an open air for 24 h. The morphologies of aggregates of **8** could not be determined by a conventional TEM technique due to the dissolution of aggregates during the sample preparation.
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