Supporting Information

for

A Wulff-type boronate for bornoate affinity capture of cis-diol compounds at medium acidic pH condition

Hengye Li, Yunchun Liu, Jing Liu and Zhen Liu *

* *Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, China.*

* To whom correspondence should be addressed: Dr. Zhen Liu, phone/fax 86-25-8368 5639. E-mail: zhenliu@nju.edu.cn

Experimental procedures

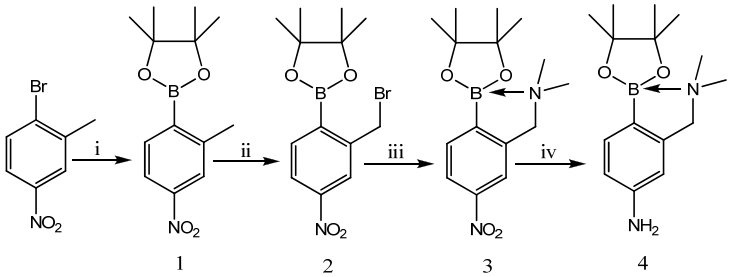
Materials. 2-bromo-5-nitrotoluene (98%) was purchased from Fuxin Xinrui Fine Chemical Research Institute (Fuxin, China) and passed through a silica column for further purification before use. All other reagents and solvents were purchased from commercial suppliers and used without further purification. Water used in all of the chromatographic experiments was purified by a Milli-Q Advantage A10 Ultrapure Water Purification System (Millipore, Milford, MA, USA).

Analyses. The synthesized compounds were characterized by nuclear magnetic resonance (NMR) and mass spectroscopy (MS). ^{1}H NMR, ^{13}C NMR and ^{11}B NMR spectra were recorded on a Bruker Avance DMX 500 MHz instrument (Bruker Biospin GmbH, Rheinstetten, Germany) using the solvent residual as peak as reference (7.26 ppm). $BF_3 \cdot OEt_2$ was used as an external reference for ^{11}B NMR spectra. Mass spectra were recorded on a Micromass GC-TOF mass spectrometer (Manchester, England，or Waters/Micromass UK Ltd., Manchester, UK) with electron ionization (EI) energy set at 70 ev. Scanning electron microscopy (SEM) analyses were performed on a Hitachi FE-SEM S-4800 (Tokyo, Japan). Nitrogen adsorption-desorption measurements were conducted at 77 K on an ASAP2020 instrument (Micromerities, Norcross, GA, USA). The FT-IR spectrum was acquired on a Thermo Nicolet iS10 FT-IR spectrometer (Waltham, MA, USA). The ^{11}B Magic Angle Spinning (MAS) NMR spectra was recorded on a Bruker AVANC III 400 MHz instrument (Bruker Biospin GmbH, Rheinstetten, Germany) using $B(OH)_3$ as reference. X-ray photoelectron spectra (XPS) were collected with a Thermo Fisher Scientific K-Alpha spectrometer with monochromatic Al Kα radiation (1,486.6 eV).

All chromatographic experiments were carried out on a TriSep-2100pCEC instrument (Unimicro Technologies, Pleasanton, CA, USA) with a UV-absorbance detector. A flow rate of 0.1 or 0.15 mL min^{-1} was used with the splitting ratio of 100:1. The UV wavelength was set at 260 nm. Micellar electrokinetic chromatographic (MEKC) analyses were carried out on a Beckman Coulter P/ACE MDQ system (Beckman Instruments, Fullerton, CA, USA). An untreated fused silica capillary of 56.5 cm (50 cm to detector) × 50 μm I.D. was used for the

separation. The running buffer was 25 mM borate-50 mM phosphate buffer containing 300 mM sodium dodecyl sulfate (SDS), pH 6.7. The applied voltage for the separation was 9.0 kV, The capillary temperature was set at 30 ^{o}C. Sample was injected at 0.5 psi for 5 s. The UV absorbance detection wavelength was set at 256 nm.

1 The synthesis of amino-functionalizationed wulff- type boronate 4



Scheme S1. The synthesis of the amino-functionalized wulff-type boronate.

Conditions: (i) bis(pinacolato)diborane, KOAc, Pd(dppf)Cl_2, dioane, 100 ^{o}C ; (ii) NBS, AIBN, CCl_4, reflux; (iii) dimethylamine, ether, 0 ^{o}C; (iv) Fe, NH_4Cl, ethanol-H_2O, 100 ^{o}C.

(a) The synthesis of 5-nitrotoluene-2-pinacol boronate (1)

Compound 1 was prepared by a method by Prieto et al[1] with modification. 2-bromo-5-nitrotoluene (10.02 g, 46.62 mmol), bis(pinacolato)diborane (13.00 g, 51.14 mmol), KOAc (12.80 g, 130.62 mmol), Pd(dppf)Cl_2 (0.42 g, 0.57 mmol) were charged in a round bottom flask. The flask was vacuumed and refilled with nitrogen three times. Dry 1,4-dioxan (220 mL) was added and the suspension was stirred and heated at 100 ^{o}C under nitrogen atmosphere. Five minutes later, triethylamine (1 mL) was added into the mixture. Then the mixture was stirred and heated continually at 100 ^{o}C for 12 h. After cooling the mixture was diluted by adding 220 mL of ethyl acetate. The mixture was filtered and the solvent was extracted successively with water twice and brine once. The organic layer was collected and dried with anhydrous $MgSO_4$. The filtered solution was evaporated. The resulting crude product was purified by silica gel column chromatography (Hexane/EtOAc 50:1 v/v). A light brown oil was obtained. Yield: 8.62 g (70%). ^{1}H NMR (500 MHz, $CDCl_3$)

δ8.02 (1H, s), 7.98 (1H, dd, J = 1.5 and 1.5 Hz), 7.92 (1H, d, J = 8Hz), 2.64 (3H, s), 1.39 (12H, s). ^{13}C NMR (500 MHz, $CDCl_3$) δ136.7, 123.7, 119.3, 77.3, 24.7, 21.9. ^{11}B NMR (500 Hz, $CDCl_3$) δ33.95. MS (EI^+) *m/z*: [249.2 (1.30), 248.1 (100), 247.1 (37.2)] (M^+-CH_3).

(b) The synthesis of pinacol 2-bromomethyl-4-nitrophenylboronate (2)

Compound 2 was prepared following the method reported by Jin et al.[2] with modification. Compound 1 (5.30 g, 20.14 mmol) and CCl_4 (150 mL) was charged in a three neck round bottom flask equipped with a reflux condenser. Then N-bromosuccinimide (4.75 g, 26.69 mmol) and azobisisobutyronitrile (0.30 g, 1.83 mmol) was added to the flask resulting into a suspension. The reaction mixture was stirred under reflux for 4 h. After cooling to room temperature the reaction was washed successively with water twice and brine once. The organic layer was collected and dried with anhydrous $MgSO_4$. The filtered solution was concentrated under reduced pressure. The crude product was purified by recrystallization from 10mL of hexane. A write powder was obtained. Yield: 5.47 g (80%). ^{1}H NMR (500 MHz) δ8.25 (1H, d, J = 2Hz), 8.13 (1H, dd, J = 2 and 2 Hz), 8.01 (1H, d, J = 3Hz), 4.95 (2H, s), 1.42 (12H, s). ^{13}C NMR (500 MHz, $CDCl_3$) δ137.5, 124.3, 121.7, 77.3, 31.7, 24.8. ^{11}B NMR (500 MHz, $CDCl_3$) δ33.56. MS (EI^+) *m/z*: [340.1, (0.14), 342.1 (0.25)] (M^+).

(c) The synthesis of pinacol 2-dimethylaminomethyl-4-nitrophenylboronate (3)

Compound 3 was prepared according the method by Zhong and Anslyn.[3] A round bottom flask was charged with NaOH (20 g) and ether (100 mL) and cooled in an ice-salt bath. With stirring a solution of dimethylamine (20 mL, 33% aqueous solution) was added into the flask. The resulting mixture was stirred for 2h under ice-salt bath. The upper layer was poured into another flask carefully which also was cooled in an ice-salt bath. Then a solution of compound 2 (5.00 g, 14.66 mmol) in ether (100 ml) was added drop-wise into the second flask with stirring through a dropping funnel. After stirring for 2 h, the precipitate was filtered and the filtrate was evaporated under reduced pressure obtaining a bright yellow solid. The crude product was mixed with 75 mL of hexane and heated under reflux for 30 min with stirring. The upper clear layer was poured into a beaker immediately and cooled to room temperature. The light yellow crystal was collected by filtration and dried under vacuum at 40

°C. Yield: 4.04 g (89%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 (1H, d, J = 7.5 Hz), 7.90 (1H, s), 7.72 (1H, d, J = 7.5 Hz), 3.97 (2H, s), 2.64 (6H, s), 1.33 (12H, s). ^{13}C NMR (500 MHz, $CDCl_3$) δ 132.0, 122.6, 117.9, 77.2, 64.5, 45.7, 26.7. ^{11}B NMR δ 16.50. MS (EI^+) m/z: [305.2 (2.29), 306.2 (8.75), 307.2 (0.11)] (M^+).

(d) The synthesis of 3-(dimethylaminomethyl) aniline-4-pinacol boronate (4)

Compound 4 was prepared by the method by Wang et al.[4] with modification. Compound 3 (4.00 g, 13.06 mmol) was dissolved in a mixture of ethanol (120 mL) and water (30 mL). The solution was transferred into a round bottom flask to which iron (3.60 g, 64.29 mmol) and ammonium chloride (1.10 g, 20.75 mmol) was added. The mixture was stirred mechanically and heated at 100 °C for 4 h. After cooling, the reaction mixture was filtered and the residue was washed with ethanol (10 mL) five times. The combined filtrate was mixed with anhydrous sodium carbonate (2.20 g, 20.75 mmol) and concentrated under vacuum. The residue was mixed with dichloromethane (50 ml) and dried over anhydrous Na_2SO_4. After filtration, the filtrate was mixed with molecular sieves (6 g, 3.4 Å, activated at 150 °C for 2h before use) and stirred over night. Molecular sieves were removed by filtration and the filtrate was dried under high vacuum. A yellow powder was obtained. Yield: 3.21 g (89%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48 (1H, d, J = 8 Hz), 6.69 (1H, s), 6.62 (1H, d, J = 4.5 Hz), 3.91 (2H, s), 3.79 (2H, s), 2.53 (6H, s), 1.32 (12H, s). ^{13}C NMR (500 MHz, $CDCl_3$) δ 135.5, 114.6, 113.7, 77.0, 62.8, 44.5, 25.7. ^{11}B NMR (500 MHz, $CDCl_3$) δ 22.54. MS (EI^+) m/z: [275.2 (4.82), 276.2 (18.72), 277.2 (0.24)] (M^+).

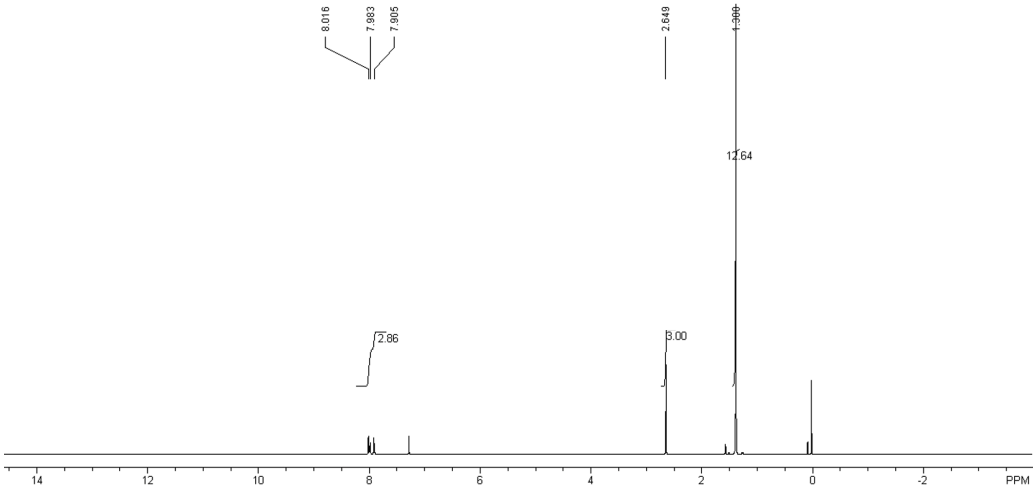


Fig. S1. ^{1}H NMR spectrum of compound 1 in $CDCl_3$.

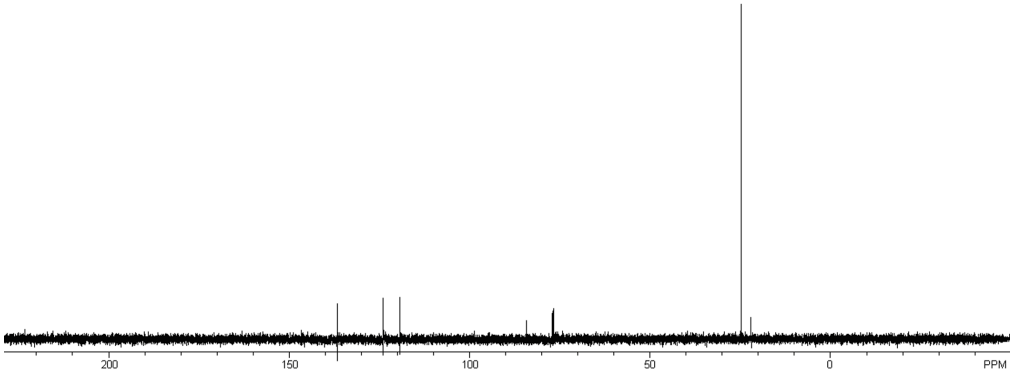


Fig. S2. ^{13}C NMR spectrum of compound 1 in $CDCl_3$.

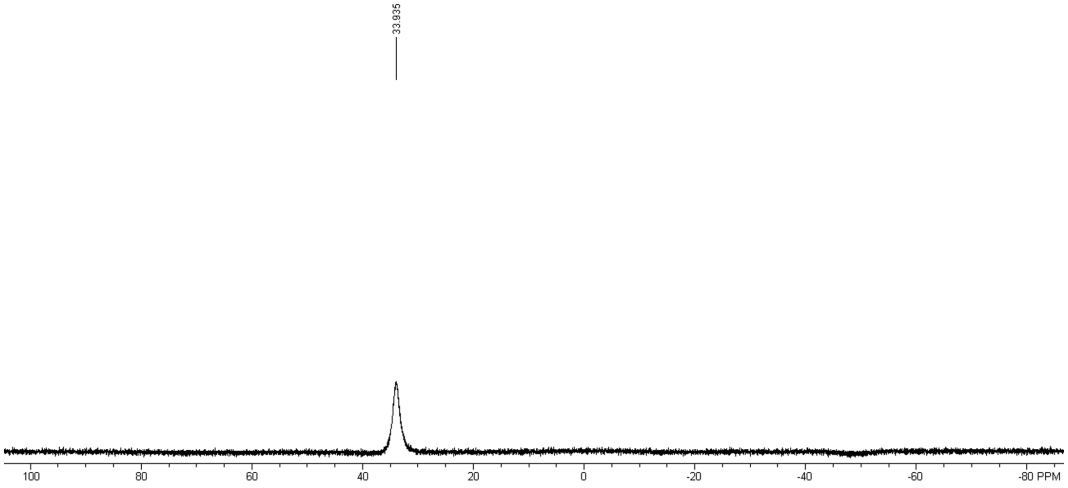


Fig. S3. ^{11}B spectrum of compound 1 in $CDCl_3$.

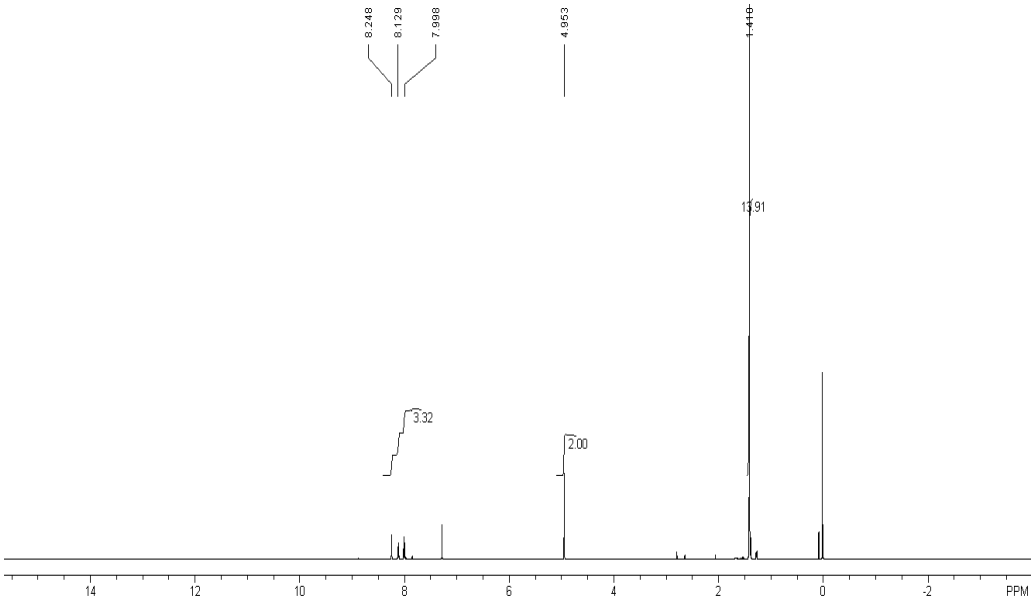


Fig. S4. ^{1}H NMR spectrum of compound 2 in $CDCl_3$.

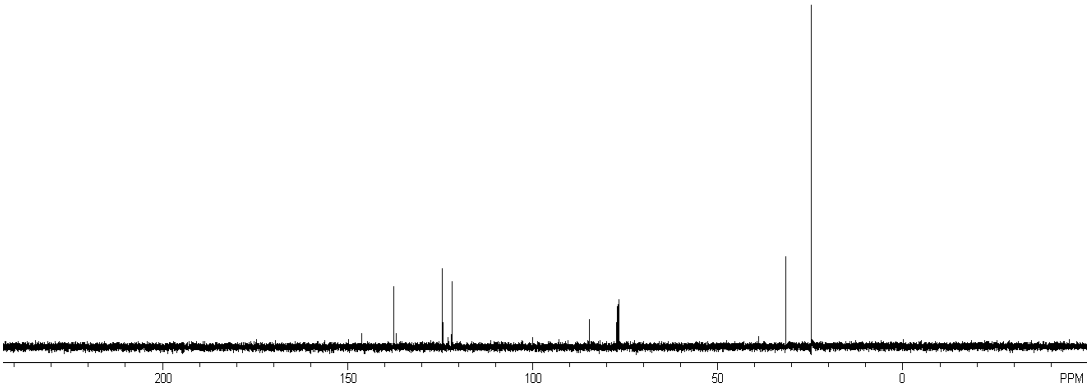


Fig. S5. ^{13}C NMR spectrum of compound 2 in $CDCl_3$

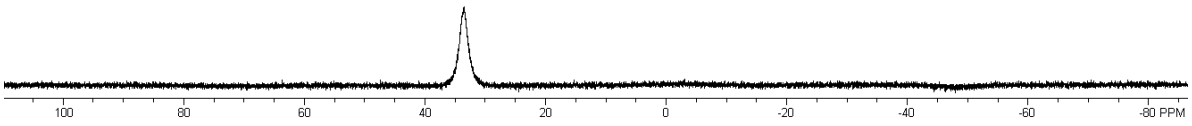


Fig. S6. ^{11}B spectrum of compound 2 in $CDCl_3$.

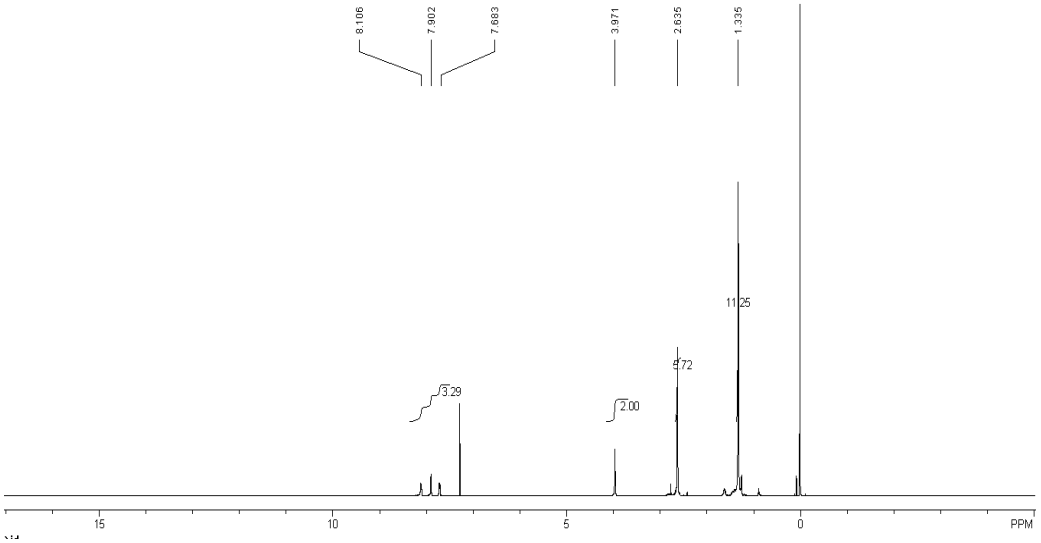


Fig. S7. ^{1}H NMR spectrum of compound 3 in $CDCl_3$.

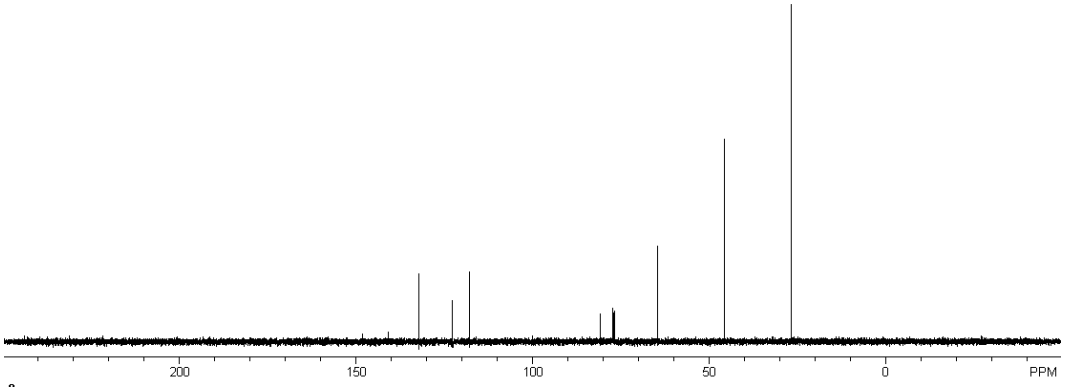


Fig. S8. ^{13}C NMR spectrum of compound 3 in $CDCl_3$

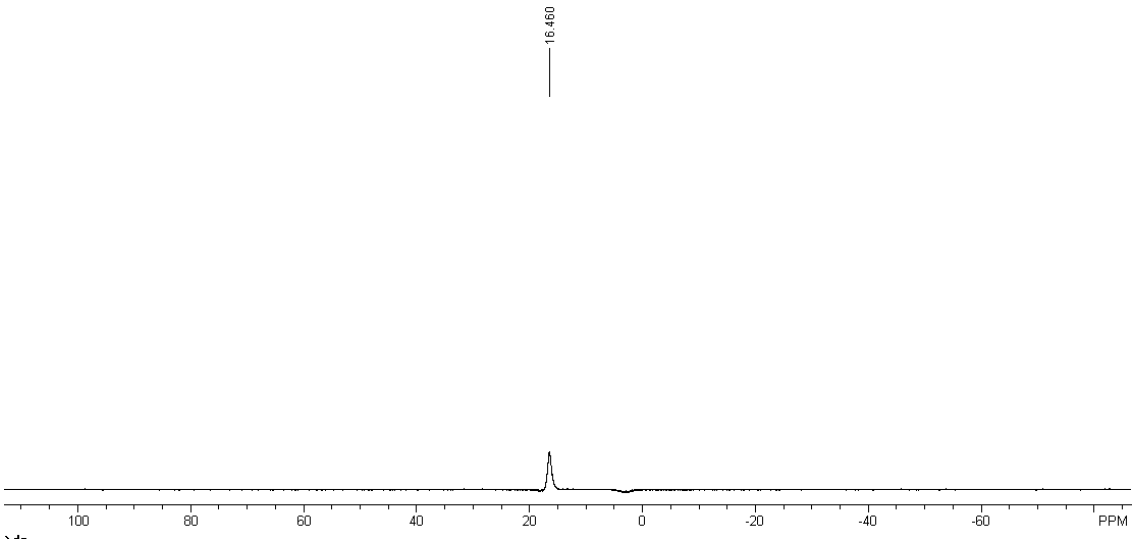


Fig. S9. ^{11}B spectrum of compound 3 in $CDCl_3$

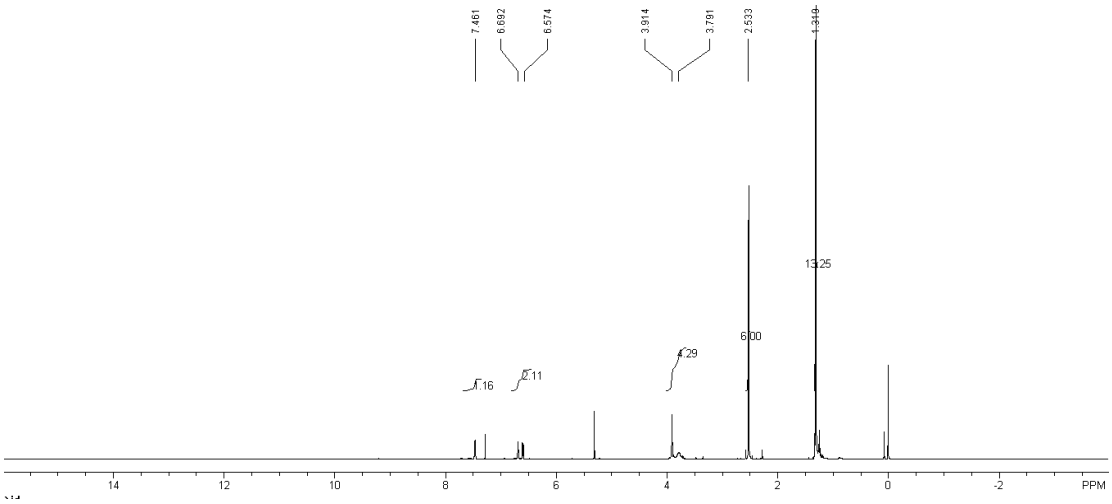


Fig. S10. ^{1}H NMR spectrum of compound 4 in $CDCl_3$.

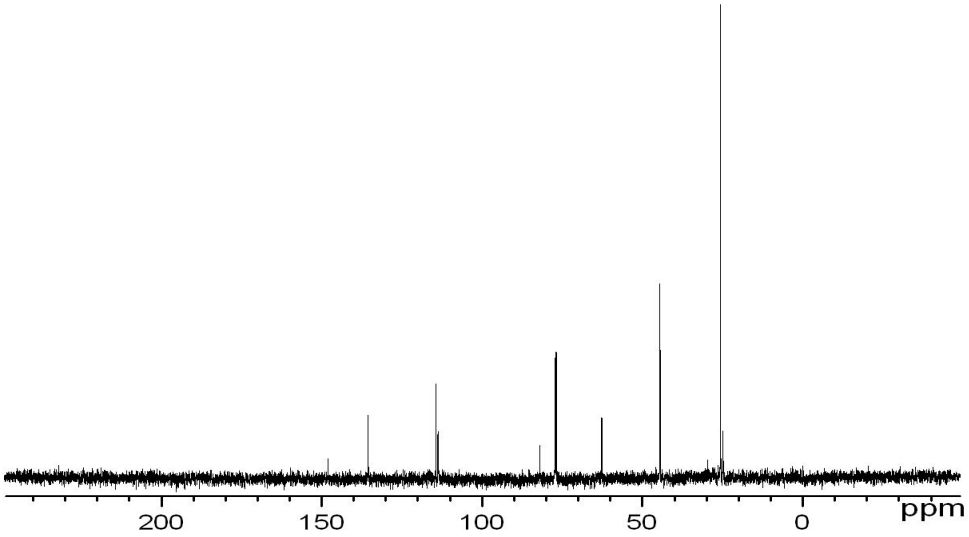


Fig. S11. ^{13}C NMR spectrum of compound 4 in $CDCl_3$.

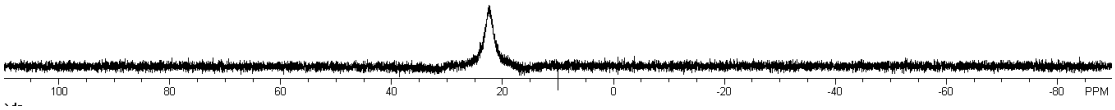


Fig. S12. ^{11}B spectrum of compound 4 in $CDCl_3$.

2 Preparation and characterization of poly (GMA-co-PEGDA) and boronic acid functionalized poly (GMA-co-PEGDA) monoliths

2.1 (a) Preparation of poly (GMA-co-PEGDA) monolithic capillary

Prior to polymerization, pretreatment of the fused-silica capillary with an inner diameter of 150 μm was executed as the following procedure.[5] First, the capillary was successively washed with NaOH (0.1 M), water, HCl (0.1 M), water and methanol for 30 min respectively and then dried by passage of nitrogen gas for 12 h. A solution of r-MAPS (50% v/v in

methanol) was injected into the capillary, and then the capillary was sealed with rubber at both ends and submerged in a water bath at 50 ^{o}C for 12h. Finally, the capillary was washed with methanol to flush out the residual reagent and dried by passage of nitrogen gas overnight. The polymerization reaction of poly (GMA-co-PEGDA) monolith is shown in Fig. S13. A solution consisting of GMA (240 mg), PEGDA (160 mg), DMSO (200 mg), 1,4-butanediol (400 mg) and AIBN (10 mg) was supersonicated for 30 min to obtain a homogeneous solution, then the solution was injected into the pretreated capillary. The capillary was sealed at both ends and submerged into a water bath at 75 ^{o}C for 18 h. The resulting monolith was washed with methanol.

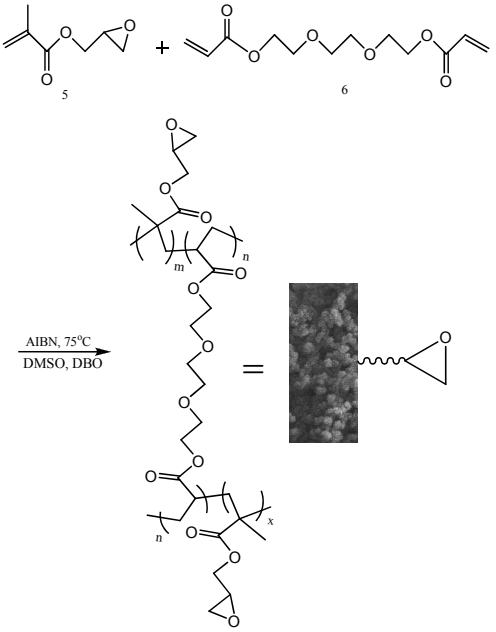


Fig. S13. Polymerization reaction of the poly (GMA-co-PEGDA) monolith.

2.1(b) Post-modification of the poly(GMA-co-PEGDA) monolithic capillary with compound 4

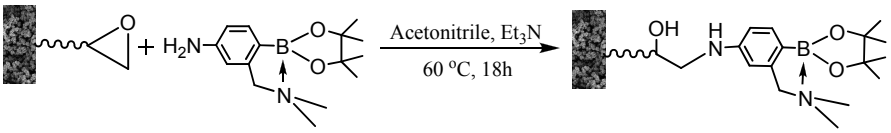


Fig. S14. The modification of the poly (GMA-co-PEGDA) monoliths.

The poly (GMA-co-PEGDA) monolithic column was modified through nucleophilic attack of the epoxide with compound 4 according to the method reported by O.G. Potter et al.[6] The modification reaction is shown in Fig. S14. Poly (GMA-co-PEGDA) monolith was rinsed with acetonitrile (ACN) for 4 h. Then, dissolving 0.20 g of compound 4 and 0.18 g of triethylamine in 5 mL of ACN, the resulting solution was flushed continuously through the monolithic column for 18 h at 65 ^{o}C. Finally, the monolithic capillary was washed with ACN and water for 1 h respectively to obtain a boronate-functionalized monolithic column. Before chromatographic experiment, the boronate-functionalized column was conditioned by 100 mM HOAc (pH 2.7) and sample loading buffer successively.

2.2(a) Preparation of large-sized monolith for characterization

A large-sized monolithic column was synthesized in an empty regular HPLC column for FT-IR characterization of the monolith. The monolith was washed with methanol to flush out the residual reagent. Then the monolith was moved out of the column and dried under vacuum at 50 ^{o}C for 24 h before characterization.

2.2(b) Post-modification of the large-sized monolith with compound 4

The large-sized monolith synthesized in an empty regular HPLC column was moved out of the column after polymerization and cut into pieces. The pieces were washed with methanol using a Soxhlet extractor for 24 h. After dried under vacuum at 50 ^{o}C for 24 h, a part of the pieces was submerged into a solution containing compound 4 (0.40 g) and triethylamine (0.36 g) dissolved in 10 mL ACN. The resulting mixture was heated at 65 ^{o}C with stir for 18 h. The modified monolithic pieces was washed with methanol using a Soxhlet extractor for 24 h and dried under vacuum at 50 ^{o}C for 24 h.

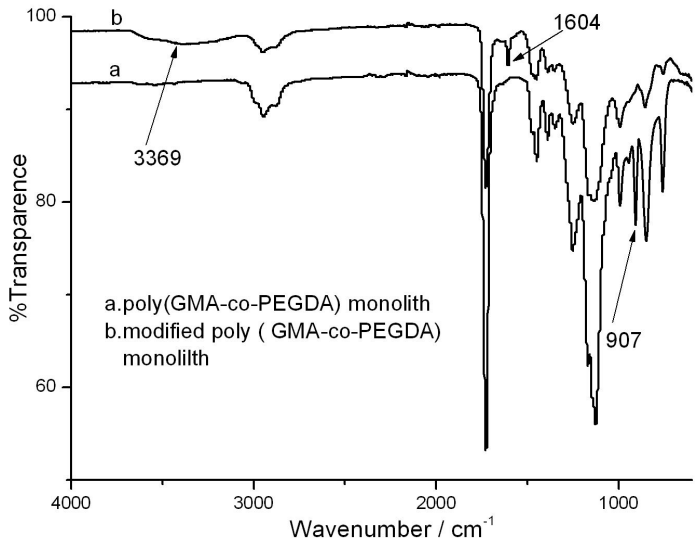


Fig. S15. FT-IR spectra of the (a) poly(GMA-co-PEGDA) monolith, (b) compound 4 modified poly(GMA-co-PEGDA) monolith.

The FT-IR spectra for the unmodified and modified monoliths are shown in Fig. S15. From the contrast of spectrum a and spectrum b, three differences are obvious: 3,369 cm^{-1}, 1,604cm^{-1} and 907 cm^{-1}, which can be attributed to –OH vibration, skeleton vibration of benzene ring and epoxy group vibration, respectively. The disappearance of peak at 907 cm-1 in the spectra a and the appearance of peaks at 3,369 cm^{-1} and 1,604cm^{-1} indicate the presence of compound 4 on the modified monolith.

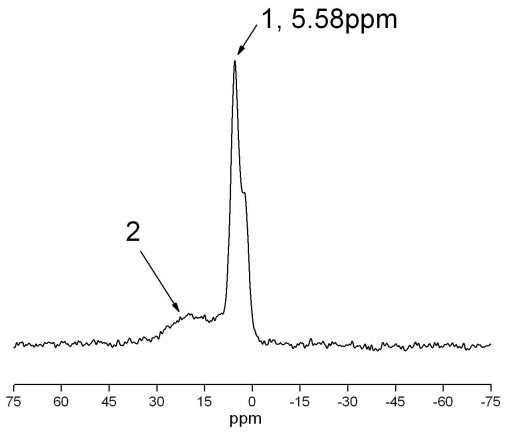


Fig. S16 The ^{11}B MAS-NMR spectra of the modified monolith.

Fig. S16 shows the ^{11}B MAS-NMR spectra of the modified monolith. Peak 1 suggests the

presence of intramolecular B-N interaction in most of the molecules of compound 4 attached to the poly(GMA-co-PEGDA) monolith while peak 2 indicates that a small portion of molecules of compound 4 did not exhibit intramolecular B-N interaction.

2.2(c) The quantification of the boronate loading of modified monolith with XPS

The boronate loading of the modified monolith was quantified by XPS. By comparing spectrum a and b in Fig. S17, it can be seen that there is an additional N1S peak in spectrum b which indicates compound 4 has been successfully attached to the monolith. The boron element can not observed in spectrum b and this may be due to the low atom concentration of boron element in the modified monolith. In addition, the atom concentration ratio of C, O, B and N elements in the modified monolith is 72.19:24.94:1.01:1.85. The atom concentration ratio of B and N was 1.01:1.85 and this is basically consistent with that of compound 4 (B:N=1:2).

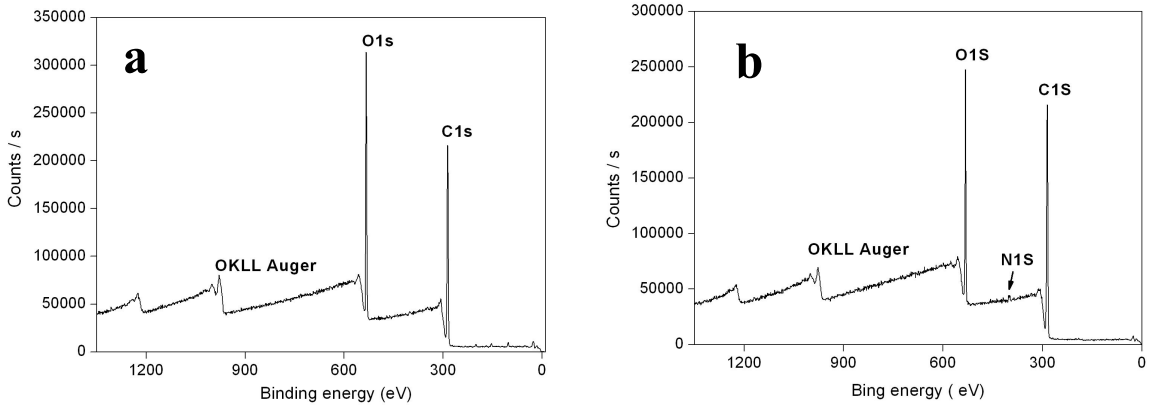


Fig. S17 XPS survey scan of the non-modified (a) and the modified monolith (b).

2.3 The measurement of the binding capacity

The cis-diol binding capacity of the boronic acid functionalized monolithic column was measured using frontal chromatography with adenosine and deoxyadenosine as the test compounds. Deoxyadenosine was not retained by the monolithic column and eluted first as the dead time marker while adenosine was captured by the monolithic column and eluted until

the column was saturated. The binding capacities measured in the pH range from 5.5 to 7.0 are listed in Table. S1.

Table S1 The binding capacity of the boronate affinity monolithic column.

pH	Cis-diol binding capacity (μmol/mL)
5.5	2.63
6.0	3.28
6.5	5.40
7.0	6.82

3 Chromatographic retention behavior of quinol (7) and catechol (8) on the boronate affinity monolithic column

Quinol (7) and catechol (8) were additionally used as analytes to testify the retention mechanism of the modified poly(GMA-co-PEGDA) monolith column. Because of the hydrophobic property of the benzene ring, ACN was added to the mobile phase to suppress the non-specific interaction between benzene ring and the backbone of the monolith. A modified monolith column with an effective length of 20 cm was used in this section of experiment. The flow rate was set at 0.15 mLmin^{-1} with the splitting ratio of 100:1.

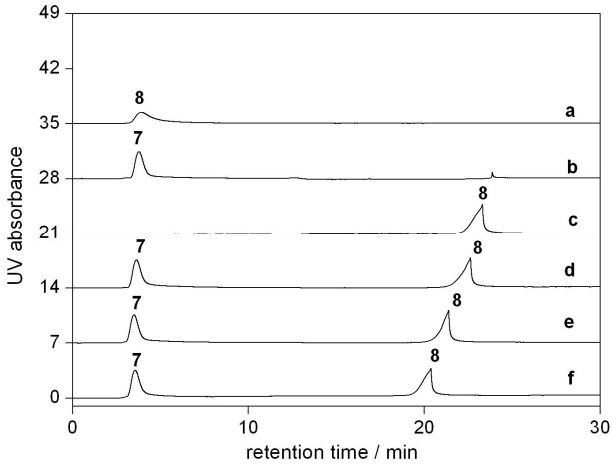


Fig. S18 The chromatographic retention of quinol (7) and catechol (8) on the modified poly(GMA-co-PEGDA) monolithic column. Mobile phase: a) 70:30 (v/v) ACN/100 mM

phosphoric acid; b-f) 70:30 (v/v) ACN/10 mM sodium phosphate buffer at pH 7.0, 7.0, 7.0, 6.0, 5.5, respectively, switched to 70:30 (v/v) ACN/100 mM phosphoric acid at 8 min. Sample: a) 0.2 mg mL^{-1} catechol dissolved in 70:30 (v/v) ACN/100 mM phosphoric acid; b) 0.5 mg mL^{-1} quinol dissolved in 70:30 (v/v) ACN/10 mM sodium phosphate buffer at pH 7.0; c) catechol dissolved in 70:30 (v/v) ACN/10 mM sodium phosphate buffer at pH 7.0; d-f) 0.2 mg mL^{-1} catechol and 0.5mg mL^{-1} quinol dissolved in the corresponding loading buffer at pH 7.0, 6.0 and 5.5 respectively.

From Fig.S18 we can see that quinol showed no retention on the modified monolithic column at all tested conditions. In contrast, catechol showed no retention when the loading buffer was 70:30 (v/v) ACN/100 mM phosphoric acid (trace a in Fig.S18) while it was retained and enriched at pH 7.0 (trace c in Fig.S18). Meanwhile, catechol was selectively retained and enriched under the interference of quinol at and above pH 5.5 (trace d-f in Fig.S18).

4 Selective enrichment of nucleosides from human urine

Furthermore, selective enrichment of nucleosides from human urine (pH 6.5) was carried out on the modified monolithic column. Spontaneous urine sample was collected from an adult male volunteer. The collected urine was frozen immediately and stored at -20 ^{o}C. Prior to analysis, the sample was thawed and brought to room temperature. A 1-mL aliquot of urine was centrifuged for 10 min at 12,000 r/min. The supernatant was used as sample. A 15 cm long piece of the modified monolithic column was used as the extraction column. 200 μL of the supernatant was passed thought the extraction column at a flow rate of 1 μL min^{-1}. Then, the column was washed with water for 30 min in order to completely flush out the un-retained components. Next, ACN was used to wash the column for 30 min to flush out the non-specific retained components. Finally, the retained components were washed out by 100 mM HOAc and collected into tubes. For each tube, 5 μL eluate was collected and analyzed by MEKC according to a literature approach.[7] Identity for some peaks was confirmed by comparison the electropherogram with that for known standards.

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