Supporting Information

Boronic Acid-Based Peptidic Receptors for Pattern-Based Saccharide Sensing in Neutral Aqueous Media, an Application in Real-Life Samples

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Abbreviations. Ac, acetyl; Boc, tert-butyloxycarbonyl; Fmoc, 9-fluorenyloxycarbonyl; HOBt, 1-hydroxybenzotriazole; DMF, N,N-dimethylformamide; NMM, N-methylmorpholine; TBTU, 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluroborate; NMP, N-Methylpyrrolidinone; TEOF, Triethyl orthoformate; MeOH, methanol.

Materials and Methods. Reactions were generally run under a blanket of argon and when necessary standard Schlenk techniques were employed. Anhydrous solvents (NMP, TEOF) were purchased from Aldrich and used as received. CH_2Cl_2 was dried via distillation over CaH_2 whereas MeOH and and DMF were dried via passage through an alumina column prior to usage. Solvents were deoxygenated via 4 freeze-pump-thaw cycles when required. Tentagel-NH_2 resin (NovaSyn TG amino resin, loading capacity: 0.26 mmol/g) was purchased from NovaBiochem and Tentagel-NH_2 resin containing a photolabile linker (Fmoc-Photolabile Resin SS, 100-200 mesh, loading capacity: 0.6 mmol/g) was purchased from Advanced Chem. Fmoc- and Boc-protected amino acids were purchased from NovaBiochem. All other solvents and reagents were purchased form Fisher or Aldrich and used as received. The synthesis of N-aceylated blank resin followed that previously described in the literature. Mass spectra were recorded on a Waters ZMD with a quadruple mass analyzer and electronic absorption spectra on a Beckman DU640 spectrometer and HPLC analysis done on a Gemini Chromasil C18 reverse phase column.

General Synthetic Procedures are as follows:

Fmoc deprotection on the solid phase: The Fmoc-protected resin was suspended in a solution of 20% piperidine in DMF (20 ml per gram of resin) and agitated for 30 minutes. The solution was then drained from the resin and washed with DMF (3 x 10 ml x 3 min), CH_2Cl_2 (3 x 10 ml x 3 min), MeOH (3 x 10 ml x 3 min), DMF (3 x 10 ml x 3 min) and CH_2Cl_2 (3 x 10 ml x 3 min). The progress of the deprotection was monitored by the ninyhdrin test.

Deprotection of Orthogonally-Protected Side-Chains on the solid phase: The resin possessing orthogonally-protected side chains was suspended in a mixture of 95% TFA, 2.5 % H_2O and 2.5% TES (pre-mixed for an hr) (20 ml per gram of resin) and stirred for 1hr. The mixture was then drained from the resin and washed with DMF (3 x 10ml x 3 min), MeOH (3 x

10 ml x 3 min), CH_2CL_2 (3 x 10 ml x 3 min), DMF (3 x 10 ml x 3min) and CH_2Cl_2 (3 x 10 ml x 3 min). The progress of the deprotection was monitored by the ninhydrin test.

General procedure for the preparation of resin-bound peptides: Activated ester solutions of amino acids were prepared using the protected amino acid (5 eq), HOBT (4 eq.), TBTU (4 eq.) and NMM (9 eq.) in DMF and stirred for 10 min. Coupling of the amino acids to the resin proceeded by suspending the resin (1 eq.) in the activated ester solution and gently agitating this mixture for 2-4 hr. Successful completion of each amino acid coupling (as monitored by the ninhydrin test) was followed by washing cycles DMF (3 x 10ml x 3 min), MeOH (3 x 10 ml x 3min), CH_2CL_2 (3 x 10ml x 3 min), DMF (3 x 10 ml x 3min) and CH_2Cl_2 (3 x 10 ml x 3min) and deprotection of the Fmoc group and orthogonally-protected side chains were then done in a manner that was described above. Amino acid sequences were confirmed by Edman degradation characterization

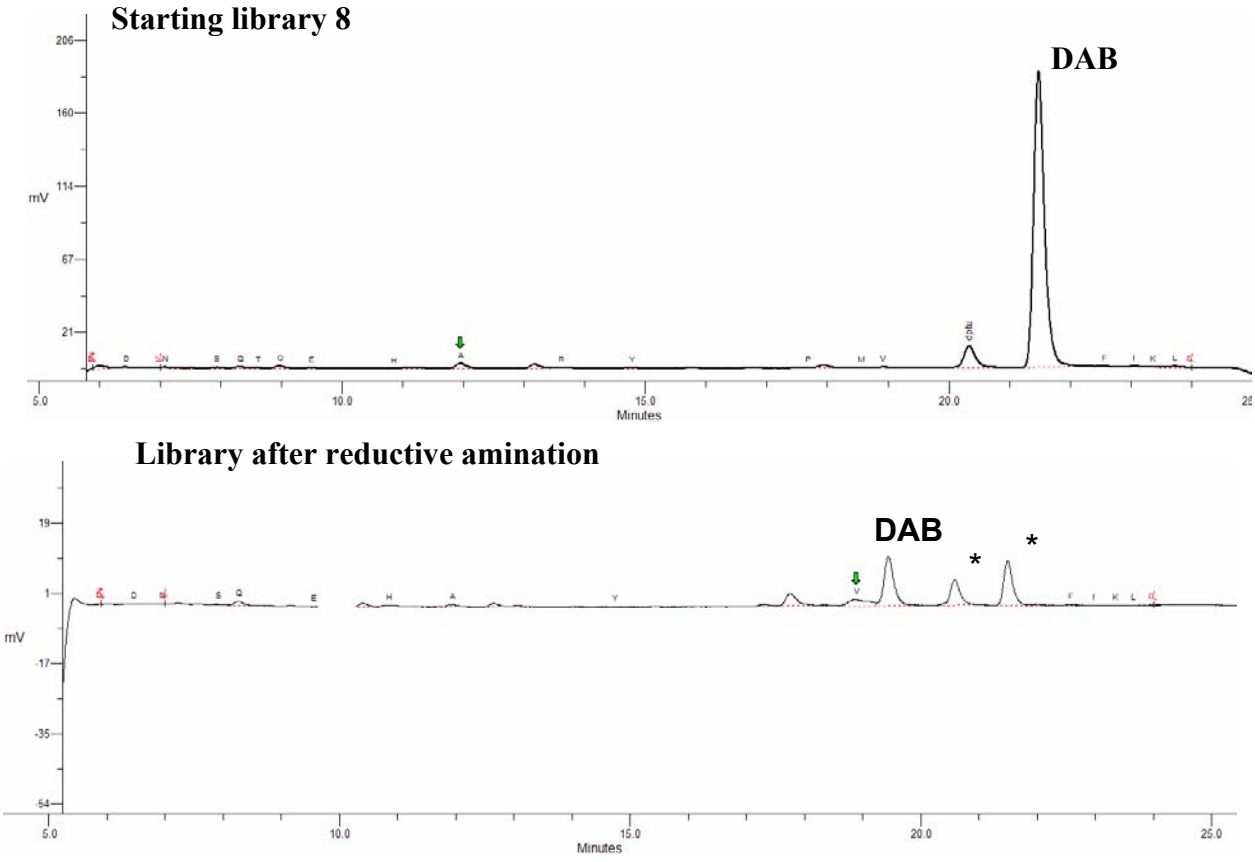
Synthesis of Pentapeptide Library 8: Tentagel-NH_2 resin (0.08g, 2.45 x 10^{-5} mol) was swollen in DMF, split into 21 equal portions and placed in conical vials. Solutions of the activated esters of the amino acids (21 total solutions; 18 solutions corresponded to the natural amino acids – cysteine and lysine; 3 identical solutions of DAB) were prepared in a manner as described above. Following the coupling of the amino acids to the resin, all reaction mixtures were combined, mixed well, activated amino acid solutions drained, washed, Fmoc deprotection effected, washed and split into 21 equal portions. This process was repeated 4 times to achieve the desired pentapeptide library. Amino acid sequences were resolved by Edman degradation characterization.

Peptide 12: A solution of 2-formylphenyl boronic acid (0.032 g, 7 eq.) in 1:1:1 (NMP:MeOF:TEOF) - 1% AcOH was added to peptide **5** (0.047 g, 1 eq.) (pre-swollen in TEOF-1% AcOH for 0.5 hr) and the reaction mixture gently agitated for 1hr. The solution was then drained from the resin, the resin washed with fresh 1:1:1 NMP:MeOF:TEOF) - 1% AcOH (1 x 5ml) and the coupling step repeated twice. $NaBH_3CN$ (0.02 g, 10 eq.) in 1:1:1 (NMP:MeOF:TEOF) - 1% AcOH was then added to the resin and the reaction mixture agitated overnight. Washing cycles (DMF (3 x 10ml x 3 min), MeOH (3 x 10 ml x 3 min), CH_2CL_2 (3 x 10ml x 3min), DMF (3 x 10 ml x 3 min) and CH_2Cl_2 (3 x 10 ml x 3 min)) of the resin were then done. A product which was negative to the ninhydrin test was obtained and the amino acid

sequence and modification of DAB corresponding to **5** confirmed by Edman degradation (cycle 3).

Peptide 13: Peptide **5** (20 mg) was suspended in deoxygenated dioxane, hydrazine (2 µL) added and the suspension photolyzed for 5 hr. The formation of peptide **6** was confirmed by HPLC, ESI-MS and MALDI-TOF MS/MS. MS (MALDI-TOF) *m/z*: 880.4 m/z $[M-42]^+$. MS (ESI-MS) 922.4 m/z $[M\ ]^+$ 880.4 m/z $[M-42]^+$

Examples of Edman Degradation Characterization Results



***** hydrophobically shifted-peaks

DAB appears at higher retention time after modification due a slight difference in the gradient used in the C18 column. Peak assigned to DAB based on the standard compound.

II Acquisition and Analysis of Data from the Taste-Chip Platform

Instrumentation. Thirty randomly-selected resin-bound receptors from library 8 and 5 N-acetylated blank beads were placed in individually-addressable pyramidal pits in a silicon wafer chip. This was done using a pair of ultrafine tweezers. The chip loaded with the microbeads was then placed in a microfluidics flow cell. Details of the preparation of the silicon wafer chip and the microfluidics flow cell have been previously described.[1]The microfluidics flow cell is secured to the stand of an Olympus SZX 12 stereoscope using common lab tape. The stereoscope allows for illumination of the beads in the array using a General Elecctic Quartzline lamp as the illumination source. Image capture of the indicator uptake was performed with a 12-bit DVC 1312C (DVC, Austin, TX) charge-couple device positioned on the stereoscope and interfaced with Image Pro Plus 4.0 software (Media Cybernetics) for data extraction. Introduction of solutioins (saccharide, buffer, acid and base) to the microfluidics flow cell was effected using an Amersham Pharmacia Biotech AKTA Fast Protein Liquid Chromatograph (FPLC) controlled by Unicorn 3.0 Software.

Single Run of an Indicator Uptake Assay. Each assay was performed at room temperature under continuous flow conditions. Saccharide solution (80 μM in 500 μM HEPES Buffer at pH =7.4 in water) was delivered to the flow cell at a rate of 0.25 ml/min. This was followed by a brief buffer wash (5 minutes at 1.2 ml/min) and delivery of indicator (8 μM in 500 μM HEPES buffer at pH=7.4 in water) at a flow rate of 0.5 ml/min. Images of the array were taken at 5s intervals during the indicator delivery stage using the CCD camera. Macros developed within the ImagePro software controlled the timing and frequency of the image capture. Extensive acid and base washes (300 mM HCl for 80 min at 2 ml/min and 150mM NaOH for 10 min at 2ml/min) were then done to remove saccharide and indicator bound to the RBRs.

Data Analysis. Images of the array which were captured by the CCD camera were analyzed by drawing areas of interest (AOI) in the central region of each microbead. Red, green and blue (RGB) intensities were then extracted for AOI. RGB intensities were then converted to absorbance values.

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[1] (a) Lavigne, J. J.; Savoy, S.; Clevenger, M. B.; Ritchie, J. E.; McDoniel, B.; Yoo, S.-J.; Anslyn, E. V; McDevitt, J. T.; Shear, J. B.; Neikirk, D. *J. Am. Chem. Soc.* **1998**, *120*, 6429-6430. (b) Goodey, A.; Lavigne, J. J.; Savoy, S. M.; Rodriquez, M. D.; Curey, T.; Tsao, A.; Simmons, G.; Wright, J.; Yoo, S.-J.; Sohn, Y.; Anslyn, E. V.; Shear, J. B.; Neikirk, D. P.; McDevitt J. T. *J. Am. Chem. Soc.* **2001,** *23*, 2559-2570.