Monolithic Materials

DOI: 10.1002/anie.200902469

Ring-Opening Polymerization with Synergistic Co-monomers: Access to a Boronate-Functionalized Polymeric Monolith for the Specific Capture of *cis*-Diol-Containing Biomolecules under Neutral Conditions**

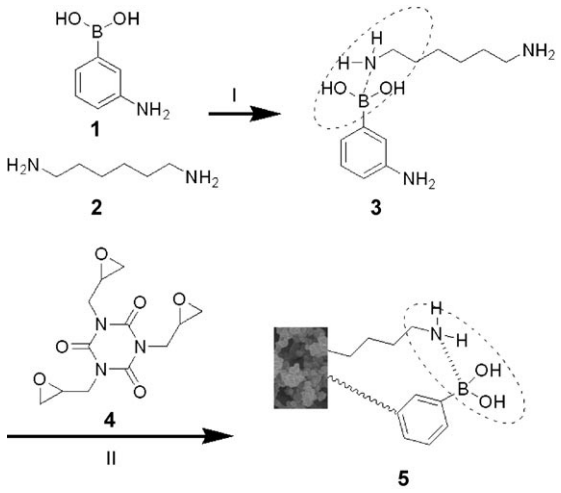
Lianbing Ren, Zhen Liu, Yunchun Liu, Peng Dou, and Hong-Yuan Chen*

Polymeric monoliths, which show fast convective mass transfer between the monolith bed and the surrounding solution, have already become important materials in separation science and (bio-)catalysis.[1] Although some purely polymeric monoliths (such as acrylate-based monoliths) are excellent separation media, functionalization is of utmost importance in most situations. Conventional functionalization can be classified into two strategies: 1) copolymerization of functional monomers[2] and 2) postpolymerization functionalization.[3] In both cases, the designed functionalities depend on the structure and properties of functional monomers. To obtain a certain functionality, a high-purity monomer with appropriate structure and properties is indispensable.

Boronate affinity chromatography (BAC) has been a useful means for the specific capture and separation of *cis*-diol-containing biomolecules, such as saccharides, nucleosides, and glycoproteins, since the early 1980s.[4] The principle relies on reversible covalent complex formation/dissociation between boronic acids and *cis* diols in an alkaline/acidic aqueous solution. Recently, boronate-functionalized monoliths were synthesized by copolymerization[5] and postpolymerization functionalization.[6] However, as for other BAC-based techniques, an apparent disadvantage is that the chromatography in aqueous solution has to be performed in alkaline media and can lead to the degradation of labile compounds. Thus, boronate-functionalized monoliths that function at neutral pH would be highly desirable for physiological samples.

A conventional solution to this problem is to decrease the pK_a value of the ligands by synthesizing novel boronic acids with exquisite structures through: 1) the introduction of an electron-withdrawing group, such as a sulfonyl group, into the ligand molecules,[7] 2) the introduction of a neighboring amino group capable of B–N coordination into the ligand molecules (Wulff-type boronic acids),[8] or 3) the replacement of intramolecular B–N coordination with intramolecular B–O coordination (improved Wulff-type boronic acids).[9] According to these strategies, to make a boronate-functionalized monolith that is functional under neutral conditions, a boronic acid monomer with a low pK_a value, if not commercially available, must be synthesized and purified first through tedious procedures. Herein, we present a new approach—ring-opening polymerization with synergistic co-monomers—for the preparation of a boronate-functionalized polymeric monolith that functions under neutral conditions, without the synthesis and purification of a single functional monomer.

The main synthetic route is based on the ring-opening polymerization protocol established recently by Tanaka and co-workers.[10] An epoxy resin, a diamine curing agent, and a porogenic solvent are required for the preparation of the monolith. To obviate the inconvenience of the synthesis of a new diamine monomer, we took advantage of the coordination of *m*-aminophenylboronic acid (mPBA, **1**) with 1,6-hexamethylenediamine (HMDA, **2**) to form a stable complex **3** with a B–N bond (Scheme 1). The B–N-coordinated complex was used as a diamine curing agent in a ring-opening polymerization reaction with the epoxy resin tris(2,3-epoxypropyl)isocyanurate (TEPIC, **4**) to form a macroporous monolith. Since the conformation of the coordinated complex is "frozen" by the polymerization, the coordinated complex



Scheme 1. I) B–N coordination and II) ring-opening polymerization with synergistic co-monomers.

[*] L. Ren, Dr. Z. Liu, Y. Liu, P. Dou, Prof. H. Y. Chen
Key Laboratory of Analytical Chemistry for Life Science
School of Chemistry and Chemical Engineering
Nanjing University
22 Hankou Road, Nanjing 210093 (China)
Fax: (+86) 25-8368-5639
E-mail: zhenliu@nju.edu.cn

[**] We gratefully acknowledge the financial support of a General Grant (No. 20675038) from the National Natural Science Foundation of China, a Key Grant of 973 Program (No. 2007CB914102), an International Science & Technology Cooperation Grant (2008DFA01910) from the Ministry of Science and Technology of China, and a General Grant (No. KB2008258) to Z.L. from the Natural Science Foundation of Jiangsu Province.

on the resulting monolith **5** can function like a Wulff-type boronic acid ligand and was expected to be capable of capturing *cis*-diol-containing molecules under neutral conditions.

The mechanism for reversible capture/release upon changes in the pH value of the medium is illustrated in Scheme 2. In an aqueous medium at pH 7, the nitrogen-coordinated boronic acid reacts covalently with a *cis*-diol-containing compound to form a five- or six-membered ring. When the medium is made more acidic, the amine group is protonated, and the B–N coordination is broken, which results in the release of the *cis* diol from the monolith. When the medium is neutralized again, the protonated amine group is deprotonated, with recovery of the B–N coordination. Thus, the pH-controlled capture/release mechanism can be employed for affinity chromatographic separation at physiological pH values.

To verify the expected intermolecular B–N coordination, ^{11}B NMR spectroscopic measurements were carried out for

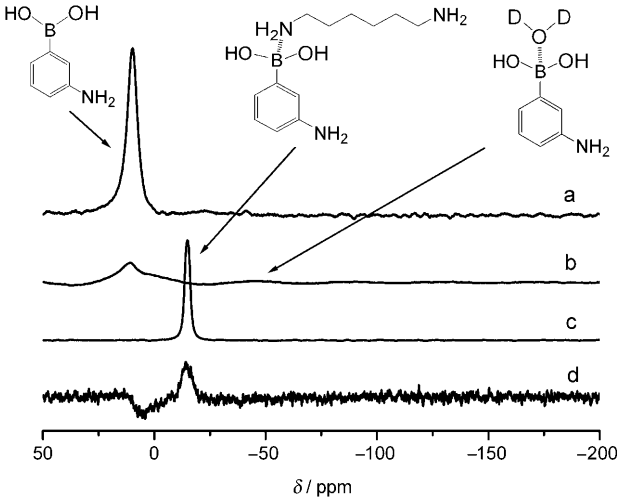
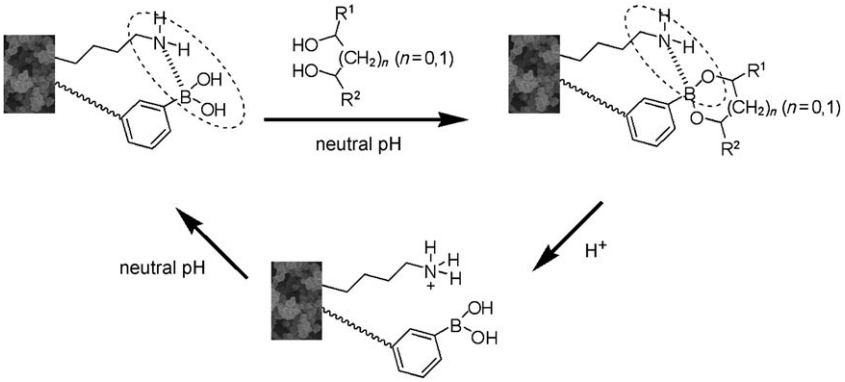


Figure 1. ^{11}B NMR spectra of mPBA dissolved in a) $CDCl_3$, b) D_2O, c) $CDCl_3$ containing HMDA, and d) D_2O containing HMDA.



Scheme 2. Intermolecular synergistic action for the reversible capture/release of *cis*-diol-containing compounds.

mPBA in different media. mPBA was first dissolved in PEG 200 (the porogenic solvent) with and without the addition of HMDA in a 1:1 molar ratio. The mixture was then dissolved in an aprotic ($CDCl_3$) or a protic solvent (D_2O). A signal was observed at $\delta = 9.8$ ppm in $CDCl_3$, and the same signal was observed along with another signal at $\delta = -42.1$ ppm in D_2O (D_2O-solvated mPBA; Figure 1). Upon the addition of HMDA, the chemical shifts of mPBA at $\delta = 9.8$ and -42.1 ppm disappeared, and a new peak at -14.2 ppm was observed. This peak can be assigned to the nitrogen-coordinated aryl boronic acid. These results indicate that the expected intermolecular B–N coordination did occur under conditions similar to those used for the ring-opening polymerization.

With coordinated mPBA and HMDA as the co-monomers, TEPIC as the crosslinker, and PEG 200 as the porogenic solvent, boronate-functionalized monolithic capillaries were prepared by the proposed approach. The obtained monoliths exhibited a well-controlled skeletal network and a well-distributed macroporous open-channel network. Scanning electron microscopy (SEM) images of a representative monolith are shown in Figure 2. A mercury intrusion measurement indicated that macropores of approximately 3 μm in diameter contributed a total intraparticle porosity of 64.3%, whereas mesopores contributed a total interparticle porosity of 18.6%. The average size of mesopores was estimated to be 9.6 nm by a Brunauer–Emmett–Teller (BET) measurement corresponding to a pore volume of 0.047 $cm^3 g^{-1}$ and a specific surface area of 19.9 $m^2 g^{-1}$. These results suggest that the prepared monolith followed the typical bicontinuous pore-distribution model.[11] The loading capacity of the prepared monolithic capillaries at pH 7.0 was measured to be 1.56 μmol mL^{-1} by frontal chromatography with catechol

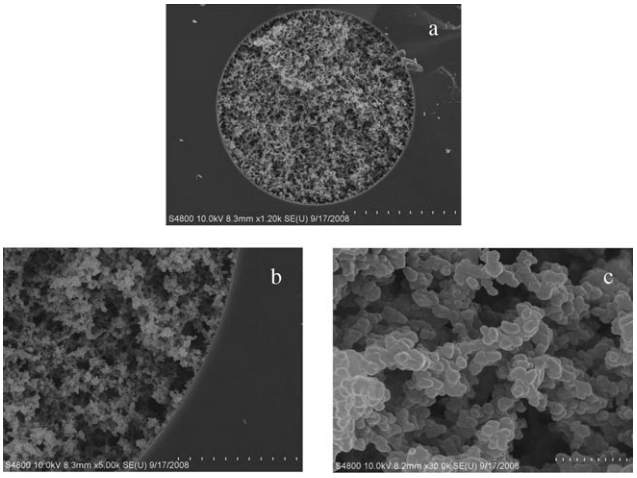


Figure 2. Scanning electron microscopy photographs of the cross-section of the prepared monolithic capillary at different magnifications: a) 1200× (distance between scale lines bottom right: 40.0 μm); b) 5000× (distance between scale lines: 10.0 μm); c) 30000× (distance between scale lines: 1.00 μm).

as the test compound. For comparison, the value for a boronate-functionalized monolithic column without intermolecular coordination was zero.[5]

The expected functionality of the prepared monolithic column was first evaluated on the basis of the affinity chromatographic separation of catechol and quinol. Catechol is a *cis* 1,2-diol, whereas quinol is a non-*cis* diol analogue for control experiments. Quinol showed no retention on the monolithic column at all the pH values investigated. In contrast, catechol was not retained at pH 2.7, but its retention was enhanced significantly when the pH value was increased (Figure 3). At pH 7.0 and 7.5, catechol was completely trapped by the column, and its elution needed an acidic

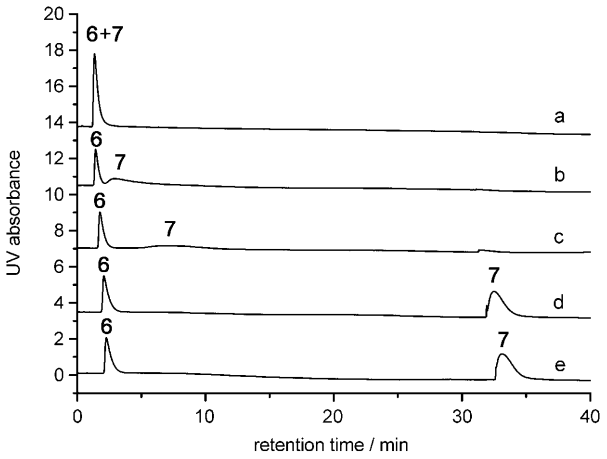


Figure 3. Chromatographic retention of quinol (**6**) and catechol (**7**) on the boronate-functionalized monolithic column. Mobile phase: a) 100 mM HAc (pH 2.7); b–e) 10 mM sodium phosphate buffer at pH 6.0, 6.5, 7.0, and 7.5, respectively; the mobile phase was changed to 100 mM HAc (pH 2.7) at 30 min. Sample: Quinol and catechol were dissolved in the mobile phase at a concentration of 1 mg mL^{-1} each.

mobile phase (pH 2.7). In contrast, it was reported that commercial mPBA–agarose affinity materials and a poly(4-vinylphenylboronic acid-co-ethylene glycol dimethacrylate) monolith did not capture catechol at pH < 8.0.[5,12] Thus, the pH value at which capturing was possible was decreased by at least one pH unit by the monolith with synergistic co-monomers.

The enrichment of adenosine (a *cis* 1,2-diol) demonstrates the usefulness of the boronate-functionalized monolith. When an acidic mobile phase (pH 2.7) was used, adenosine showed no retention and exhibited a relatively broad peak (Figure 4). When the sample was prepared with a PBS buffer (10 mM sodium phosphate, pH 7.4), loaded onto the column with the same buffer, and then eluted with the acidic mobile phase, adenosine was enriched, and the peak became sharper (the enrichment of catechol needs a mobile phase containing an organic solvent at a certain concentration to offset the hydrophobic retention).

In summary, a new approach to the preparation of boronate-functionalized monoliths has been developed: ring-opening polymerization with synergistic co-monomers. The prepared boronate-functionalized monoliths were able to

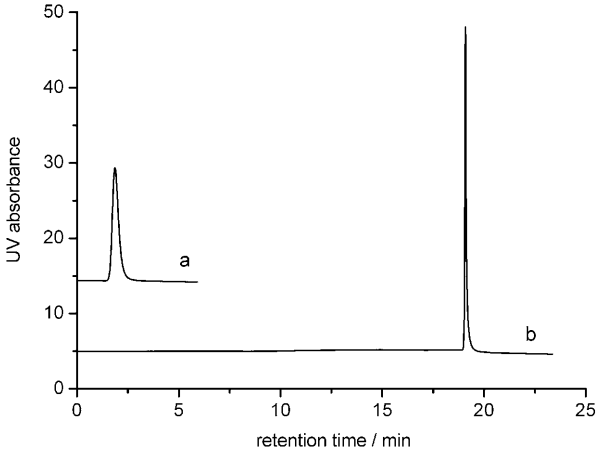


Figure 4. Chromatographic retention and enrichment of adenosine on the boronate-functionalized monolithic column. Mobile phase: a) 100 mM HAc (pH 2.7); b) 10 mM sodium phosphate buffer (pH 7.4); the mobile phase was changed to 100 mM HAc (pH 2.7) at 15 min. Sample: adenosine (1 mg mL^{-1}) in 10 mM sodium phosphate buffer (pH 7.4).

capture *cis*-diols under neutral conditions, which should facilitate the application to physiological samples. Unlike conventional strategies for monolith preparation and the lowering of the pH values at which they function, the proposed approach substitutes two coordinated functional co-monomers for a single functional monomer and obviates tedious synthesis and purification procedures. Thus, the concept of intermolecular synergism provides a new method for the functionalization of polymer monoliths and Wulff-type boronic acids. Monoliths prepared by the proposed approach may be promising for glycocapture in glycoproteomics and glycomics.

Experimental Section

A capillary with an inner diameter of 75 or 150 μm was rinsed sequentially with 1M NaOH, water, 1M HCl, and water for 30 min each. The capillary was then dried with a stream of nitrogen in a GC oven at 60 °C. A 1:1 (v/v) mixture of tetrahydrofuran and (3-aminopropyl)triethoxysilane were pumped through the capillary at 80 °C for 24 h. The modified capillary was then washed with methanol. TEPIC (0.32 g), mPBA monohydrate (0.055 g), and HMDA (0.041 g) were dissolved completely in PEG 200 (2.8 g). This solution was injected into the modified capillary with a syringe. The polymerization reaction was carried out at 80 °C for 12 h, and the capillary was then washed with water and methanol.

A TriSep-2100 pCEC instrument (Unimicro Technologies, Pleasanton, CA, USA) with a UV-absorbance detector was used for the chromatographic separations. A boronate-functionalized monolithic column with a total length of 50 cm (effective length: 35 cm) was used in all experiments. A flow rate of 0.15 mL min^{-1} was used with the splitting ratio set at 99:1; the UV absorbance was monitored at 275 nm. The volume of each sample was 20 nL.

^{11}B NMR spectra were recorded on a Bruker Avance DMX 500 MHz instrument at 160 MHz. A solution of BF_3OEt_2 was used as an external reference ($\delta = -18.2$ ppm). mPBA monohydrate (0.055 g) was dissolved in PEG 200 (0.5 g) with or without the addition of HMDA (0.041 g), and the solution was added to $CDCl_3$ or D_2O (0.4 mL) for measurement.

For characterization of the pore-size distribution and specific surface area, a monolith bed was synthesized in an empty regular HPLC column. The monolithic column was washed with water and methanol until the residual reagents had been flushed out. The obtained monolith bed was dried and removed from the column for characterization.

Received: May 9, 2009
Revised: June 20, 2009
Published online: July 30, 2009

Keywords: boronate affinity chromatography · intermolecular synergism · molecular recognition · monolithic materials · ring-opening polymerization

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