

Synthesis, decoding, and preliminary screening of a bead-supported split-pool library of triboronic acid receptors for complex oligosaccharides

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Accessory Publication

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1. Preparation of library of triboronic acid receptors on macrobead (250 – 300 μm) TentaGel[®] resin.

The TentaGel[®] bound tetramine library was weighed (0.35 g, 0.042 mmol at 0.12 mmol/g) into a dry 25 mL round bottom flask without a stir bar. A condenser was fitted to the round bottom and together they underwent three evacuation and argon backfill cycles until they were maintained under an argon atmosphere. The resin was then swelled in 8.5 mL of dry THF before the addition of 1,2,2,5,5-pentamethylpiperidine (PMP) (0.23 mL, 1.26 mmol) followed by 2-(bromomethyl)phenylboronate ester (Reference: T. D. James, K. R. A. S. Sandanayake, R. Iguchi, S. Shinkai, *J. Am. Chem. Soc.* **1995**, *117*, 8982-8987) (0.26 mL at 1.39 g/mL, 1.26 mmol). The reaction was heated to 65 °C for 48 hours until it was cooled and transferred into a 25 mL polypropylene filter vessel using THF. The resin was rinsed with THF (3×), water (1 minute and then 30 minutes), THF (3×), methanol (3×) and dichloromethane (3×). It was then dried under high vacuum over drierite/KOH for 16 hours to give the resin bound triboronic acid library. It should be noted that this chemistry was optimized on a number of model individual sequences prior to the library synthesis.

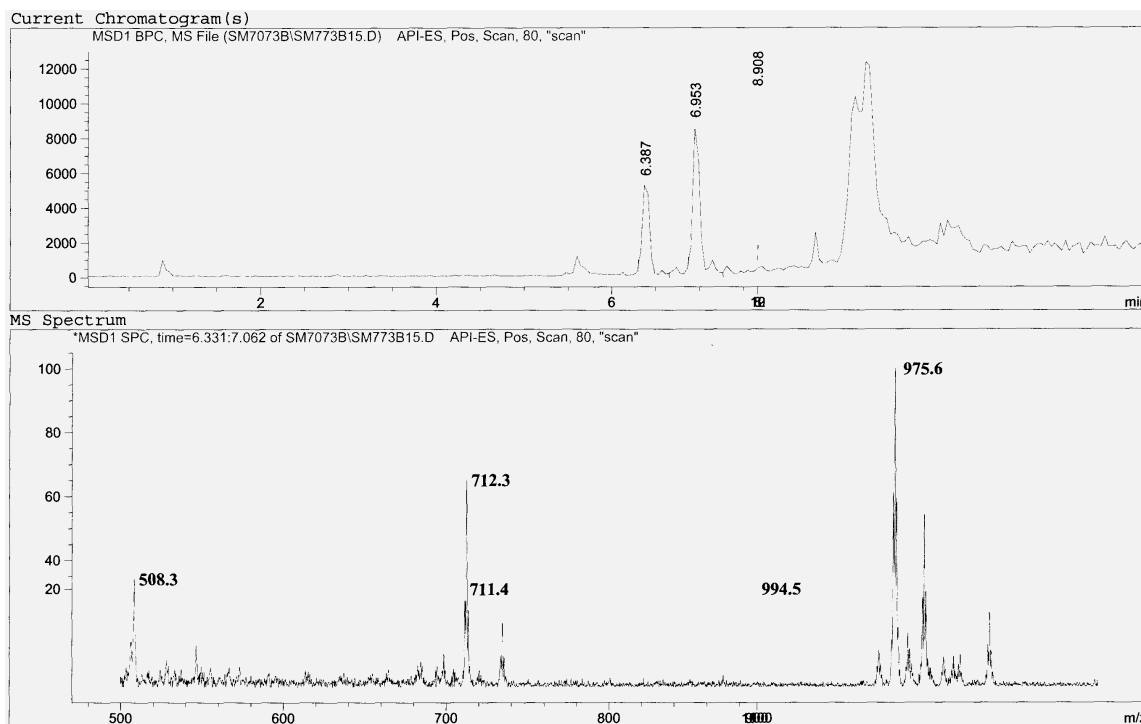
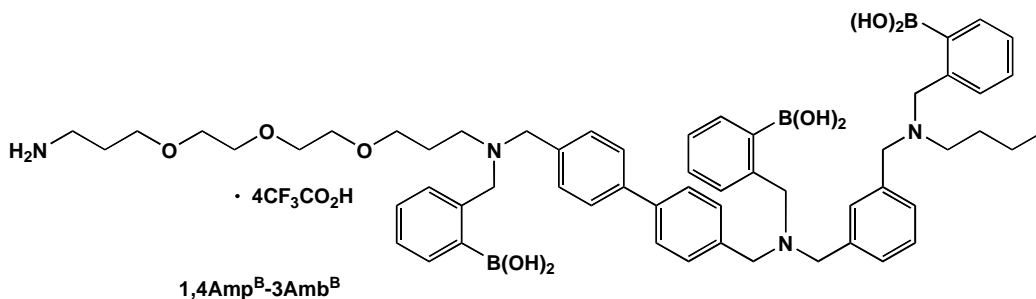
2. Procedure for single bead resin cleavage and LCMS analysis.

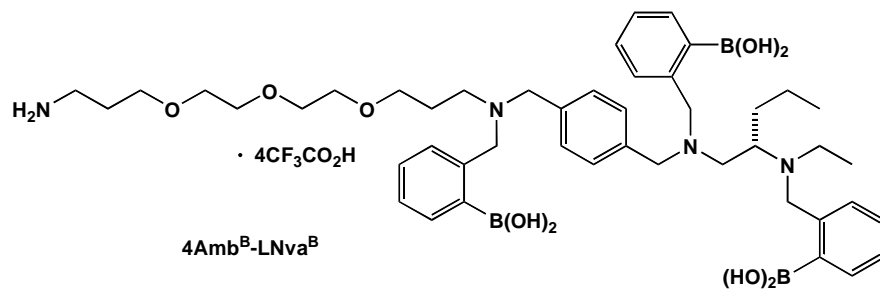
The dried beads were spread out on glass Petri dish and observed under a microscope. The beads were picked up using the tip of a 25 μL glass microsyringe containing 5 to 7.5 μL of 5% TFA in dichloromethane, and then transferred into a 200 μL glass conical microvial. The TFA solution was injected into the microvial which concomitantly removed the bead from the syringe tip into the microvial with the solution. The microvial was then placed inside an autosampler vial which was then capped. After 15 minutes the cap was removed from the autosampler vial to allow the TFA solution to evaporate. Methanol (5.0 - 7.5 μL) was added to the conical microvial and the solution injected into an LCMS. The identity of each sequence was determined by the mass differences between the partially terminated sequences and the full sequence that eluted through the LC column. LC conditions - column: Zorbax SB-C8 4.6×50 mm, 3.5 μm; eluent: 15 to 85% acetonitrile (0.1% TFA) in water (0.1% TFA) over 5 minutes

then maintained at 85% for 7 minutes at 0.7 mL/min; MSD conditions - capillary voltage: 3200 V (positive mode); fragmentor voltage: 120 V; mass scanning range: 250 – 900 amu; nebulizer pressure: 40 psig; gas temperature: 350°C; drying gas flow: 10.0 L/min.

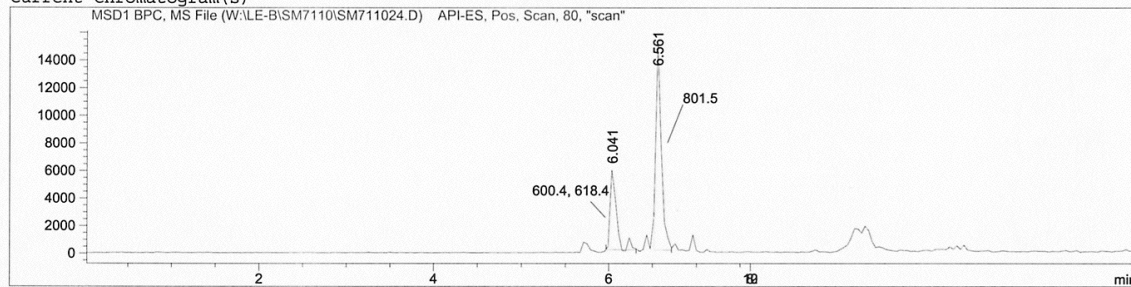
3. Additional representative examples of single-bead decoding from the library of triboronic acid receptors.

Note: These four randomly selected beads from the library clearly show the major complete fragment and the truncated fragment from termination synthesis, which allowed the positive identification of these beads.

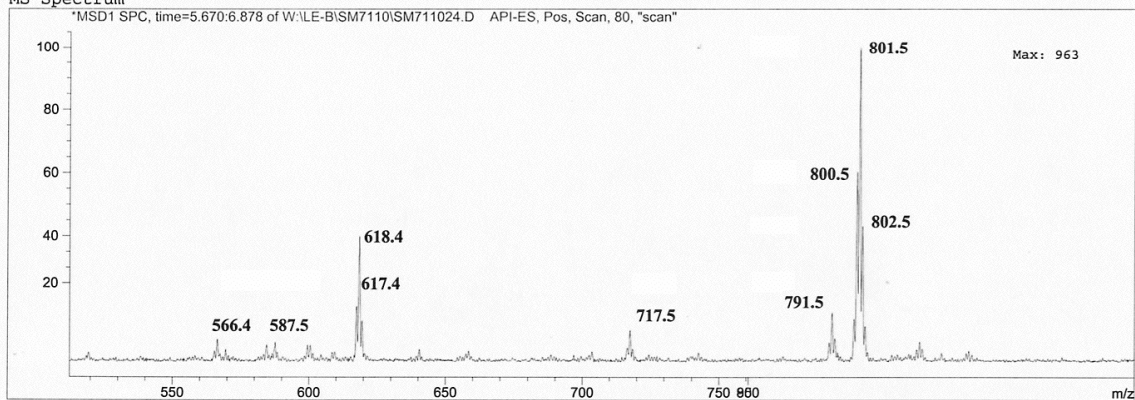


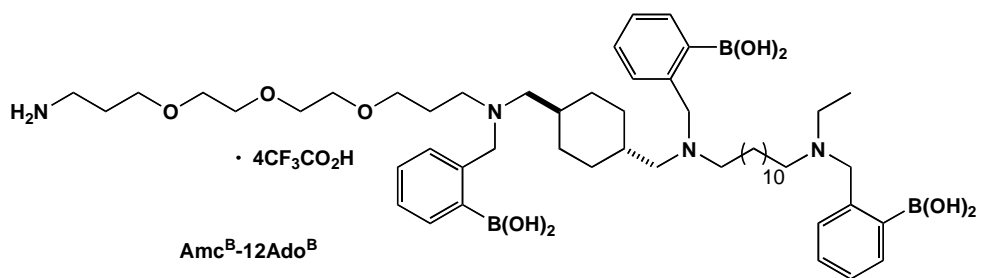


Current Chromatogram (s)

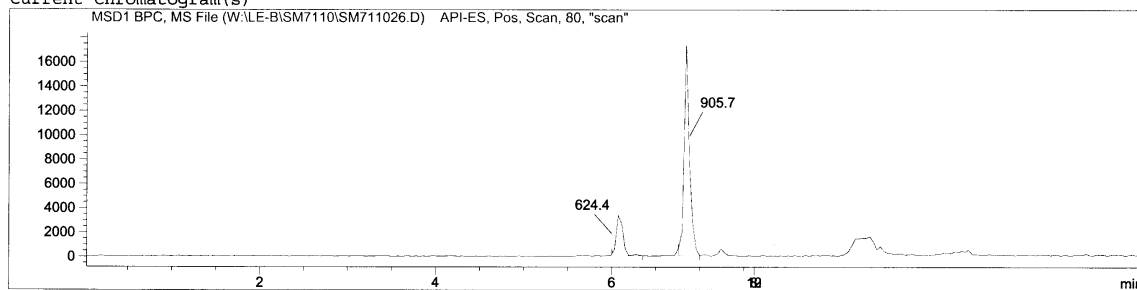


MS Spectrum

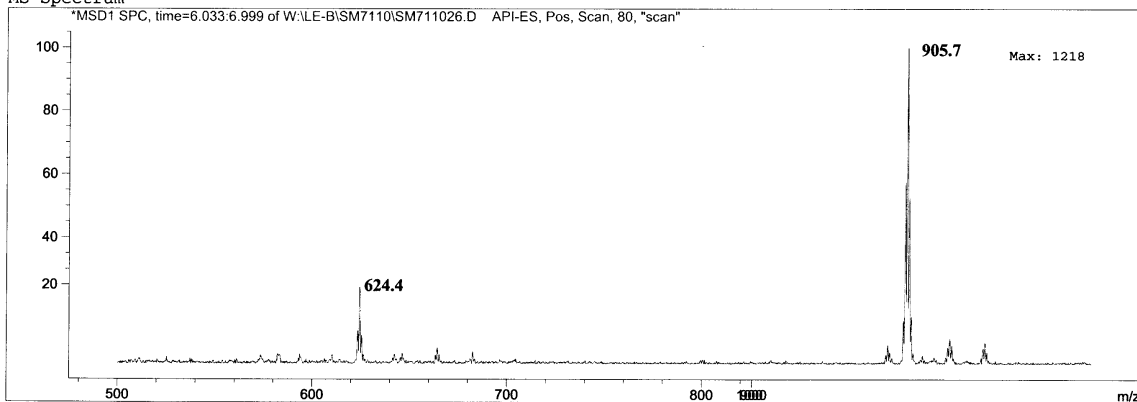




Current Chromatogram (s)



MS Spectrum



4. Preparation of conjugate 7 by labeling of Lewis-b with nitrobenzobenzodiazole.

The procedure followed, starting from the 8-methoxycarboxyloctanol glycoside of Le^b (supplied by Prof. O. Hindsgaul), was previously described by Zhang *et al.* (Y. Zhang, X. Le, N. J. Dovichi, C. A. Compston, M. M. Palcic, P. Diedrich, O. Hindsgaul, *Anal. Biochem.* **1995**, 227, 368-376.) The labeling reagent employed was 4-(7-nitro-2,1,3-benzooxadiazoyl-4-ylamino)propionic acid (from Molecular Probes, Eugene, Oregon; $\lambda_{em}(max) = 530$ nm, $\lambda_{ex}(max) = 466$ nm in methanol). After preparative TLC, using as a eluent chloroform/methanol/water (60:35:6), and lyophilization, a fluffy yellow solid was obtained which was checked by ESMS and ¹H NMR. ¹H NMR (500 MHz, D₂O) δ 8.45 (broad s, 1H), 6.46 (d, $J = 9.3$ Hz, 1H), 5.14 (d, $J = 3.7$ Hz, 1H), 5.02 (d, $J = 3.9$ Hz, 1H), 4.85 (apparent q, $J = 6.5$ Hz, 1H), 4.67 (d, $J = 8.0$ Hz, 1H), 4.36 (d, $J = 8.0$ Hz, 1H), 4.34 (apparent q, $J = 7.0$ Hz, 1H), 4.11 (dd, $J = 10.5, 10.5$ Hz, 1H), 3.96 (apparent d, $J = 10.0$ Hz, 1H), 3.93 (dd, $J = 3.0, 10.5$ Hz, 1H), 3.87 – 3.67 (m, 12H), 3.62 – 3.56 (m, 2H), 3.53 – 3.51 (m, 1H), 3.49 – 3.42 (m, 2H), 3.34 – 3.29 (m, 2H), 3.29 – 3.24 (m, 2H), 2.72 (t, $J = 6.5$ Hz, 2H), 2.06 (t, $J = 7.5$ Hz, 2H), 2.04 (s, 3H), 1.48 – 1.38 (m, 4H), 1.38 – 1.20 (m, 2H), 1.26 (d, $J = 6.5$ Hz, 6H), 1.20 – 1.10 (m, 6H). HRMS-ESMS calcd for C₄₆H₇₃O₂₄N₇Na (M+Na⁺) 1130.460467, obsd. 1130.460645. The labeled oligosaccharide was observed in the epi-fluorescent microscope using an Olympus WU cube with excitation band: 330 – 385 nm and emission band: > 420 nm, and a WIB cube with excitation band: 460 – 490 nm and emission band: > 515 nm,