# Supporting Information

# Injectable self-healing hydrogels based on boronate ester formation between hyaluronic acid partners modified with benzoxaborin derivatives and saccharides

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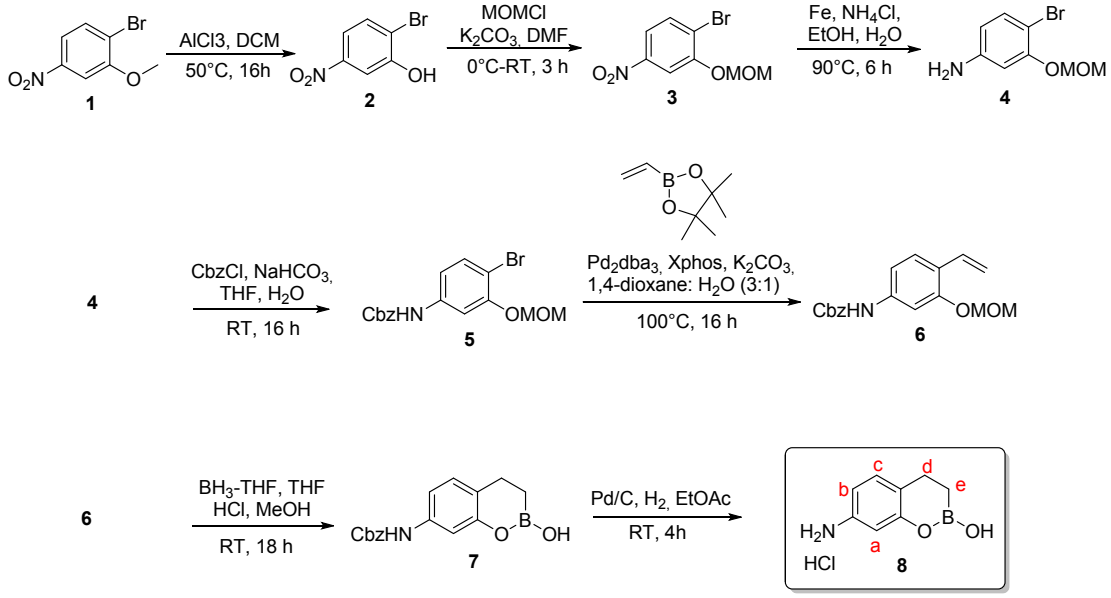
## 1. Synthesis of aminobenzoxaborin (ABORIN) compounds

### *7-amino-3,4-dihydro-2H-benzo[e][1,2]oxaborinin-2-ol (1,2-ABORIN)*




**Synthesis of 2:**
To a stirred solution of **1** (200 g, 862 mmol) in DCM (600 mL), $AlCl_3$ (334 g, 2586 mmol) was added at RT. The reaction mixture was heated to 50 °C for 16 h under $N_2$. The reaction was monitored by TLC (30 % EtOAc in *n*-hexanes). After completion of the reaction, the reaction mixture was cooled to RT and quenched with ice (2 kg). The pH was adjusted to ~4 with ammonium chloride and the aqueous layer was extracted with DCM (3 L). The organic layer was separated and washed with brine solution (1.5 L), dried over $Na_2SO_4$ and evaporated under vacuum to get **2** as a yellow solid (130 g, 70 %).

**$^1$H NMR (400 MHz, DMSO-$d_6$, 298 K):** 11.33 (s, 1H), 7.81-7.79 (d, *J* = 8.8 Hz, 1H), 7.73-7.72 (d, *J* = 2.4 Hz, 1H), 7.61-7.58 (dd, *J* = 8.8 Hz, 2.8 Hz, 1H).

**LC-MS** (ES, *m/z*): 216.28 $[M+H]^+$.

**Synthesis of 3:**
To a stirred solution of compound **2** (130 g, 596 mmol) in DMF (400 mL), was added $K_2CO_3$ (206.03 g, 1490 mmol) at RT under stirring for 10 min, followed by addition of MOM-Cl (96 g, 1192 mmol). The reaction mixture was stirred at RT for 16 h under $N_2$. The reaction was monitored by TLC (30 % ethyl acetate in *n*-hexanes). After completion of the reaction, the reaction mixture was quenched with ice (1 kg). The solids observed were filtered through Buchner funnel, washed with cold water (1 L) and dried under vacuum to yield **3** as a brown solid (98 g, 62 %).

**$^1$H NMR (400 MHz, DMSO-$d_6$, 298 K):** δ (ppm) 8.01-8.00 (d, 1H), 7.79-7.71 (m, 2H), 5.35 (s, 2H), 3.54 (s, 3H).

**LC-MS** (ES, *m/z*): 215.89 $[M+H]^+$.

**Synthesis of 4:**
To a stirred solution of **3** in a mixture of ethanol and water, iron powder was added followed by ammonium chloride at RT. The reaction mixture was heated to 90 °C for 6 h. The reaction progress was monitored by TLC (40 % ethyl acetate in *n*-hexanes). After completion of the

reaction, the medium was cooled to RT, filtered through celite bed and the filtrate was concentrated under vacuum to get a crude residue. The crude residue was portioned between ethyl acetate and water; the organic layer was dried and evaporated under vacuum to afford compound **4** as a sticky liquid (80 g, 93 %).

**$^1$H NMR (400 MHz, DMSO-$d_6$, 298 K):** δ (ppm) 7.13-7.11 (d, *J* = 8.4 Hz, 1H), 6.44-6.43 (d, *J* = 2.4 Hz, 1H), 6.18-6.16 (dd, *J* = 2.4 Hz, 8.4 Hz, 1H), 5.28 (s, 2H), 5.14 (s, 2H), 3.39 (s, 3H).

**LC-MS** (ES, *m/z*): 232 $[M+H]^+$.

**Synthesis of 5:**
To a stirred solution of **4** (80 g, 346 mmol) in THF (900 mL), a solution of $NaHCO_3$ (49.45 g, 588 mmol) in water (800 mL) was added, followed by Cbz-Cl (117.6 g, 692 mmol) at 0 °C. The reaction mixture was stirred at RT for 6 h. The reaction progress was monitored by TLC (40 % ethyl acetate in *n*-hexanes). After completion of the reaction, the medium was filtered and the filtrate was evaporated under vacuum to get a residue, which was diluted with ethyl acetate (1000 mL) and water (400 mL). The organic layer was separated and washed with water (2 x 500 mL) and brine (250 mL), dried over sodium sulfate and concentrated under reduced pressure to afford crude compound **5** as a white solid (75 g).

**$^1$H NMR (400 MHz, DMSO-$d_6$, 298 K):** δ (ppm) 7.44-7.26 (m, 8H), 6.91 (br, 1H), 6.68 (br, 1H), 5.231 (s, 2H), 5.18 (s, 2H), 3.51 (s, 3H).

**LC-MS** (ES, *m/z*): 364 $[M+H]^+$.

**Synthesis of 6:**
To a stirred solution containing **5** (65 g, 177 mmol) in dioxane (650 mL) and water (400 mL), $K_2CO_3$ (73.59 g, 531 mmol) and 4,4,5,5-tetramethyl-2-vinyl-1,3,2-dioxaborolane (44.47 g, 301.9 mmol) were added. The reaction mixture was purged with $N_2$ gas for 30 min, followed by addition of $Pd_2(dba)_3$ (16.25 g, 17.7 mmol) and X-phos (8.45 g, 17.7 mmol). The reaction mixture was heated to 100 °C for 16 h. The reaction progress was monitored by TLC (30 % ethyl acetate in *n*-hexanes). After completion of the reaction, the medium was filtered through celite bed and washed with EtOAc (500 mL). The filtrate was evaporated under vacuum to get crude residue **6** as a thick brown syrup (100 g, crude). The crude product was purified by column using 60-120 silica gel. The product was eluted at 15 % EtOAc in petroleum ether, and the combined fraction was evaporated under vacuum to yield **6** as a brown solid (38 g , 69 %).

**$^1$H NMR (400 MHz, DMSO-$d_6$, 298 K):** δ (ppm) 7.39-7.34 (m, 5H), 7.02-6.95 (m, 2H), 6.67 (br, 1H), 5.69-5.64 (d, *J* = 17.6 Hz, 1H), 5.22-5.19 (m, 5H), 3.49 (s, 3H).

**LC-MS** (ES, *m/z*): 314.19 $[M+H]^+$.

**Synthesis of 7:**
To a stirred solution of compound **6** in THF (400 mL), was added $BH_3$-THF in THF at 0 °C. The reaction mixture was stirred for 3 h at RT. The reaction progress was monitored by TLC (40% EtOAc in *n*-hexanes). Then, the medium was cooled to 0 °C, quenched with cold water (250 mL) and stirred for 30 min. Volatiles were evaporated under vacuum to get crude, diluted with methanol (250 mL) and 6 N HCl (190 mL) at 0 °C and stirred at RT for 16 h. The reaction mixture was concentrated under reduced pressure. The residue was portioned between water (500 mL) and EtOAc (1000 mL); the organic layer was separated and washed with water (500 mL) and brine (250 mL), dried over sodium sulfate and concentrated under reduced pressure to afford crude compound **7** as a thick colourless syrup (40 g, crude). The crude product was then purified by column using 60-120 silica gel. The product was eluted at 20 % EtOAc in petroleum ether, and the combined fraction was evaporated under vacuum to get **7** as a white solid (9.5 g, 26 %).

**$^1$H NMR (400 MHz, DMSO-$d_6$, 298 K):** δ (ppm) 9.66 (s, 1H), 8.91 (s, 1H), 7.43-7.34 (m, 5H), 7.06-6.99 (m, 3H), 5.13 (s, 2H), 2.66-2.62 (t, *J* = 7.6 Hz, 2H), 1.01-0.97 (t, *J* = 8.0 Hz, 2H).

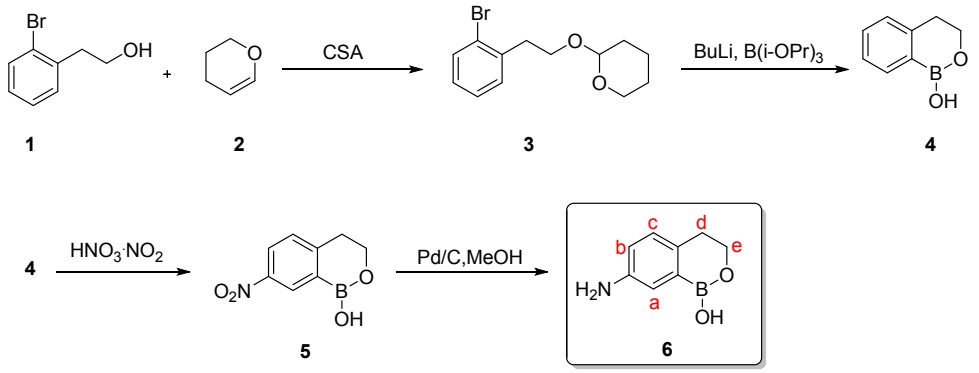
**LC-MS** (ES, *m/z*): 298.15 $[M+H]^+$.

**Synthesis of 8 (1,2-ABORIN):**
Pd/C (10% Wt) (940 mg) was added to a stirred solution of **7** (9.4 g, 316 mmol) in EtOAc (180 mL). The reaction mixture was hydrogenated under balloon pressure at room temperature for 4 h. The reaction progress was monitored by TLC (50 % ethyl acetate in *n*-hexanes). After completion of the reaction, the medium was filtered through celite bed and washed with EtOAc (500 mL). The filtrate was evaporated to 1/3 part the cooled 0 °C, and 4 M HCl in dioxane was added under stirring for 1 h at RT. Then, the reaction mixture was concentrated under reduced pressure to afford **8** as an off-white solid (5.2 g, 82 %).

**$^1$H NMR (400 MHz, DMSO-$d_6$, 298 K):** δ (ppm) 10.00 (br, 4H), 7.19-7.18 (d, *J* = 12.8 Hz, 1H, **Ha**), 6.89-6.86 (m, 2H, **Hb-Hc**), 2.74-2.70 (t, *J* = 7.6 Hz, 2H, **Hd**), 1.05-1.01 (t, *J* = 8.0 Hz, 2H, **He**).

**LC-MS** (ES, *m/z*): 164.1 $[M+H]^+$.

***7-amino-3,4-dihydro-1H-benzo[c][1,2]oxaborinin-1-ol (2,1-ABORIN)***




**Synthesis of 3:**
To a solution of compound **1** (20 g, 100.0 mmol, 1.0 eq) in DCM (400 mL) was added compound **2** (13.6 mL, 150 mmol, 1.5 eq), followed by camphor sulfonic acid (CSA, 400 mg, 0.02 eq). The mixture was stirred at room temperature for 2 h. HPLC indicated that the reaction was completed. After adding $K_2CO_3$ (1.2 g), the mixture was filtered to remove the precipitate, the filtrate was washed with $H_2O$ (200 mL) and brine (200 mL). Then, the organic phase was dried, filtered and the filtrate was concentrated under reduced pressure. The oily residue was applied to silica chromatography eluting with EtOAc/Heptanes (0:100 to 50:50) to give compound **3** as a yellow liquid (26.4 g, not pure).

**Synthesis of 4:**
To a solution of **3** (26.0 g, 91.0 mmol, 1.0 eq.) in THF (500 mL) at -78 °C, was slowly added *n*-BuLi (2.5 M in hexane, 73 mL, 182 mmol, 2.0 eq) under nitrogen atmosphere. Triisopropyl borate (68.6 g, 364 mmol, 4.0 eq) was then added and stirred at -78 °C for 30 min. The mixture was allowed to warm to room temperature gradually and stirred overnight. HPLC indicated that the reaction was completed. After carefully adding HCl (260 mL, 6 N), the yellowish solution was stirred at room temperature for another 1 h and then poured into a mixture of EtOAc (600 mL) and $H_2O$ (300 mL). The layers were separated, and the aqueous phase was extracted

with EtOAc (3 x 200 mL). Combined organic extracts was washed with $H_2O$ (200 mL), brine (200 mL), dried over $MgSO_4$, filtered and the filtrate was concentrated under reduced pressure. The oily residue was applied to silica chromatography (petroleum ether/ethyl acetate 5:1) to give compound **4** (11.5 g, 88 %) as a light-yellow solid.

**$^1$H NMR (300 MHz, DMSO-$d_6$, 298 K):** δ (ppm) 8.40 (br, 1H), 7.68 (d, *J* = 7.2 Hz, 1H), 7.38-7.36 (m, 1H), 7.25-7.18 (m, 2H), 4.07 (t, *J* = 6.0 Hz, 2H), 2.86 (t, *J* = 6.0 Hz, 2H).

**Synthesis of 5:**
To 100 mL fuming $HNO_3$ at -45 °C, compound **4** (13.2 g, 89.1 mmol) was added in small portions while maintaining the reaction temperature between -40 to -45°C. Once the addition was completed, the resulting solution was allowed to stir at -45°C for additional 10 min before poured into crushed ice (500 g). The ice mixture was allowed to warm up to room temperature gradually and the precipitate was collected by filtration and washed with $H_2O$ to afford **5** (4.5 g, 26 %) as a white powder.

**$^1$H NMR (300 MHz, DMSO-$d_6$, 298 K):** δ (ppm) 8.48 (d, *J* = 2.1 Hz, 1H), 8.25 (dd, *J* = 2.4, 8.4 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 4.12 (t, *J* = 6.0 Hz, 2H), 3.02 (t, *J* = 5.7 Hz, 2H).

**Synthesis of 6 (2,1-ABORIN):**
To a solution of compound **5** (2.5 g, 12.9 mmol, 1 eq) in MeOH (25 mL), was added Pd/C (0.25 g) under $N_2$. Then, the suspension was degassed under vacuum and purged with $H_2$ for three times. The reaction mixture was stirred at 40 °C for 5 h under $H_2$. TLC (petroleum ether/ethyl acetate 7:3) indicated that the reaction was completed. The reaction mixture was filtered to remove Pd/C and the filtrate was concentrated to dryness to give **6** (1.7 g, 81 %).

**$^1$H NMR (400 MHz, $CDCl_3$, 298 K):** δ (ppm) 7.11-6.97 (m, 2H, **Ha-Hc**), 6.79-6.73 (m, 1H, **Hb**), 4.23-4.18 (m, 2H, **He**), 2.88-2.82 (m, 2H, **Hd**).

**LC-MS** (MS, *m/z*): 164.1 $[M+H]^+$.

**2. $^1$H NMR spectra of HA-1,2-BORIN, HA-2,1-BORIN, HA-BOR and HA-PBA derivatives synthesized by amide coupling reaction ($M_w$ HA = 360 kg/mol)**

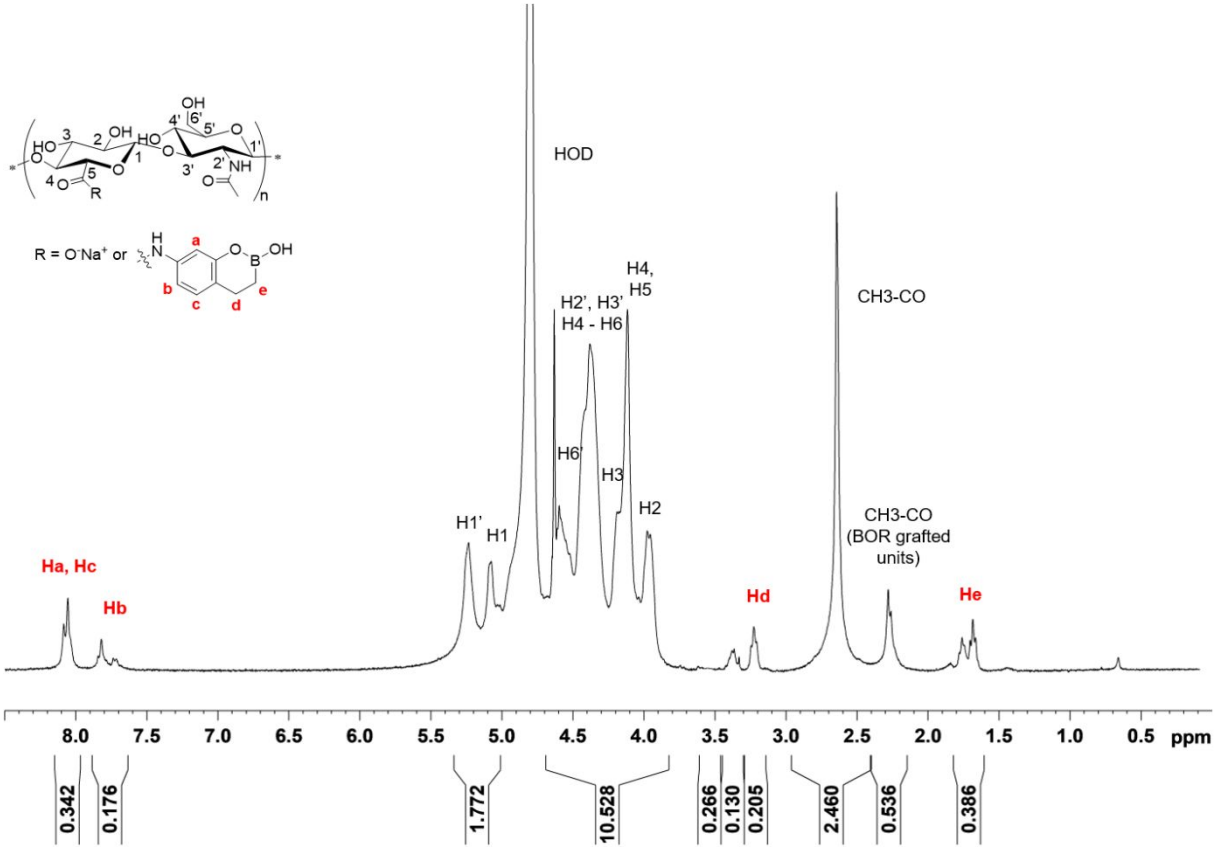



**Figure S1.** $^1$H NMR spectrum (400 MHz, $D_2O$, 6 mg/mL, 80 °C) of HA360-1,2-BORIN.

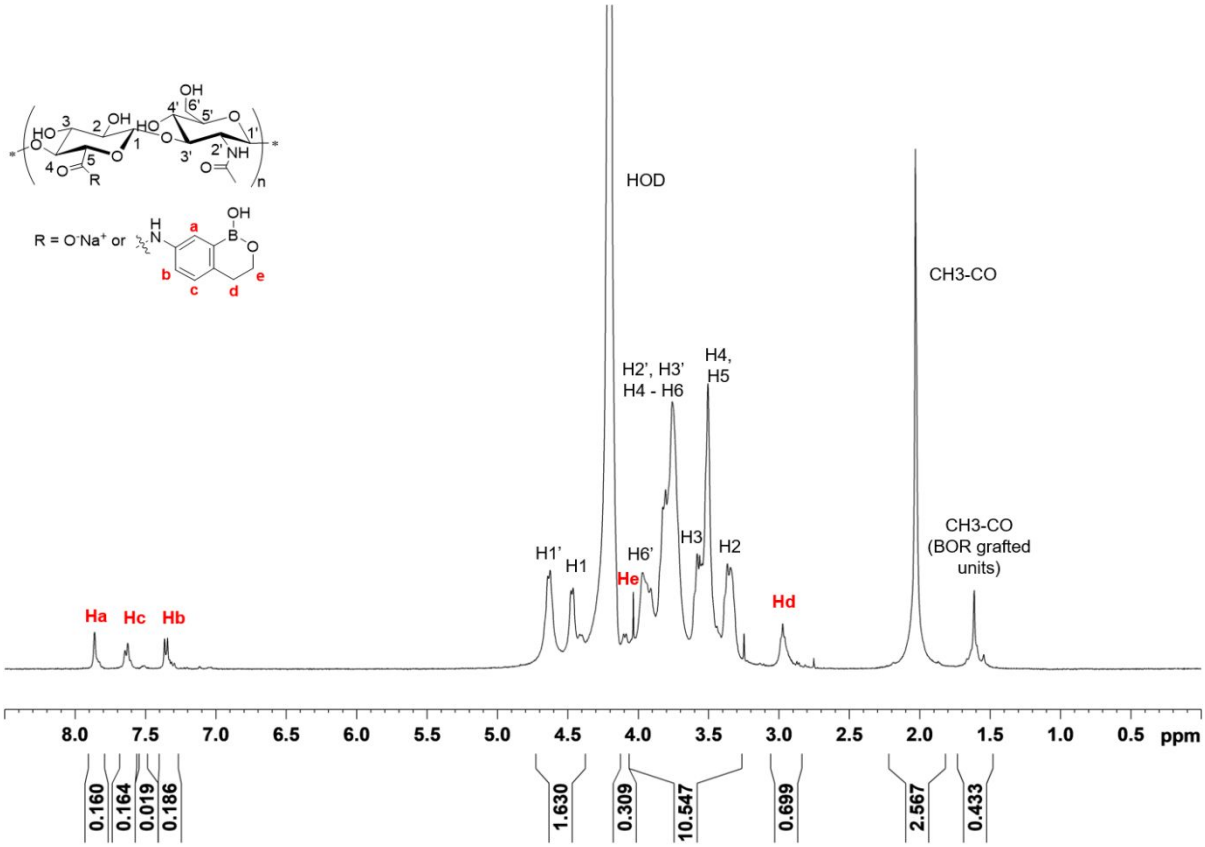



**Figure S2.** $^1$H NMR spectrum (400 MHz, $D_2O$, 6 mg/mL, 80 °C) of HA360-2,1-BORIN.

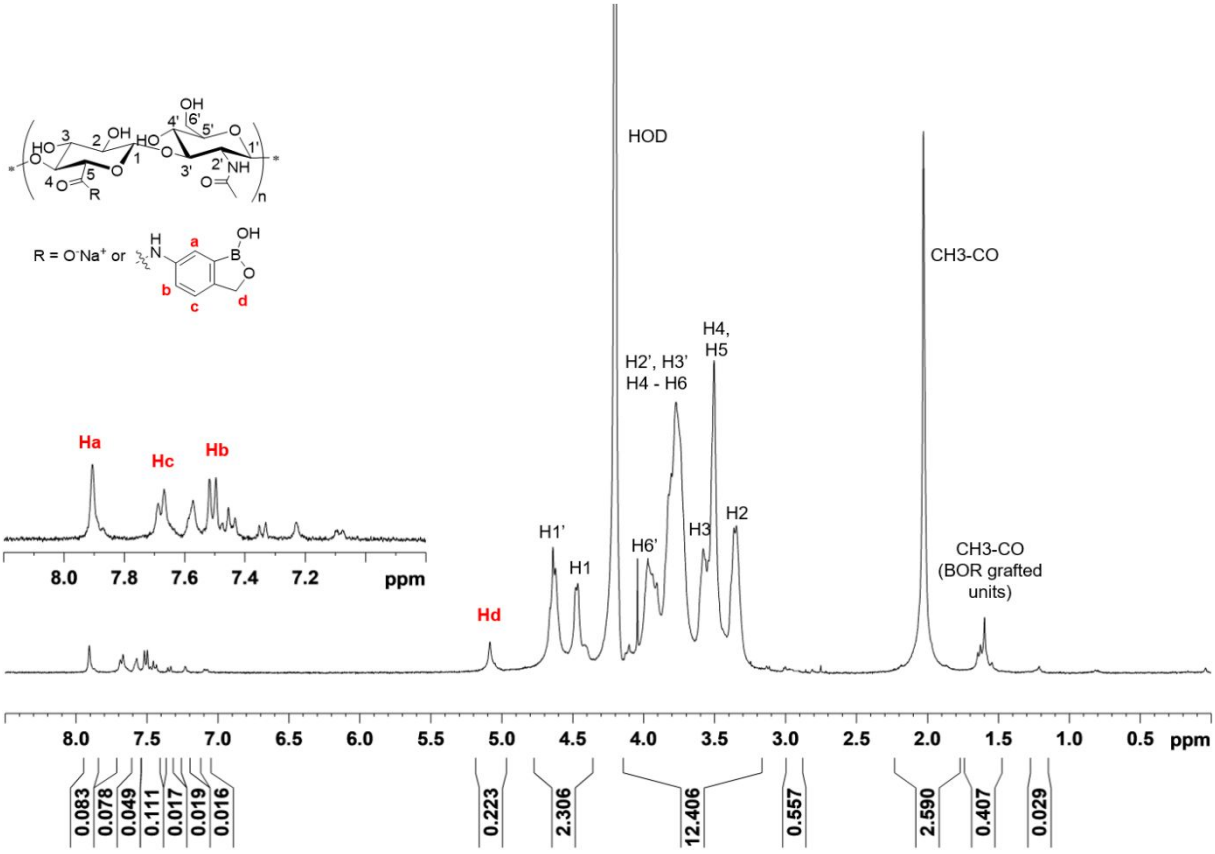



**Figure S3.** $^1$H NMR spectrum (400 MHz, $D_2O$, 6 mg/mL, 80 °C) of HA360-BOR.

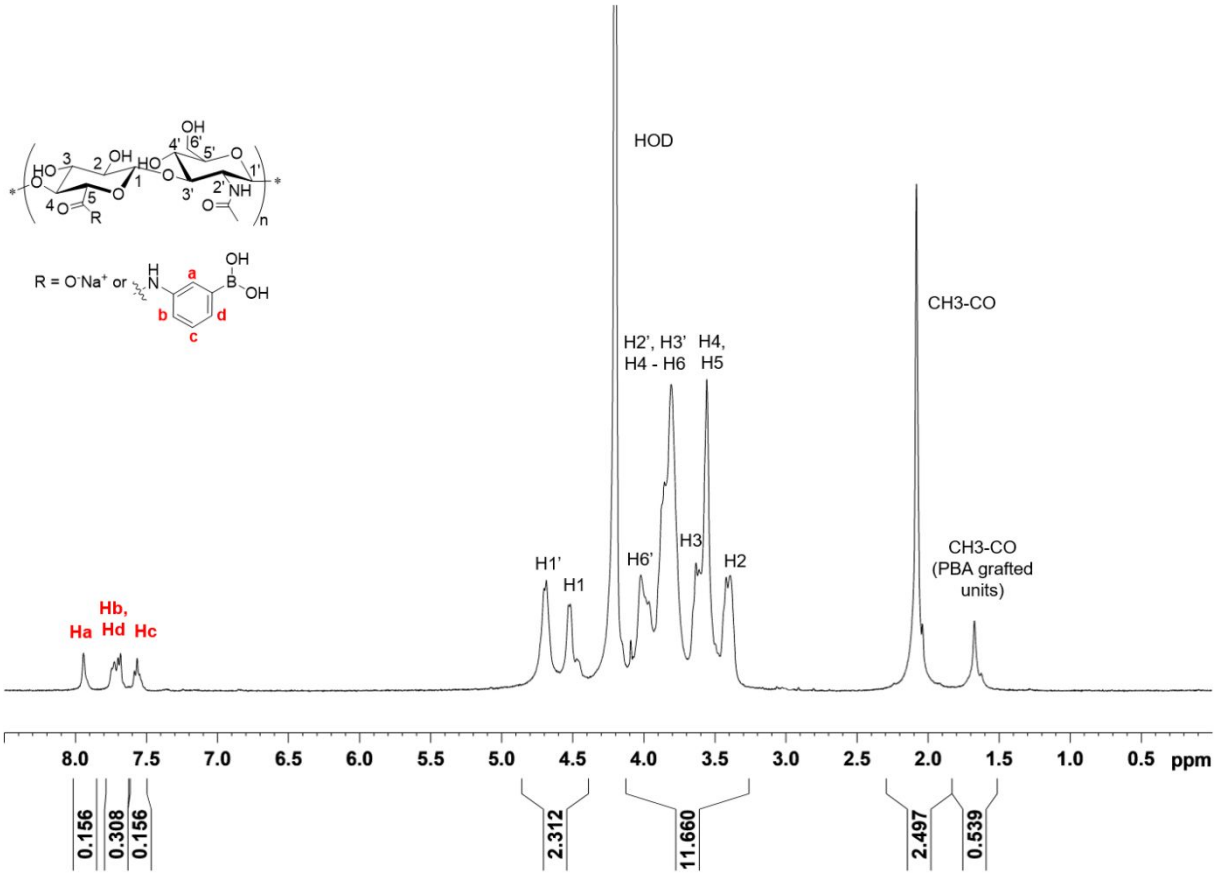



**Figure S4.** $^{1}H$ NMR spectrum (400 MHz, $D_2O$, 6 mg/mL, 80 °C) of HA360-PBA.

**3. 2D HSQC NMR characterization of HA-fructose conjugate synthesized by amide coupling reaction ($M_w$ HA = 360 kg/mol)**

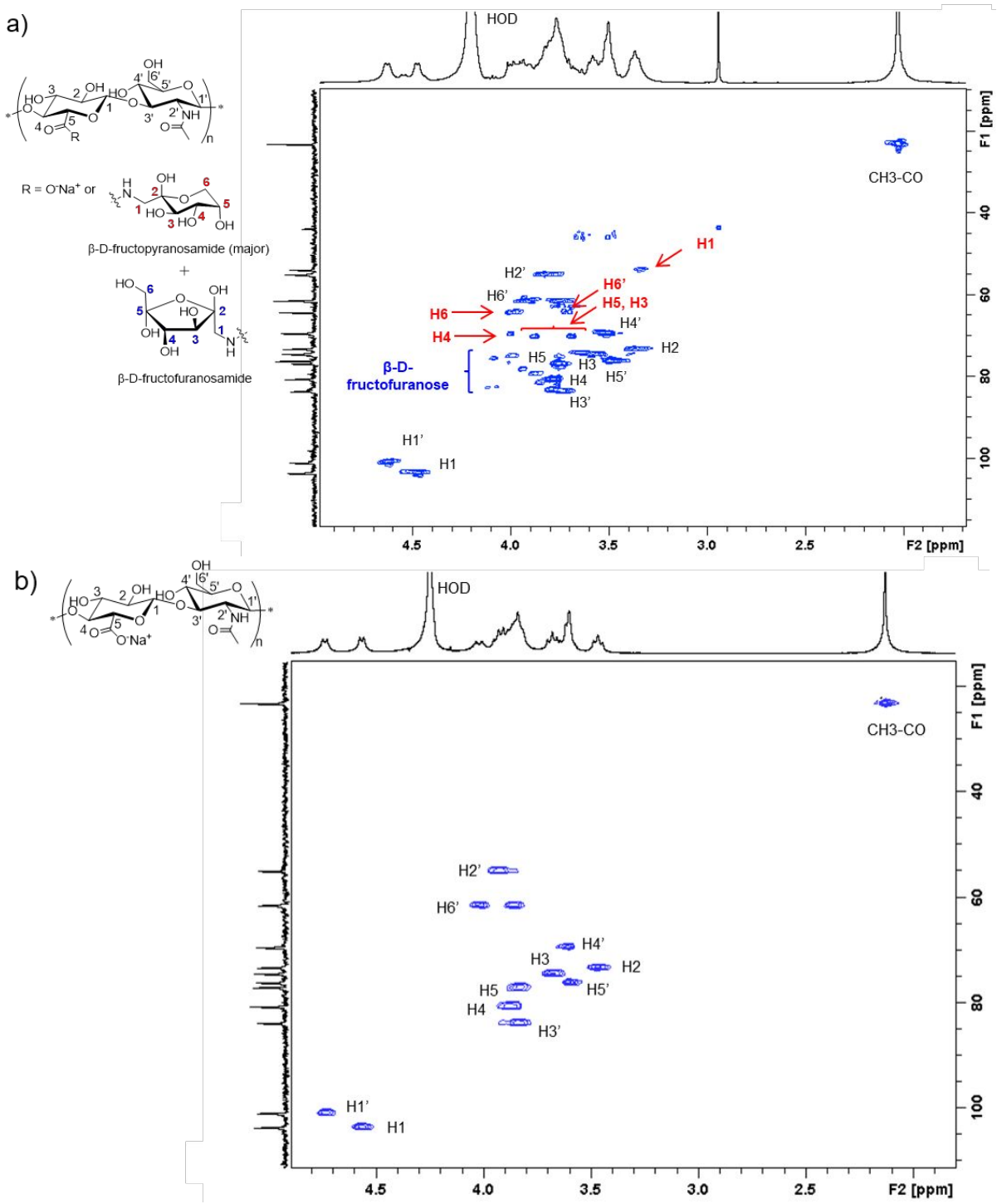
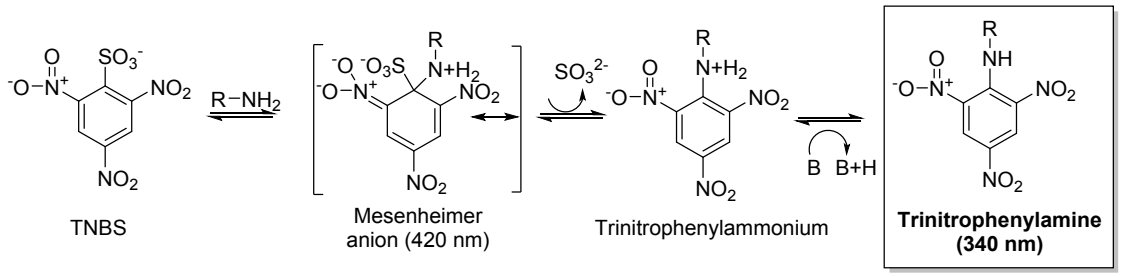



**Figure S5.** 2D HSQC NMR spectra (400 or 100 MHz, $D_2O$, 6 mg/mL, 80 °C) of HA360-fructose (a) compared to native HA360 (b). Of note, β-D-fructopyranose is the major tautomeric form observed for grafted fructose moieties, followed by the β-D-fructofuranose form.

## 4. Determination of the degree of substitution (DS) of HA-fructose from the kinetics of the amide coupling reaction ($M_w$ HA = 360 kg/mol)

As the superposition of $^1$H signals of HA and grafted fructose moieties precluded determination of the DS of HA-fructose by $^1$H NMR, their DS were estimated from the kinetics of their syntheses, by quantifying the free primary amines in the reaction medium as a function of time. This was based on the reaction of fructosamine with 2,4,6-Trinitrobenzene Sulfonic Acid (TNBS), which gives an orange-colored final product (trinitrophenylamine) that absorbs in the UV region, at around 340 nm (Scheme 1).[1,2]




**Scheme 1.** Trinitrophenylation of primary amines with TNBS.

*Procedure for the reaction kinetics by quantifying primary amines with TNBS*

The methodology to quantify primary amines with TNBS was adapted from a procedure previously described.[3] Standard curves of fructosamine were prepared by diluting 1 mg/mL stock solutions in 0.1 M sodium bicarbonate buffer pH 8.5 at known concentrations of amine (10 to 50 µg/mL). Various volumes of a fresh solution of 0.01 % TNBS (w/v) in the same buffer were added in each solution, in order to get a molar ratio of TNBS/amine of 1, and samples were incubated at 37 °C for 2 h. Then, the product of reaction between the amine and TNBS was analyzed by UV spectroscopy (from 580 to 280 nm) after addition of a small volume of 1 M HCl (150 µL) in the samples. The same procedure was used to quantify amines during the amide coupling reactions with HA, by taking small aliquots of the reaction medium as a function of time.

*Procedure for determining the DS from the kinetics of amide coupling reactions*

From the UV spectra recorded for fructosamine-TNB derivatives, a standard curve was plotted with the maximal absorbance values (curve (a) in Figure S6). It is important to note that the wavelength of the maximal absorbance depends on the primary amines. This allowed us to determine the amount of unreacted primary amines during the amide coupling reaction with HA using a fructosamine/HA molar ratio of 0.15. From the kinetic curve, we estimated approximately 100 % of conversion for HA-fructose within 24 h, which gave a value of DS of 0.15 (curve (b) in Figure S6).

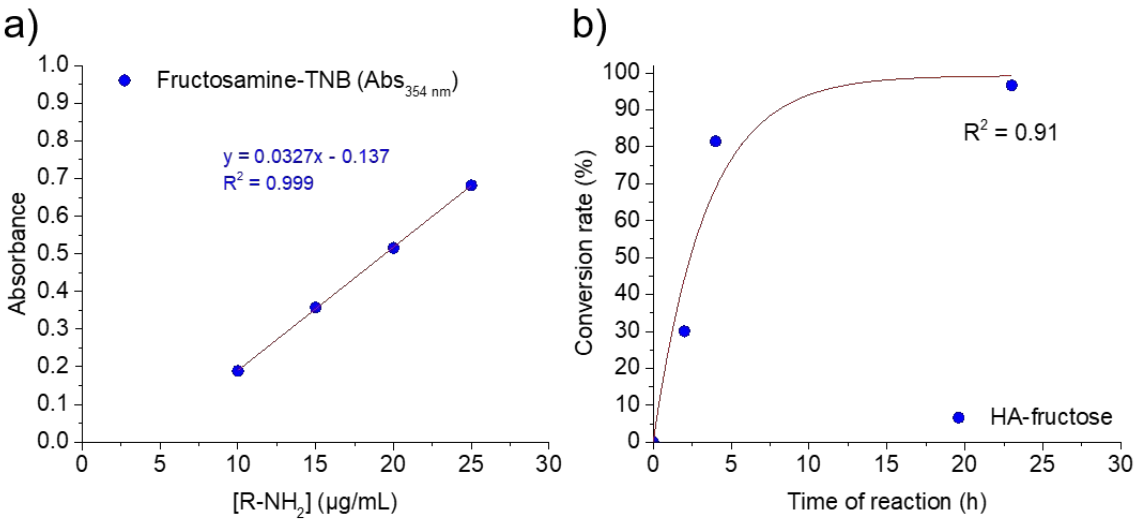



**Figure S6.** Reaction kinetics for the synthesis of HA360-fructose. Standard curve with the equation used to quantify free fructosamine (a), and kinetic curve plotted for the coupling reaction of fructosamine with HA360 (curve fitting by non-linear fit model using Origin 2015 software).

## 5. Methodology for $K_a$ measurements by $^1$H NMR spectroscopy

The procedure to determine the $K_a$ values was adapted from previous studies using free boronic acids and saccharides.[4,5] This method consisted in assuming that a boronic acid (B) and a saccharide (S) bind in one modality, BS:

$$B + S \rightleftarrows BS$$

$$K_a = \frac{[BS]}{[B].[S]} \qquad (1)$$

Where [B], [S] and [BS] are the molar concentrations of the free boronic acid, the free saccharide and the complex, respectively.

To calculate $K_a$, the [BS]/[B] ratio was determined by digital integration of the aryl protons of the boronic acid/saccharide complex and of the free boronic acid. This allowed calculation of [B], [BS] and [S], as follows:

$$[B] = \frac{[B]_0}{\frac{[BS]}{[B]} + 1} \qquad (2)$$

Where $[B]_0$ is the initial concentration of boronic acid added in the NMR tube, and

$$[BS] = \frac{[BS]}{[B]}[B] \qquad (3)$$

$$[S] = [S]_0 - [BS] \qquad (4)$$

Where $[S]_0$ is the initial concentration of saccharide added in the NMR tube.

*Procedure for the preparation of samples for NMR analysis*

Stock solutions of 1,2-ABORIN (2,1-ABORIN or APBA or ABOR) and free (grafted) fructose (HA-fructose) were prepared by solubilization in distilled water. When necessary, the pH was carefully adjusted to 7.4 by adding 1 M NaOH, using a pH-meter, and water was added to get concentrations ranging from 4 to 60 mM. Then, the solutions were diluted with 0.02 M PBS pH 7.4 in order to obtain final concentrations of 2 to 30 mM boronic acid or saccharide in 0.01 M PBS at pH 7.4. The solutions of complexes were then prepared by mixing various volumes of a stock solution of 1,2-ABORIN (2,1-ABORIN or APBA or ABOR) with a stock solution of free D-fructose or HA-fructose. This generated mixtures at pH 7.4 (± 0.1), with a boronic acid/fructose molar ratio ranging from 0.3 to 1.5. Water was removed by freeze-drying and the samples were properly dissolved in $D_2O$ prior to NMR analysis. Each value of $K_a$ was determined from two experiments of titration using freshly prepared solutions. $^1$H NMR titration was performed with at least 6 different concentrations at 25 °C.

*Procedure for determining the chemical shifts of aryl protons of bound and free boronic acids*

$^1$H NMR spectra of 1,2-ABORIN, 2,1-ABORIN, APBA and ABOR alone were first recorded (spectra (a) in Figure S7-S14). Second, $^1$H NMR spectra of the boronic acid derivatives in the presence of excess free D-fructose to induce 100 % complex formation are acquired (spectra (b) in Figure S7-S8 and S11-S12). Comparison of spectra (a) and (b) allows the assignment of the aryl protons of bound and free boronic acids. This analysis was then used to interpret the spectra from the titrations with fructose (free in solution or grafted on HA; spectra (c) in Figure S7-S8 and S11-S12 or spectra (b) in Figure S9-S10 and S13-S14).

## 6. Determination of $K_a$ by $^1$H NMR for 1,2-ABORIN (2,1-ABORIN) and fructose (free fructose and HA-fructose)

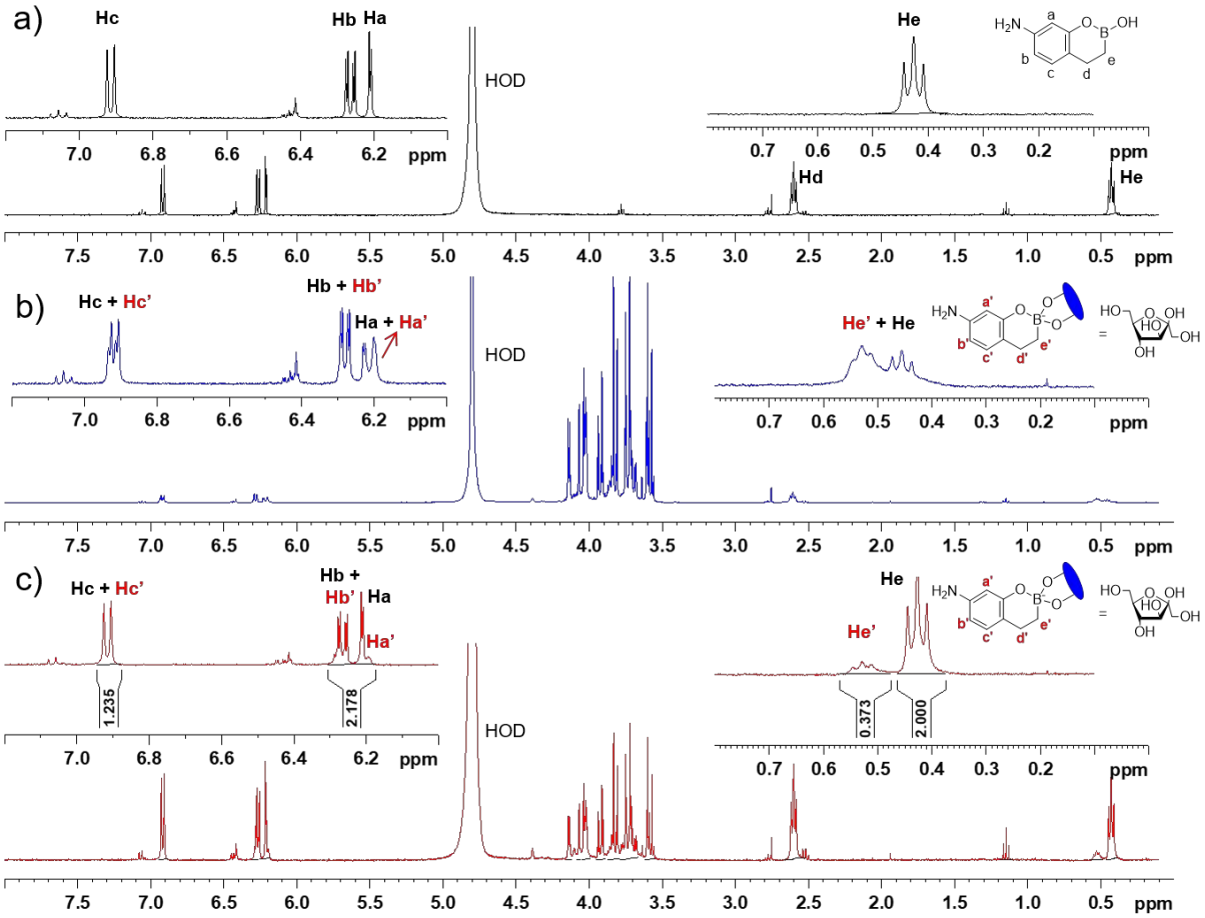



**Figure S7.** $^1$H NMR spectra in 0.01 M deuterated PBS pH 7.4 at 25 °C of 1,2-ABORIN alone (1 mM) (a), 1,2-ABORIN (1 mM) in the presence of excess D-fructose (10 mM) (b) and 1,2-ABORIN (1 mM) with D-fructose (1 mM) (c). The [BS]/[B] ratio was calculated from the digital integration of He (free 1,2-ABORIN) and He' (complexed 1,2-ABORIN) signals. Of note, the position of the oxygen of the intramolecular B-O bond between the phenyl ring and the boron atom precluded observation of $^1$H signals in the aromatic region corresponding to bound and unbound forms of 1,2-ABORIN.

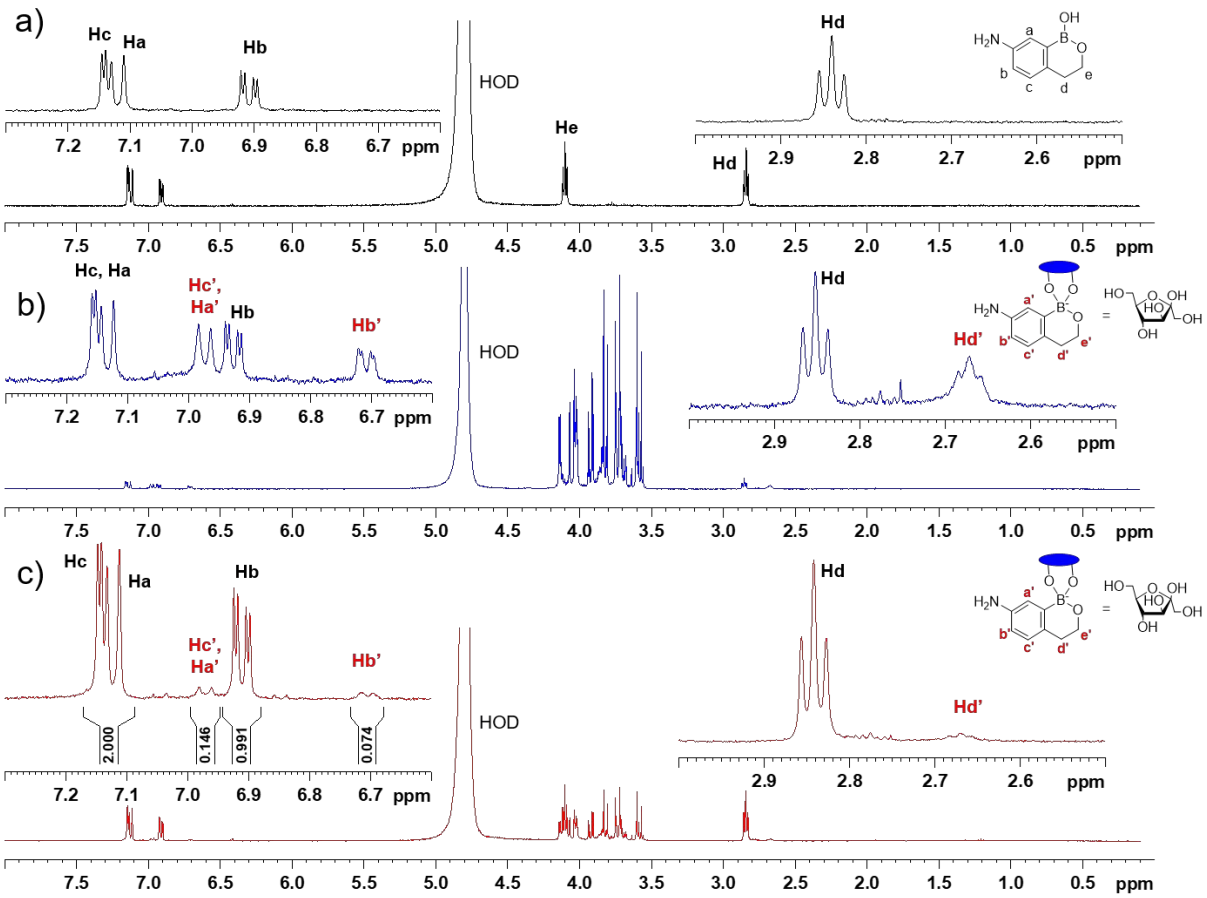



**Figure S8.** $^{1}$H NMR spectra in 0.01 M deuterated PBS pH 7.4 at 25 °C of 2,1-ABORIN alone (1 mM) (a), 2,1-ABORIN (1 mM) in the presence of excess D-fructose (10 mM) (b) and 2,1-ABORIN (1 mM) with D-fructose (1 mM) (c). The [BS]/[B] ratio was calculated from the digital integration of Hc, Ha (free 2,1-ABORIN) and Hb' (complexed 2,1-ABORIN) signals.

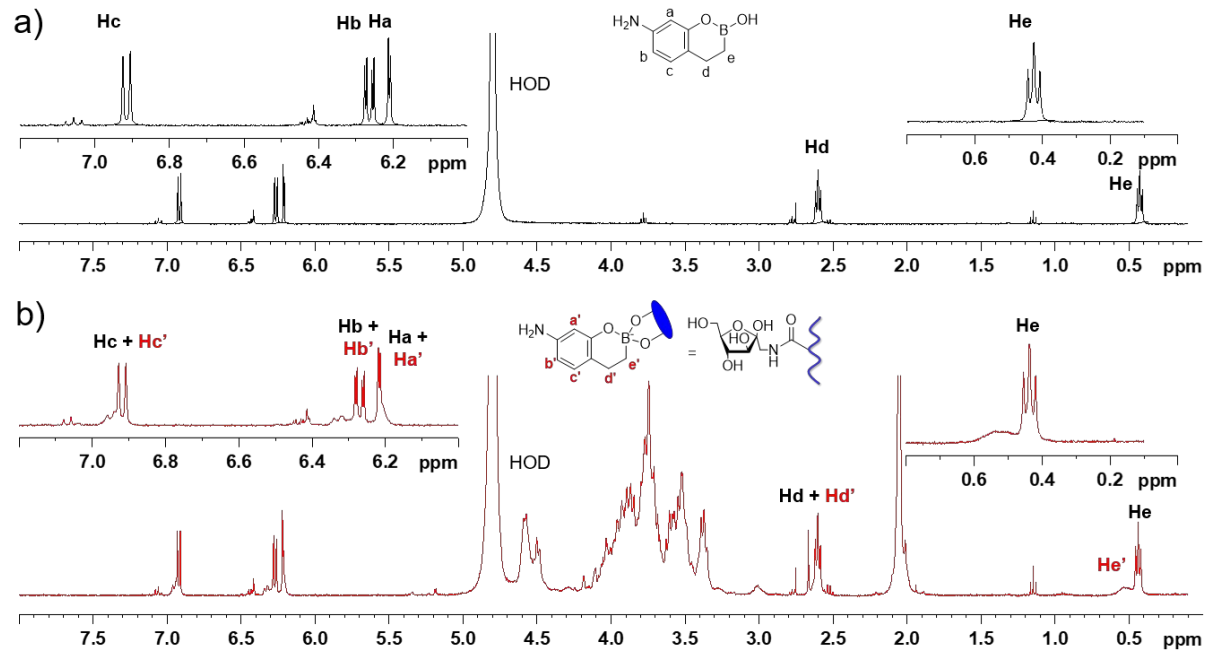



**Figure S9.** $^{1}$H NMR spectra in 0.01 M deuterated PBS pH 7.4 at 25 °C of 1,2-ABORIN alone (1 mM) (a) and 1,2-ABORIN (1 mM) with HA-fructose (1 mM of grafted fructose) (b). $K_a$ was not measured because $^{1}$H signals corresponding to free and complexed 1,2-ABORIN were not sufficiently separated to be integrated.

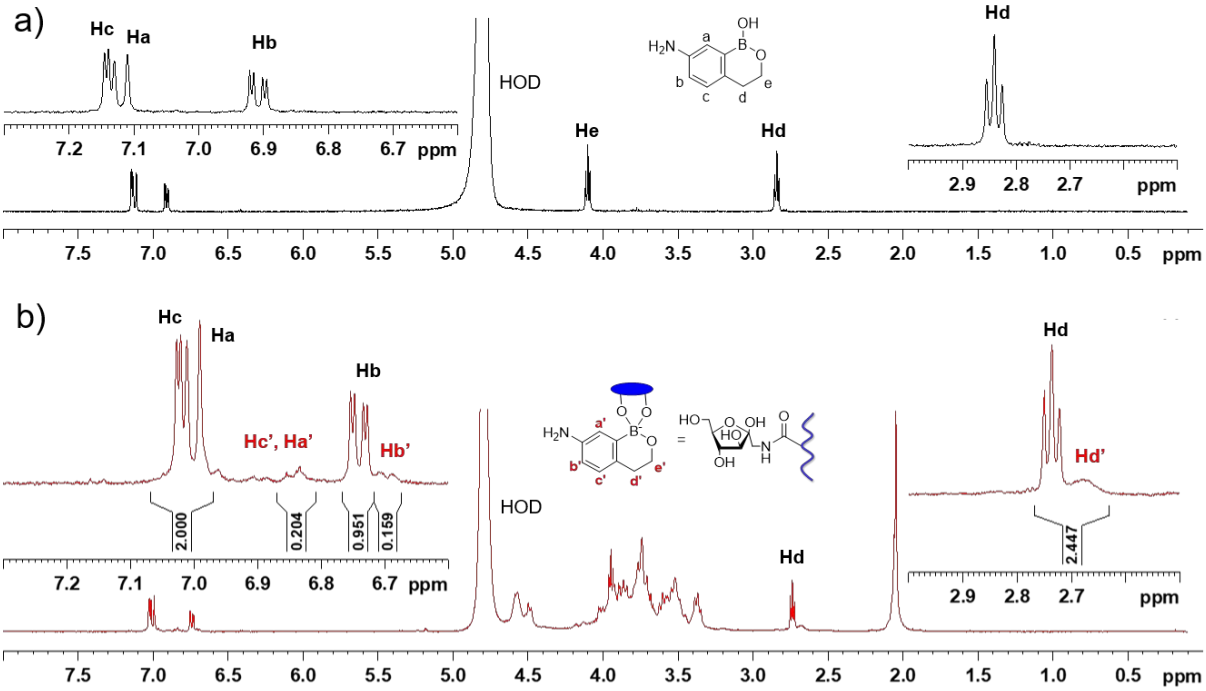



**Figure S10.** $^{1}$H NMR spectra in 0.01 M deuterated PBS pH 7.4 at 25 °C of 2,1-ABORIN alone (1 mM) (a) and 2,1-ABORIN (1 mM) with HA-fructose (1 mM of grafted fructose) (b). The [BS]/[B] ratio was calculated from the digital integration of Hc, Ha (free 2,1-ABORIN) and Hd, Hd' (4H, free and complexed 2,1-ABORIN) signals.

## 7. Determination of $K_a$ by $^1$H NMR for APBA (ABOR) and fructose (free fructose and HA-fructose)

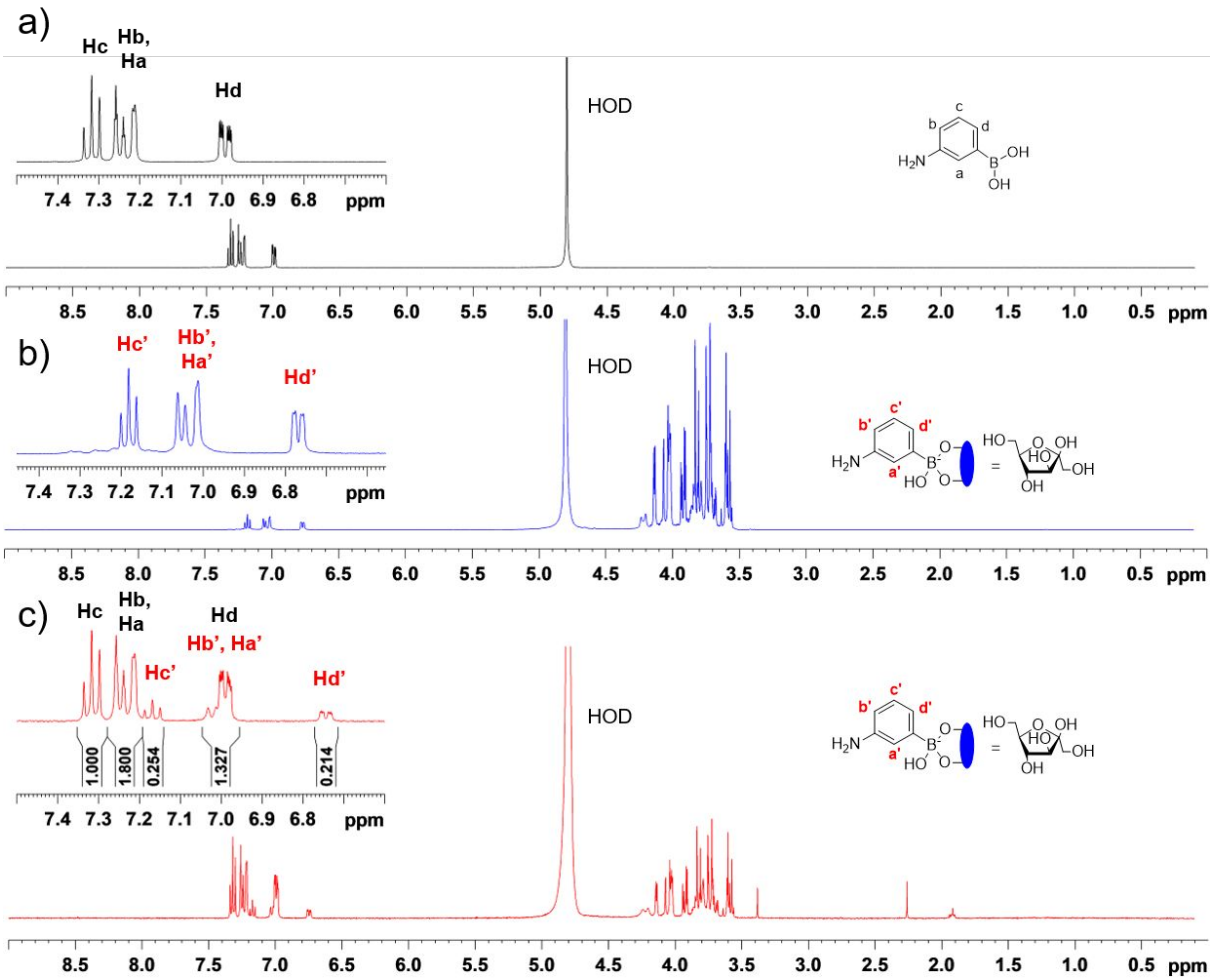



**Figure S11.** $^1$H NMR spectra in 0.01 M deuterated PBS pH 7.4 at 25 °C of APBA alone (15 mM) (a), APBA (15 mM) in the presence of excess D-fructose (150 mM) (b) and APBA (15 mM) with D-fructose (15 mM) (c). The [BS]/[B] ratio was calculated from the digital integration of Hc (free APBA) and Hd' (complexed APBA) signals.

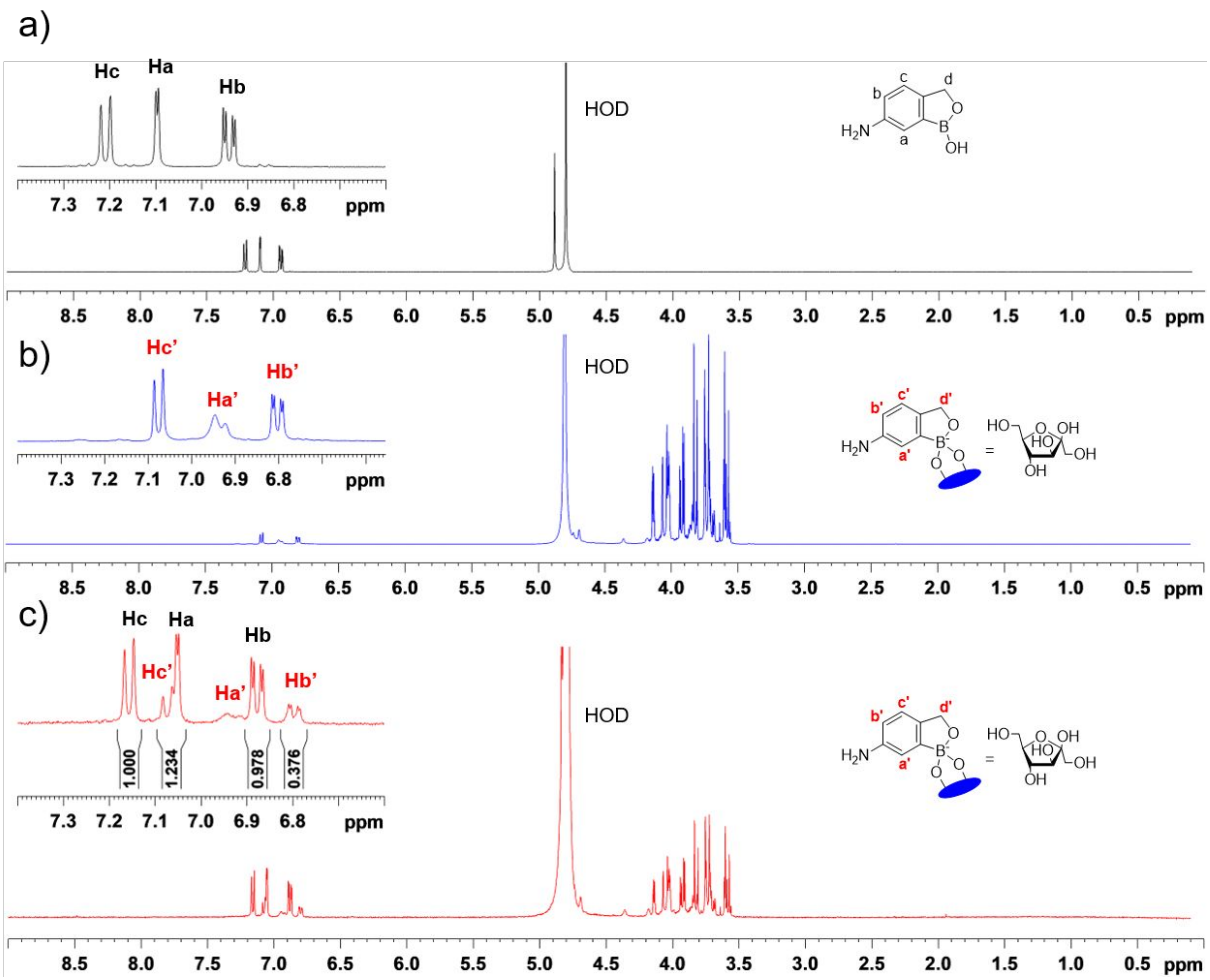



**Figure S12.** $^{1}$H NMR spectra in 0.01 M deuterated PBS pH 7.4 at 25 °C of ABOR alone (15 mM) (a), ABOR (15mM) in the presence of excess D-fructose (150 mM) (b) and ABOR (15 mM) with D-fructose (15 mM) (c). The [BS]/[B] ratio was calculated from the digital integration of Hc (free ABOR) and Hb' (complexed ABOR) signals.

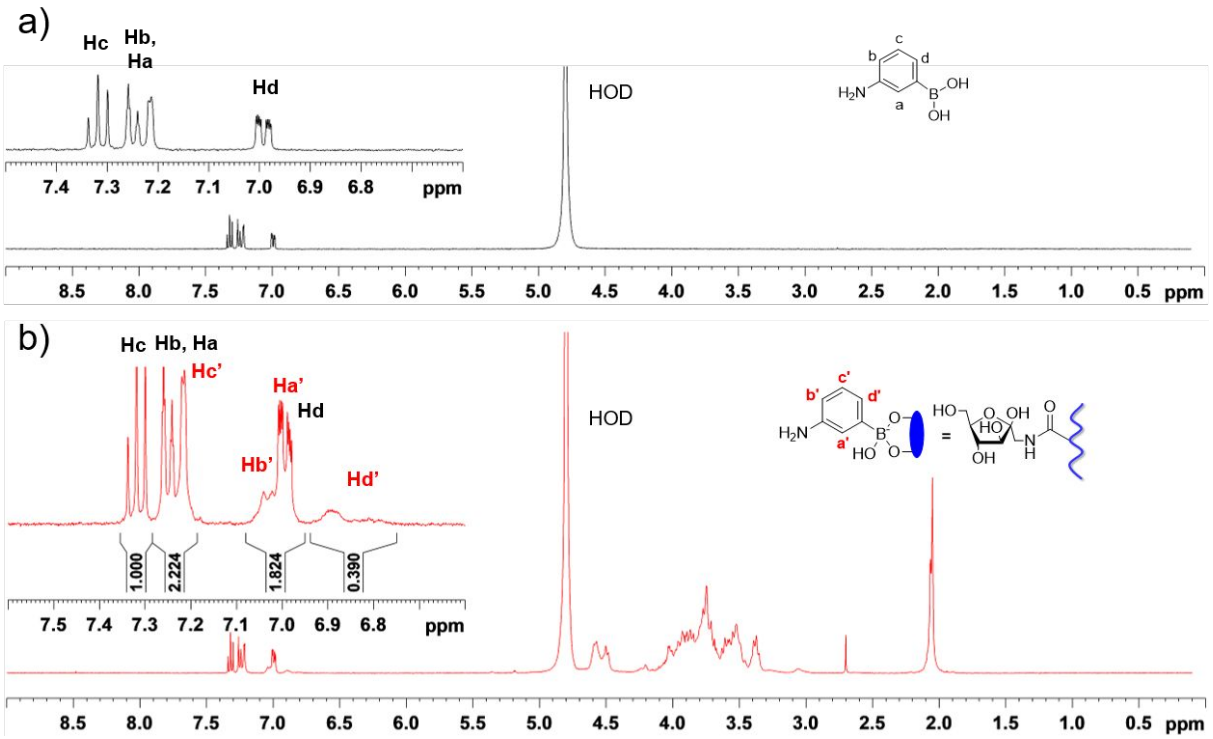



**Figure S13.** $^{1}$H NMR spectra in 0.01 M deuterated PBS pH 7.4 at 25 °C of APBA alone (1 mM) (a) and APBA (1 mM) with D-fructose (1 mM of grafted fructose) (b). Similar to free D-fructose, the [BS]/[B] ratio was calculated from the digital integration of Hc (free APBA) and Hd' (complexed APBA) signals.

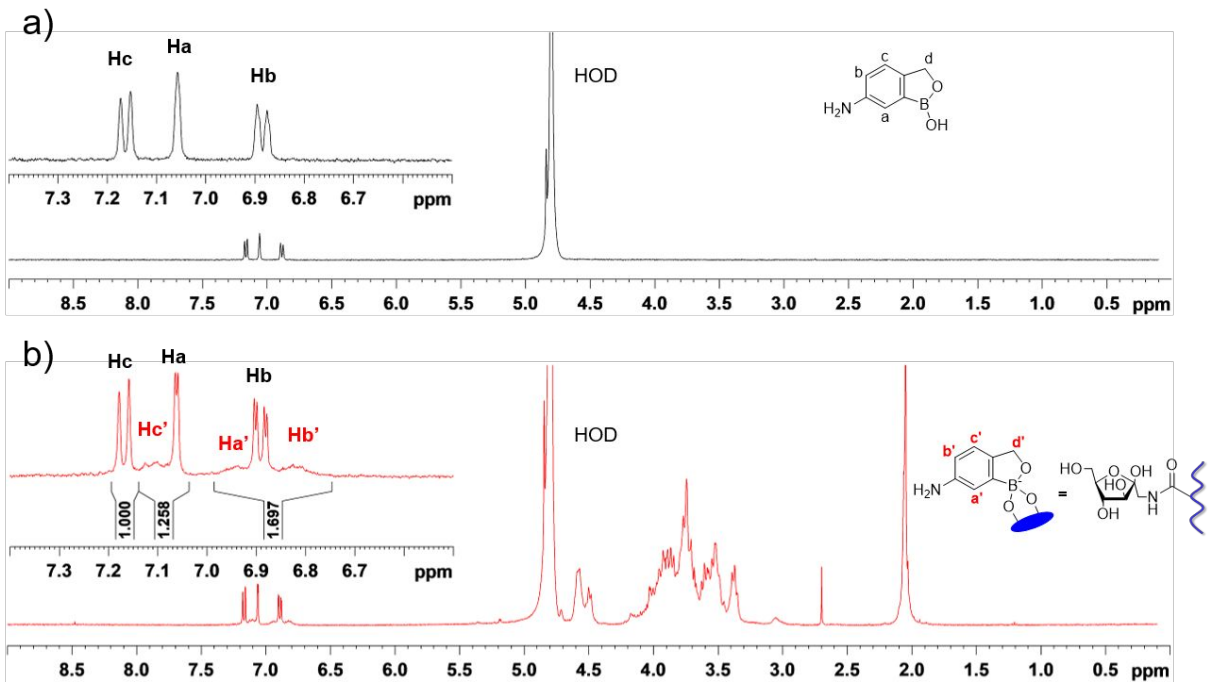



**Figure S14.** $^{1}$H NMR spectra in 0.01 M deuterated PBS pH 7.4 at 25 °C of ABOR alone (1mM) (a) and ABOR (1mM) with HA-fructose (1 mM of grafted fructose) (b). Similar to free D-fructose, the [BS]/[B] ratio was calculated from the digital integration of Hc (free ABOR) and Hb, Ha', Hb' (3H, free and complexed ABOR) signals.

## 8. Calorimetric titration of 1,2-ABORIN (2,1-ABORIN) with free D-fructose

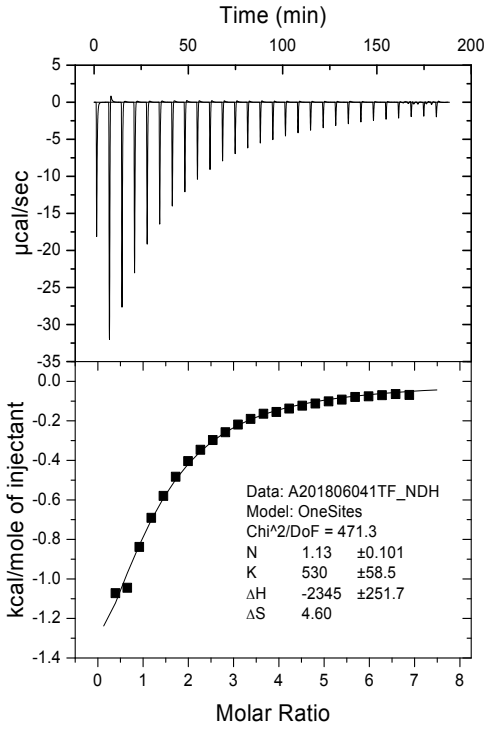



**Figure S15.** Calorimetric titration of 1,2-ABORIN (2 mM) with D-fructose (75 mM) in 0.01 M PBS (pH 7.4, 25 °C).

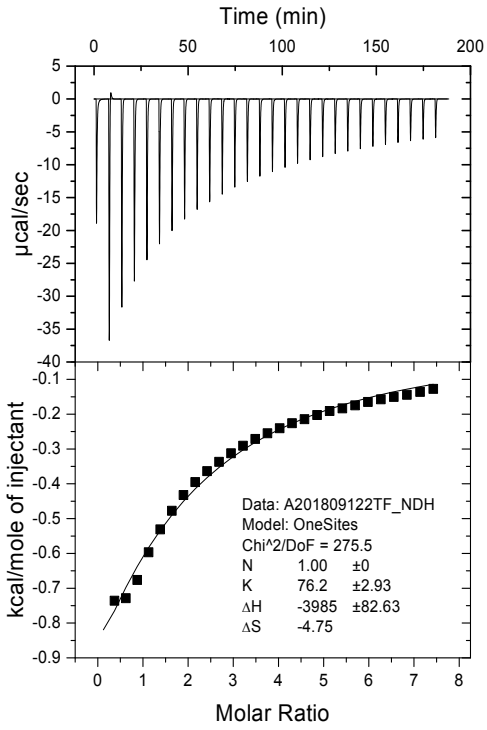



**Figure S16.** Calorimetric titration of 2,1-ABORIN (3.5 mM) with D-fructose (125 mM) in 0.01 M PBS (pH 7.4, 25 °C).

## 9. Calorimetric titration of APBA (ABOR) with free D-fructose

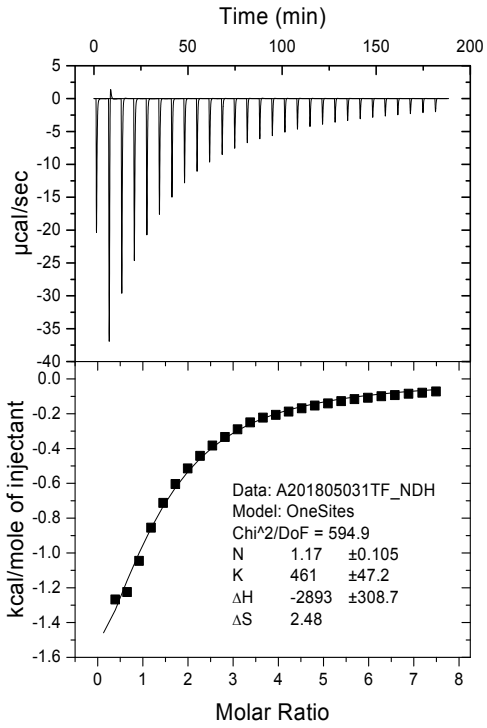



**Figure S17.** Calorimetric titration of ABOR (2 mM) with D-fructose (75 Mm) in 0.01 M PBS (pH 7.4, 25 °C).

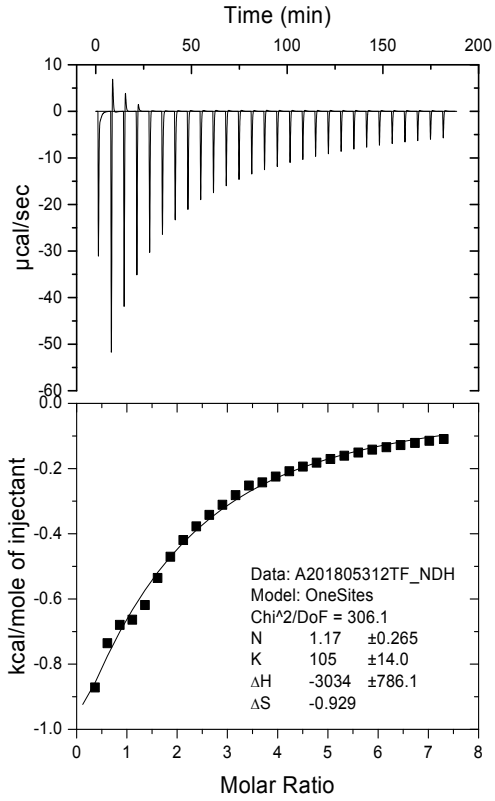



**Figure S18.** Calorimetric titration of APBA (5 mM) with D-fructose (175 mM) in 0.01 M PBS (pH 7.4, 25 °C).

## 10. Rheological analysis of the HA-1,2-BORIN (2,1-BORIN, BOR or PBA) alone at pH 7.4 ($M_w$ HA = 360 g/mol)

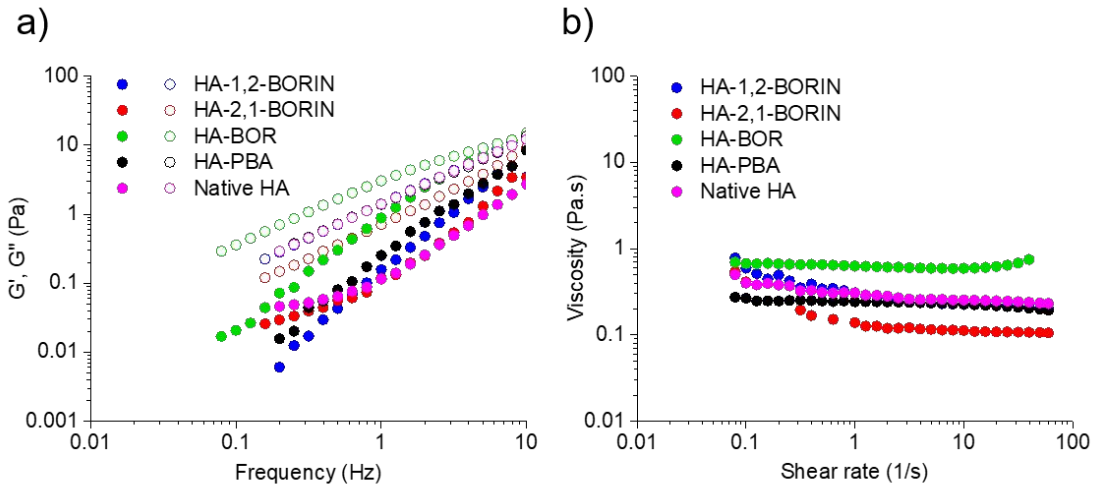



**Figure S19.** Rheological behavior of the HA360-1,2-BORIN, HA360-2,1-BORIN, HA360-BOR and HA360-PBA conjugates alone at pH 7.4 ($C_p$ = 15 g/L): (a) frequency dependence of the storage modulus (G', filled symbols) and loss modulus (G", empty symbols), and (b) viscosity .

## 11. Cytotoxicity (MTT) assay of individual solutions of HA derivatives incubated with mouse embryonic fibroblasts (MEFs)

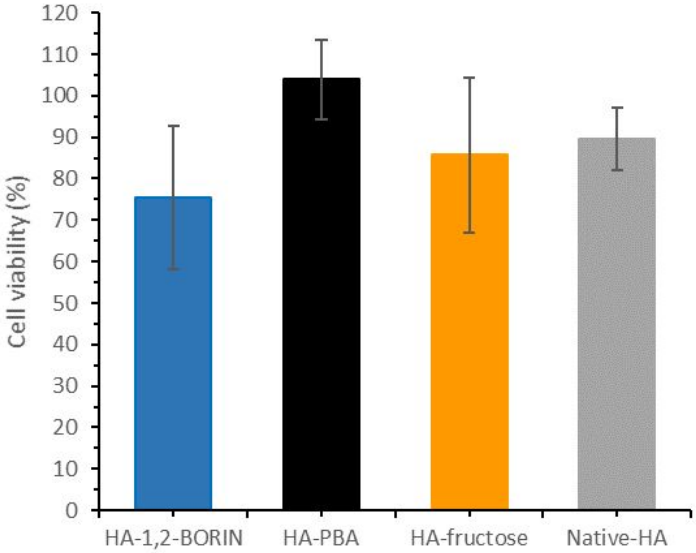



**Figure S20.** MTT assay of solutions of HA360-1,2-BORIN, HA360-PBA, HA360-fructose and native HA360 (at 7.5 g/L) incubated with MEFs in standard growth media for 72 h at 37 °C (experiment repeated 2 times independently). Error bars represent the standard errors of the mean (S.E.M.)

## References


(1) Cayot, P.; Tainturier, G. The Quantification of Protein Amino Groups by the Trinitrobenzenesulfonic Acid Method: A Reexamination. *Anal. Biochem.* **1997**, *249* (2), 184–200.

(2) Sashidhar, R. B.; Capoor, A. K.; Ramana, D. Quantitation of E-Amino Group Using Amino Acids as Reference Standards by Trinitrobenzene Sulfonic Acid. *J. Immunol. Methods* **1994**, *167*, 121–127.

(3) Hermanson, G. Bioconjugate Techniques - 3rd Edition. In *Bioconjugate Techniques*; Academic Press: San Diego, California, 1996; pp 112–113.

(4) Ellis, G. A.; Palte, M. J.; Raines, R. T. Boronate-Mediated Biologic Delivery. *J. Am. Chem. Soc.* **2012**, *134* (8), 3631–3634.

(5) Dowlut, M.; Hall, D. G. An Improved Class of Sugar-Binding Boronic Acids, Soluble and Capable of Complexing Glycosides in Neutral Water. *J. Am. Chem. Soc.* **2006**, *128* (13), 4226–4227.