Boronic Acid Recognition of Non-interacting Carbohydrates for Biomedical Applications: Increasing Fluorescence Signals of Minimally Interacting Aldoses and Sucralose

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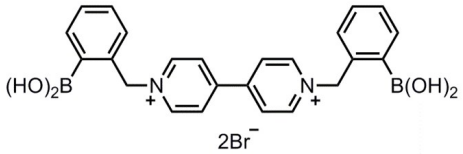
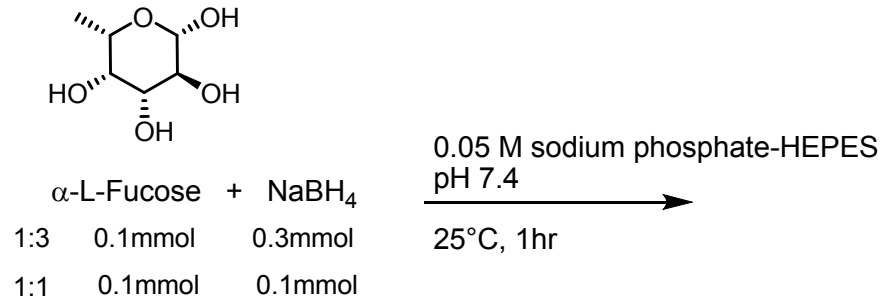
1. Synthesis

The syntheses of 4, 4'-*o*-BBV was synthesized according to previously published procedures.



Preparation of 4,4'-*o*-BBV.[1] To a solution of 2-bromomethylphenyl boronic acid (0.77 g, 3.6 mmol) in dry MeCN (10 mL), MeOH (0.65 mL) and 4,4'-dipyridyl (0.23 g, 1.5 mmol) was added, and the reaction was stirred at 55 °C for 48 hours. The reaction mixture was cooled to room temperature and acetone (10 mL) was added to induce further precipitation of a pale yellow solid. The precipitate was centrifuged, washed with acetone (2 x 10mL) and dried under a stream of argon (0.85 g, 96%yield). ^{1}H NMR (500 MHz, D_2O) δ 9.06 (d, J=10 Hz, 4H), 8.47 (d, J=5 Hz, 4H), 7.79 (d, J=10Hz, 2H), 7.56 (m, 6H), 6.11 (s, 4H); ^{13}C NMR (126 MHz, D_2O) δ 146.72, 135.51, 132.80, 130.44, 128.35, 127.85, 66.13; ^{11}B NMR (160 MHz, D_2O) δ +29.83.

2. Reaction optimization for the $NaBH_4$ reduction of aldoses



Scheme S-1. Reaction conditions used for the reduction of aldoses used to obtain optimal conditions. To Circumvent false positive signals each reaction was performed in 0.05 M sodium phosphate-HEPES buffer to maintain pH of the reaction.

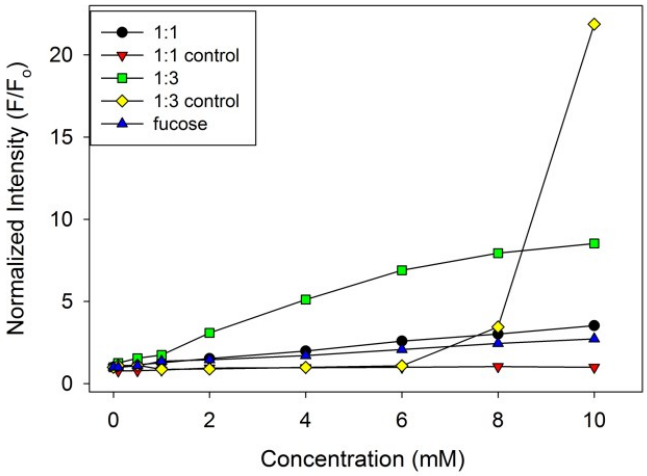


Figure S-1. Reaction optimization of $NaBH_4$ reduction of fucose. Normalized Intensity for the 4,4'-*o*-BBV (400 μM) and HPTS (4 μM) after each reaction condition. Control is the repeated condition in the absence of fucose.

Binding constant calculations:

Origin lab software was used to perform the calculation following the Benesi-Hildebrand plot where it is assumed that a 1:1 binding stoichiometry is present.[1,2] The following equation was used $\frac{F}{Fo} = \frac{\left(1 + \frac{Fmax}{Fo}\right) * Kb * A}{1 + Kb * A}$ and inputted as the following function in origin lab software: $y = \frac{1 + S * K * A}{1 + K * A}$ where S=maximum possible signal, K=binding constant, A=analyte concentration. K and S are considered the parameter variables and A is the independent variable while y is dependent.

Table S-1. Binding constants for each aldose after NaBH4 reduction compared to untreated aldose.

4,4'-*o*-BBV	Reduced Aldose (Binding constant M^{-1})	Aldose (Binding constant M^{-1})
Fucose	21 ± 2.6	5.2± 2.0
Rhamnose	67 ± 6.2	9.5± 1.5
Xylose	52 ± 8.5	2.08± 0.4

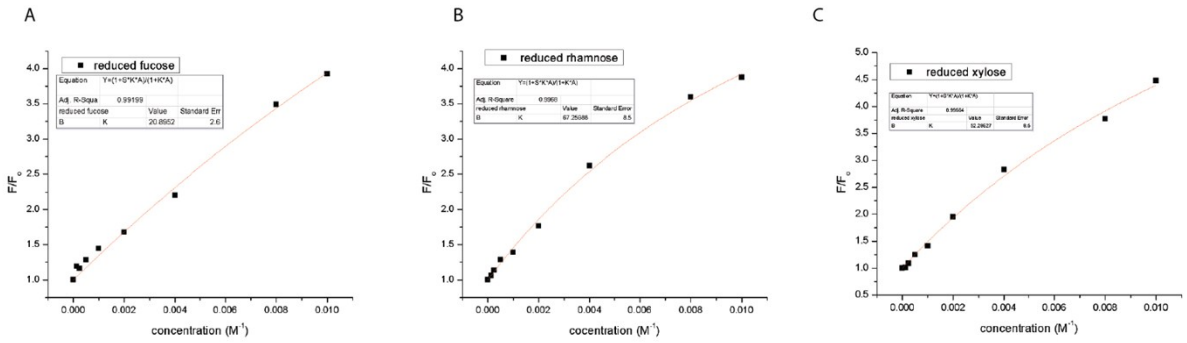


Figure S2. Non-linear fitted binding isotherms to obtain binding constants for reduced fucose (a) rhamnose (b) and xylose (c).

Table S-2. Limit of detection and quantification for reduced aldose versus unreacted aldose with 4,4'-*o*-BBV.

4,4'-*o*-BBV	Reduced Aldose (LOD, LOQ μM)	Aldose (LOD, LOQ mM)
Fucose	175,560	1.5, ND
L-rhamnose	250,550	1, ND
Xylose	260, 475	2, 6

ND: not determined

3. Fluorescence Spectra of Chemically Modified Saccharides

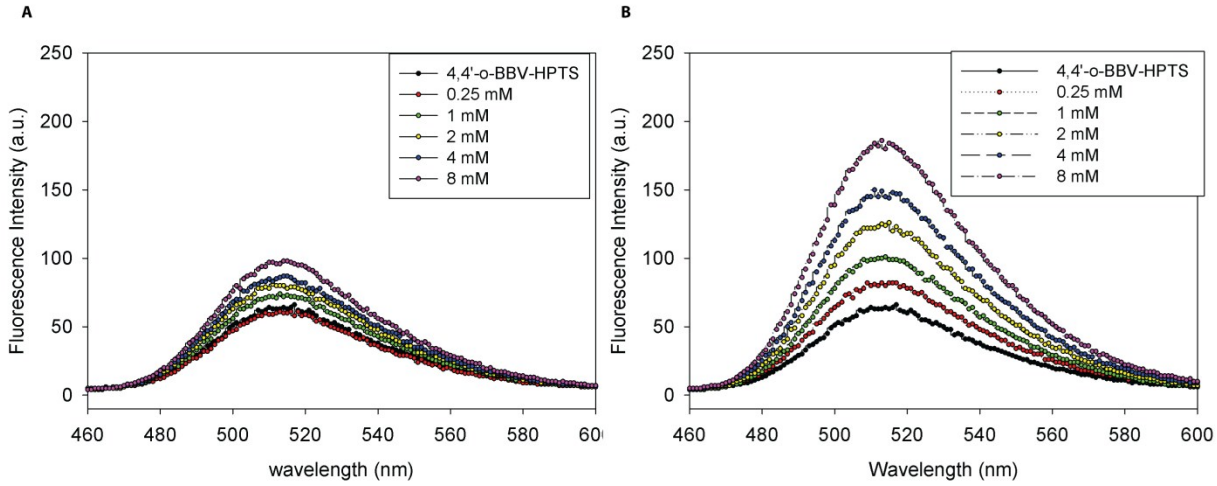


Figure S-3. Recognition of xylose (A) compared to reduced xylose (B) of 4,4'-o-BBV (400μM) with HPTS in pH 7.4 phosphate buffer, λ_{ex}=405 nm, emission scan 450-600 nm. Change in fluorescence spectrum of HPTS (4μM) in the presence of 4,4'-o-BBV (400μM) upon addition of various concentrations of aldose or reduced aldose.

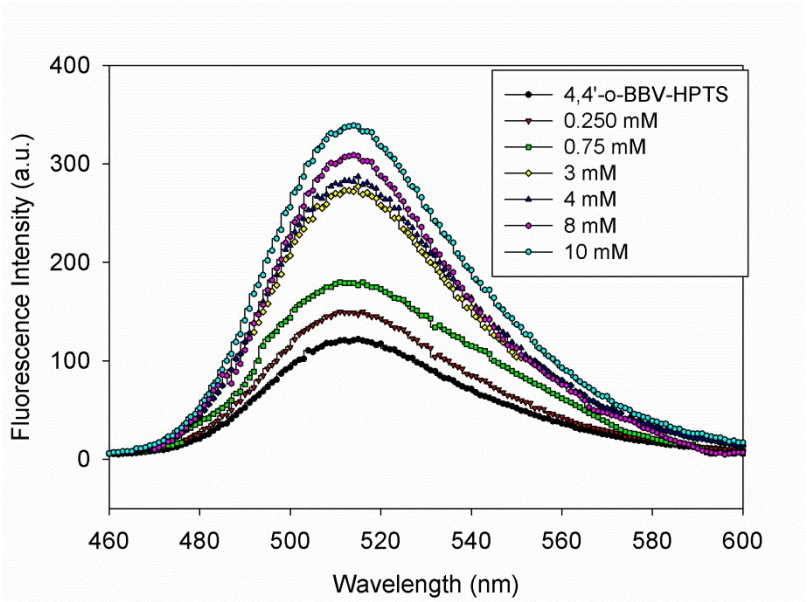


Figure S-4. Recognition of modified sucralose of 4,4'-o-BBV (400μM) with HPTS in pH 7.4 phosphate buffer, λ_{ex}=405 nm, emission scan 450-600 nm. Change in fluorescence spectrum of HPTS (4μM) in the presence of 4,4'-o-BBV (400μM) upon addition of various concentrations of modified sucralose.

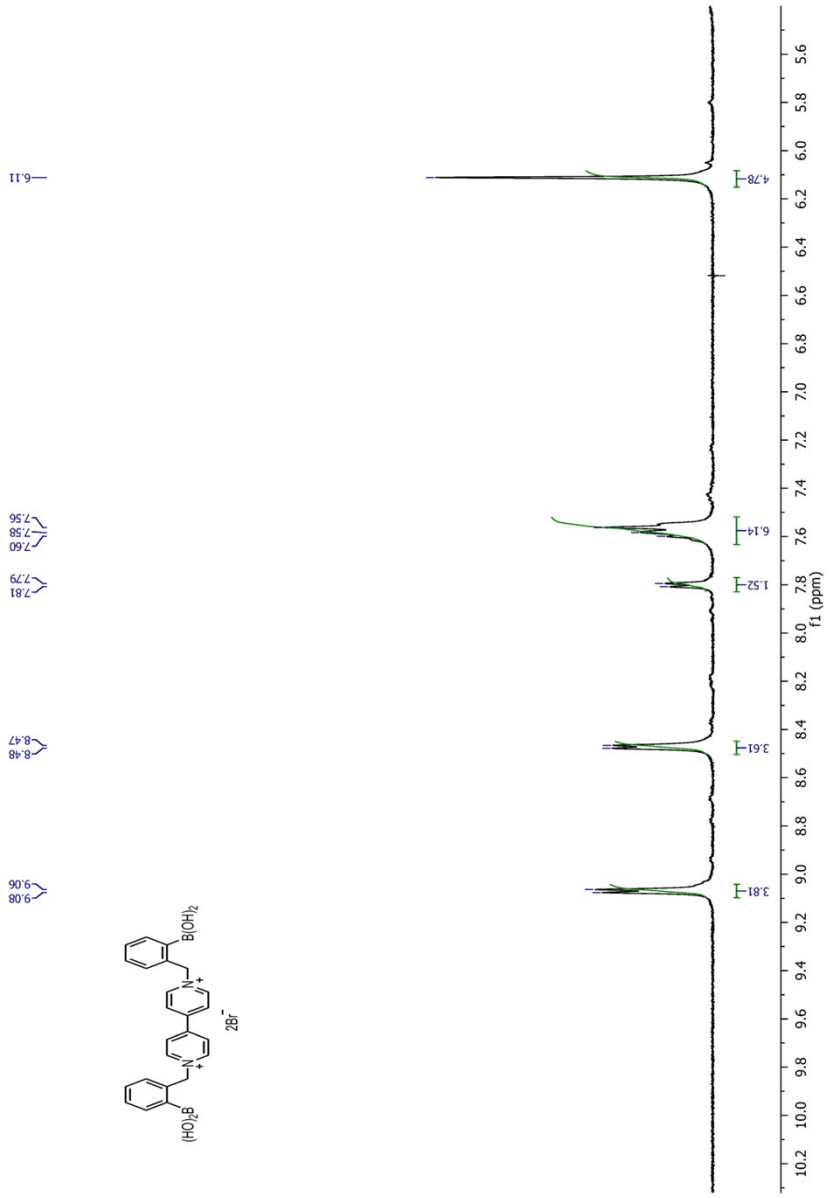


Figure S-5. ^{1}H NMR of 4,4'-*o*-BBV

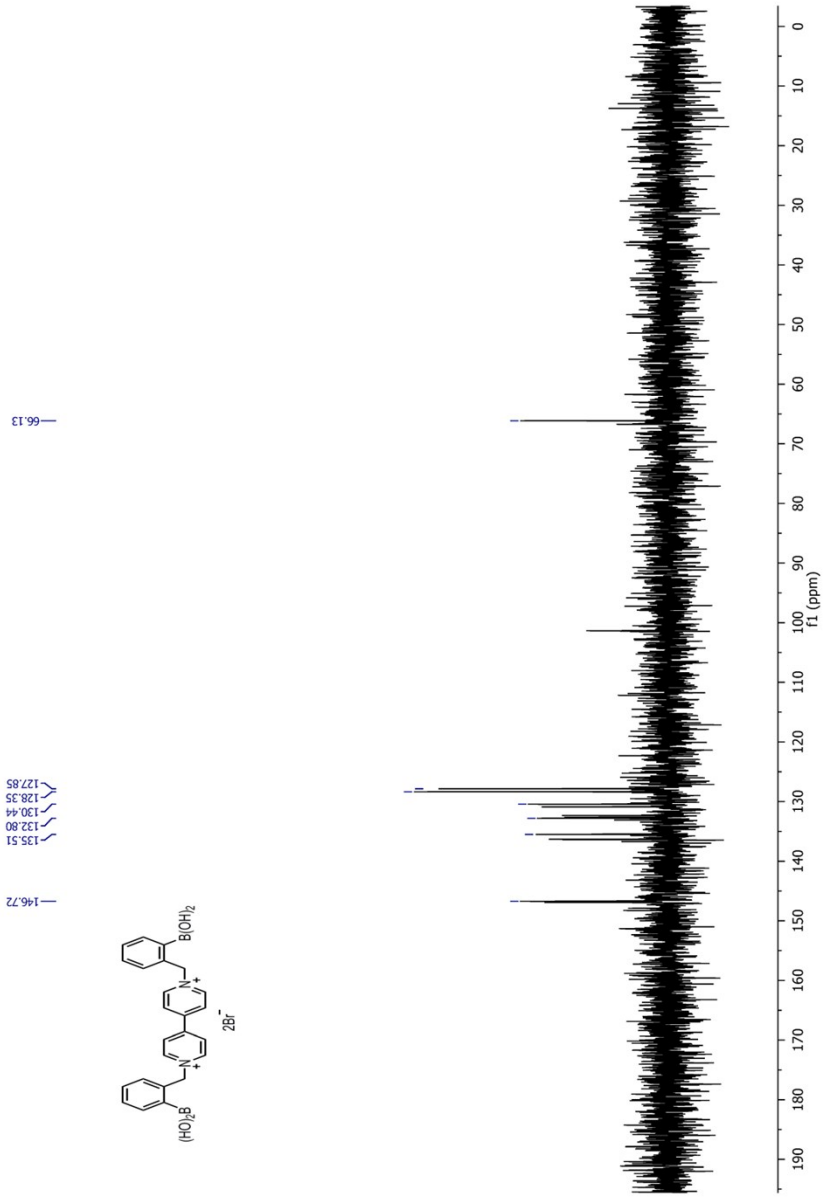


Figure S-6. ^{13}C NMR of 4,4'-*o*-BBV

4. References

1. Connors, K. A., *Binding Constants-The Measurement of Molecular Complex Stability*, John Wiley: New York, 1987.
2. S. Feryforgues, M. T. Lebris, J. P. Guette and B. Valeur, *J. Phys. Chem.*, 1988, **92**, 6233-6237.