Electronic Supplementary Information

An approach to enzyme inhibition employing reversible boronate ester formation

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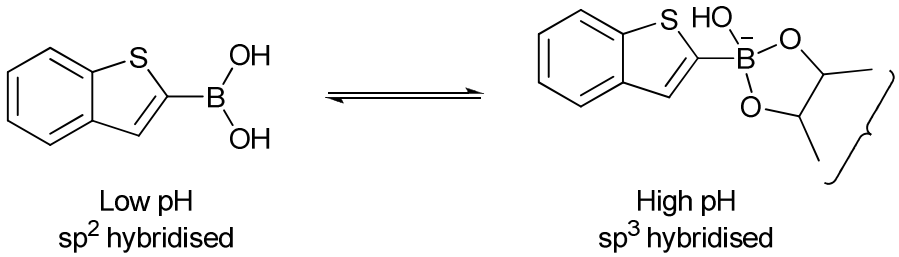
Figure S1 - ^{11}B NMR spectra showing the effect of pH on the equilibrium between **1**-D-fructose boronate ester (~8 ppm) and free boronic acid **1** (~26 ppm) (and D-fructose) in which the acid and ester undergo a slow exchange equilibrium (on the NMR timescale). In the absence of the sugar, a single fast-exchange averaged peak with a pH dependent chemical shift is observed. The concentration of **1** is 10 mM and D-fructose is 100 mM.



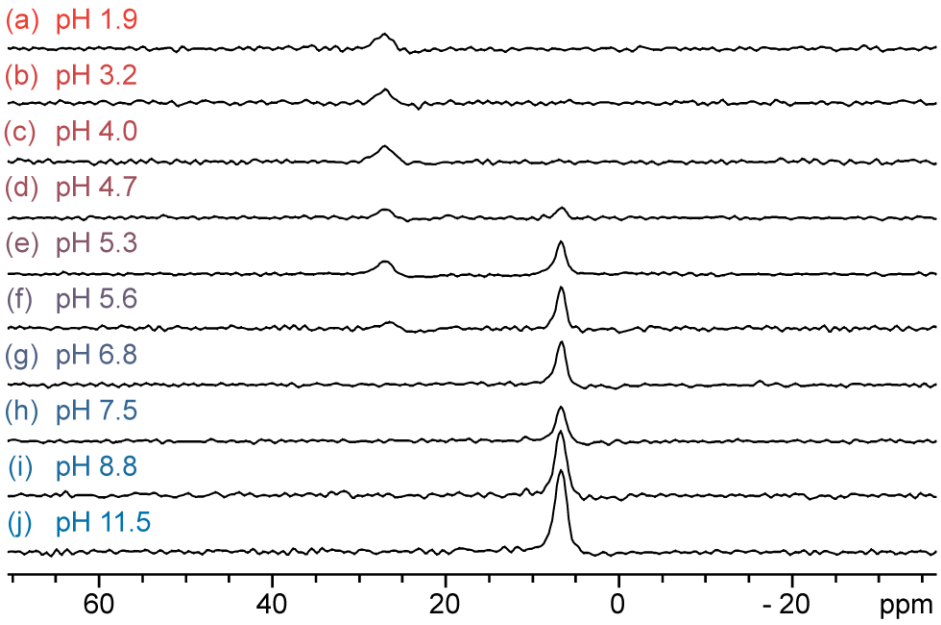


Figure S2: The structures of the sugars used in this study. Only the cis-diol geometry is shown.

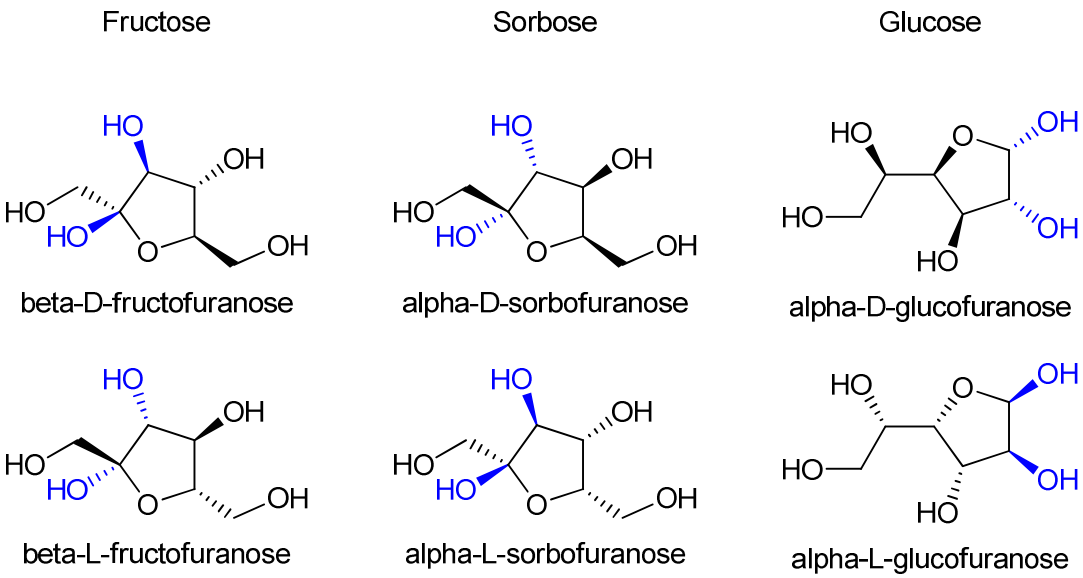
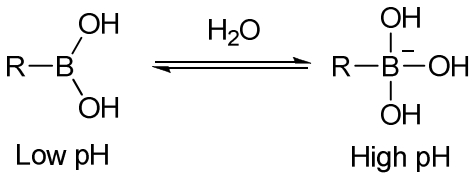


Figure S3: The effect of pH on the chemical shift of the boron resonance (δ_B), as used to determine the pK_a values of the boronic acids **1**, **2** and **3** in D_2O. The pD was calculated from the observed pH using the formula pD = pH + 0.4.[1]



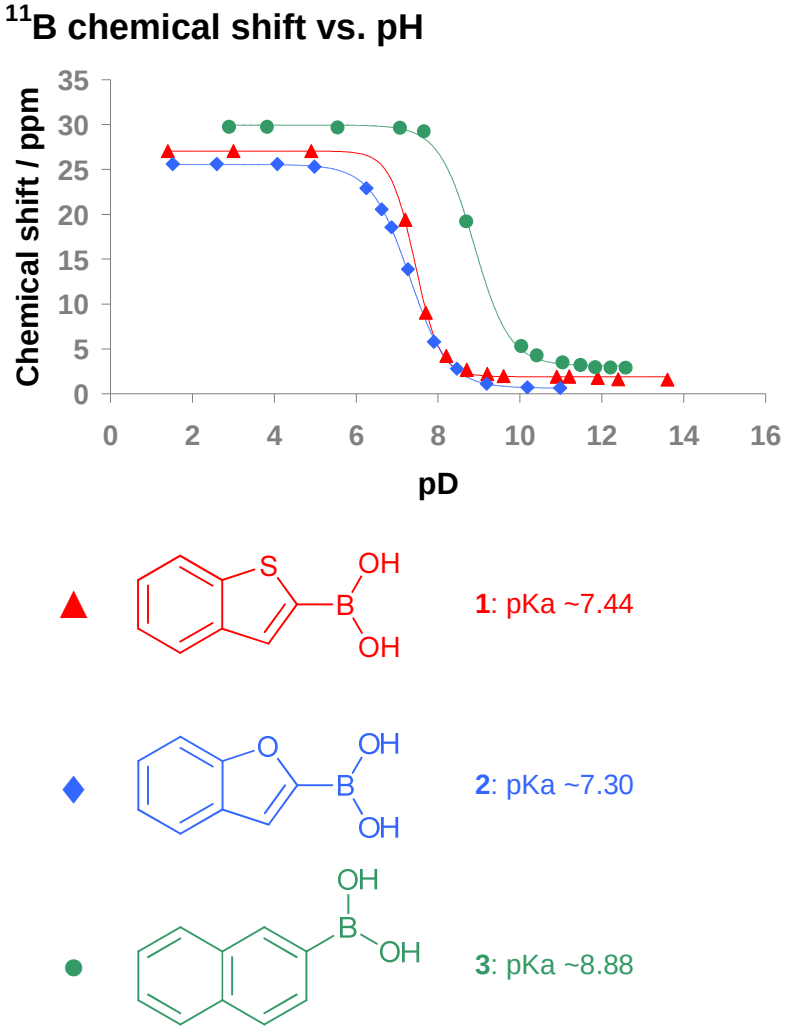


Figure S4. Monitoring ternary enzyme-boronic acid-sugar complex formation by ^{11}B NMR: (a) 5 mM boronic acid **1** alone; (b) 5 mM boronic acid **1** + 100 mM D-fructose; (c) 1 mM boronic acid **1** + 33 mg/ml (~1.3 mM) αCT; (d) 1 mM boronic acid **1** + 33 mg/ml (~1 mM) αCT + 2 mM D-fructose. The resonances correspond to 1-benzothiophen-2-ylboronic acid **1** (peak A), the **1**-D-fructose boronate ester (peak B), the αCT-**1**-D-fructose ternary complex (peak C) and the αCT-**1** binary complex (peak D).

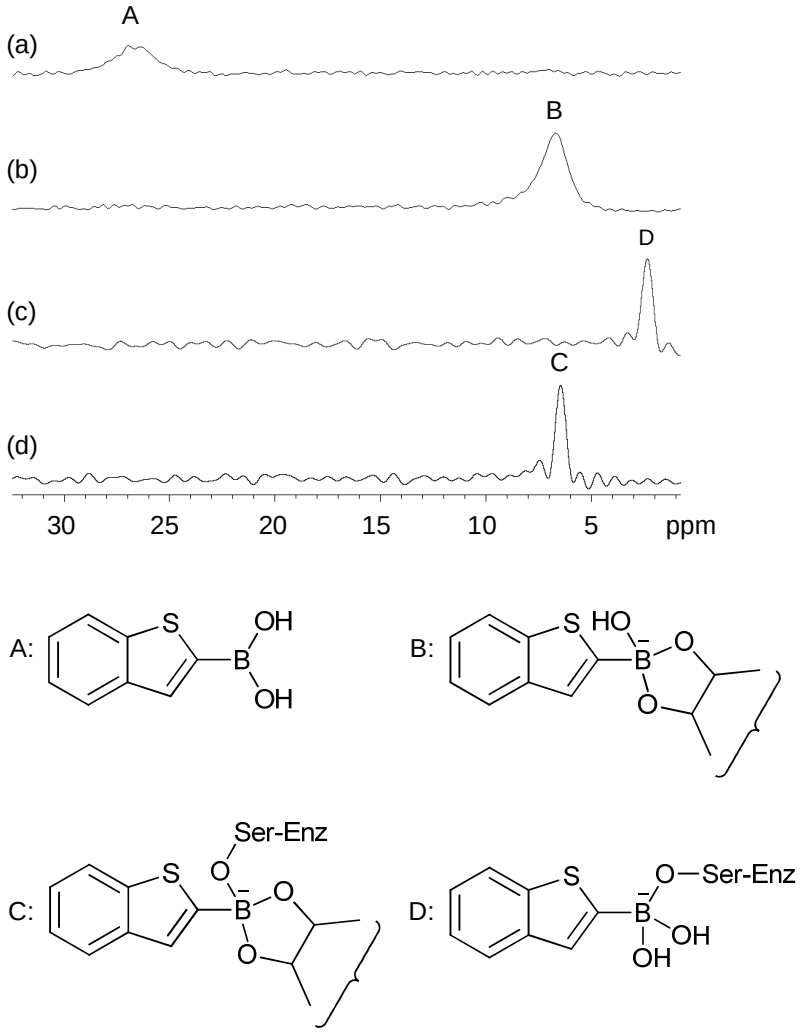
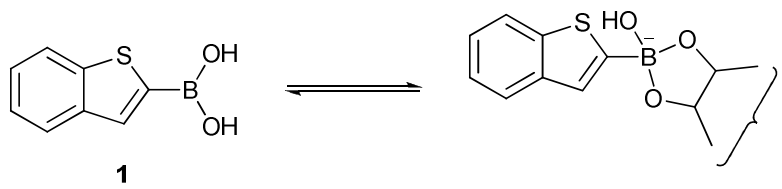


Figure S5: The propensity of boronate esters to form in solution as assayed by ^{1}H NMR. Binding curves for D- and L-fructose with **1** (K_A ~ 52 M); D- and L-glucose with **1** (K_A < 2 M); and D- and L-sorbose with **1** (K_A ~ 49 M) are shown (Concentration of **1** is 1 mM).



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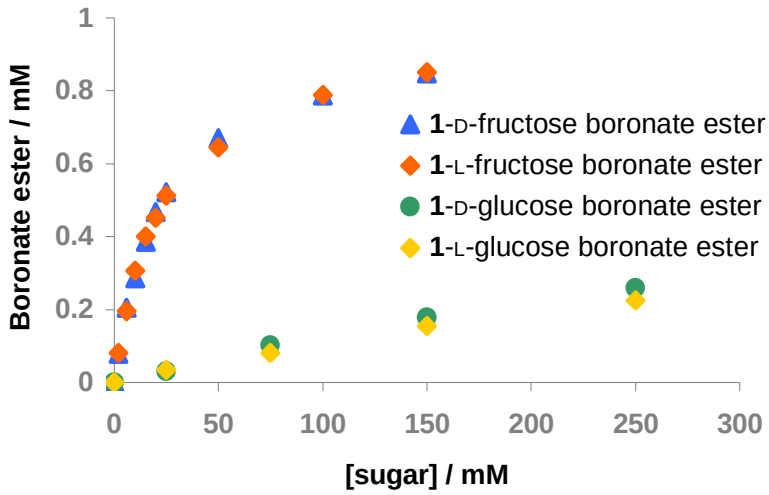
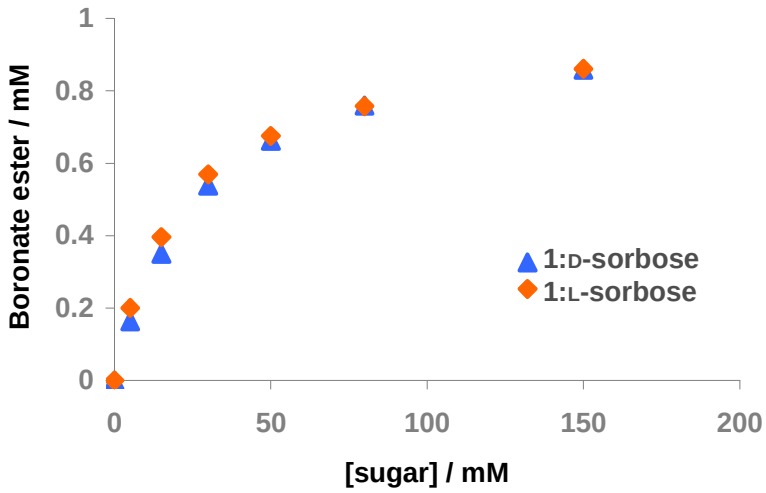
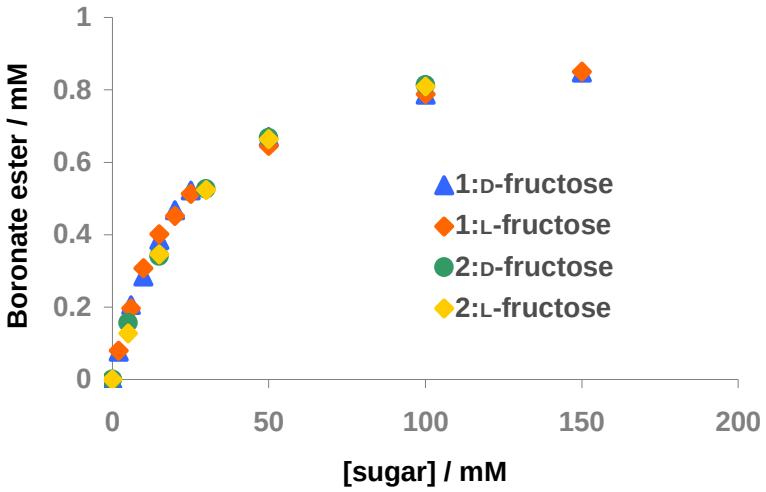




Binding curves for D-fructose and L-fructose with **1** and **2** (K_A ~ 52 M); and for D-glucose and L-glucose with **1** and **2** (K_A < 2 M) are shown (Concentrations of **1** and **2** are 1 mM).



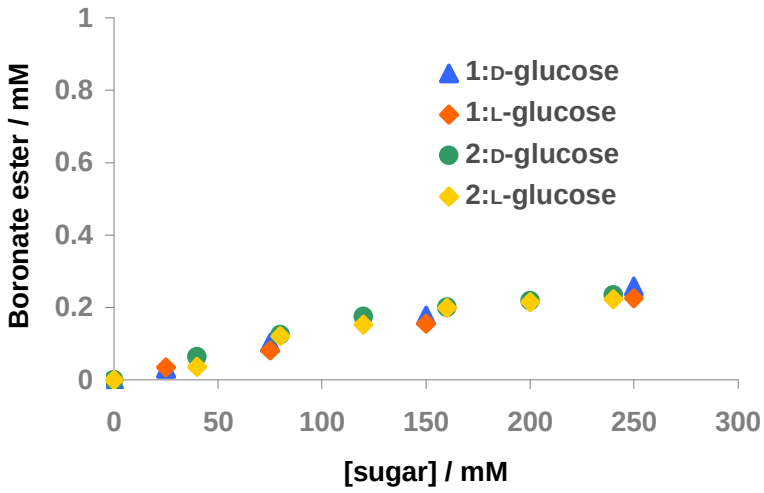


Figure S6: Formation of an αCT-**1**-sorbose ternary complex as monitored by ^{11}B NMR. More D-sorbose than L-sorbose is required to form the ternary complex. The concentrations of **1** (1 mM) and αCT (1.3 mM).

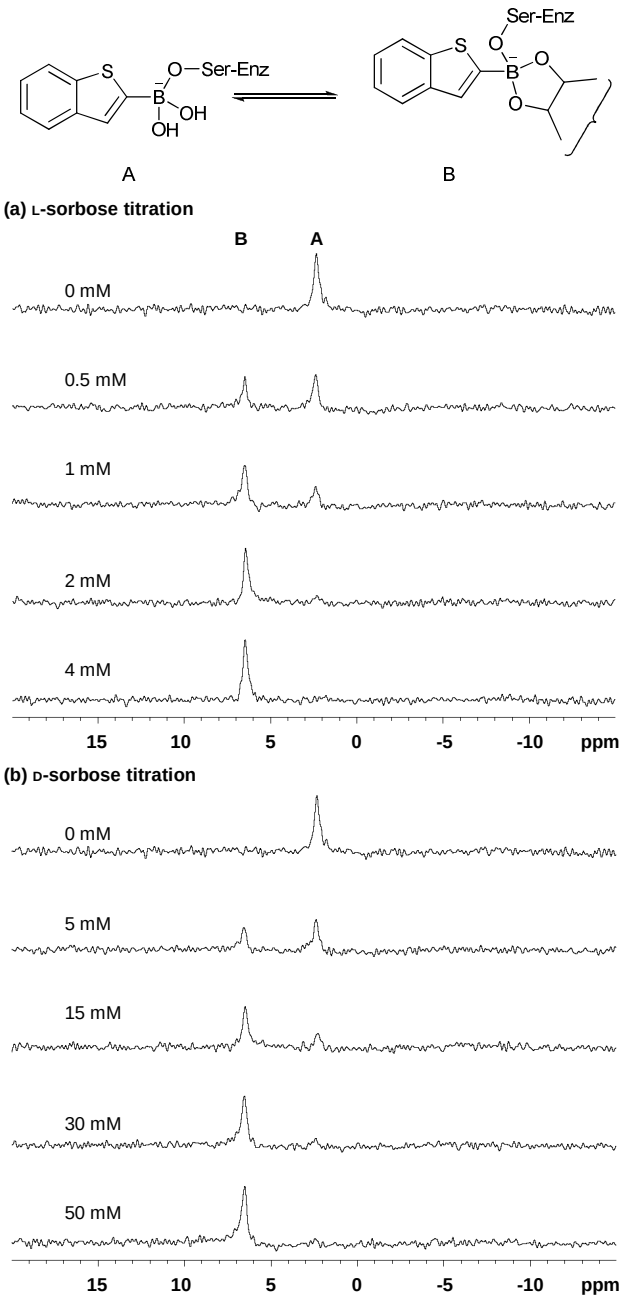
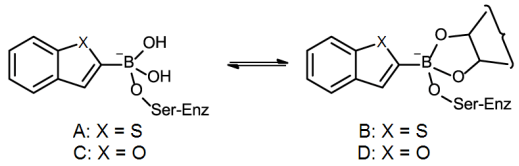
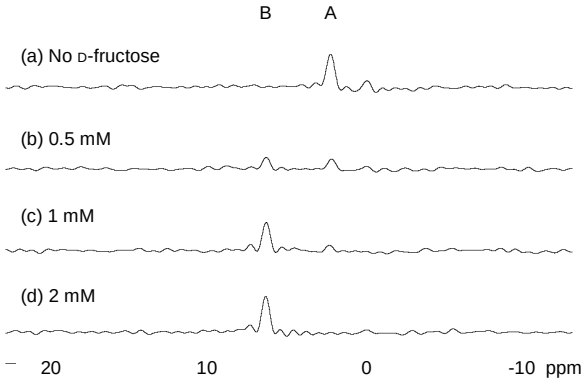


Figure S7: The formation of a ternary enzyme–boronic acid–sugar complex on titration of D-fructose (at the concentrations shown) into a mixture of **1** or **2** (1 mM) and αCT (1.3 mM).



D-fructose titration to 1 (1 mM) + αCT (1.3 mM)



D-fructose titration to 2 (1 mM) + αCT (1.3 mM)

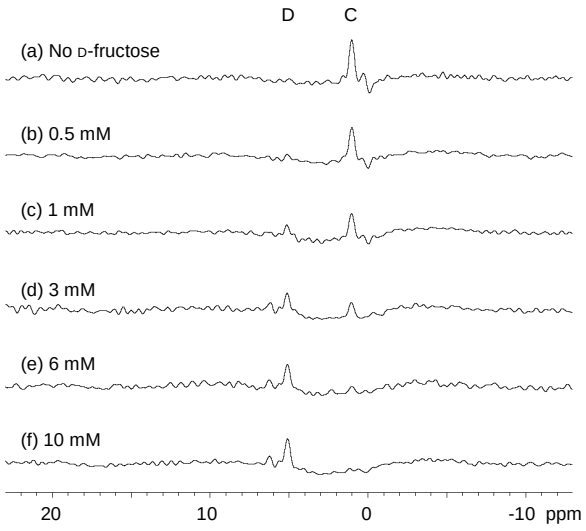
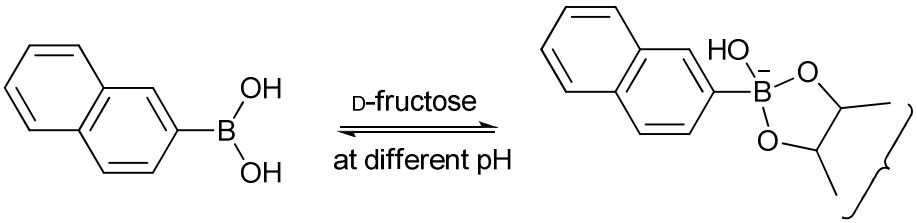


Figure S8: The dynamics of boronate ester formation depends on the pK_a of the boronic acids (naphthalen-2-ylboronic acid (**3**): pK_a ~8.8). At pH 5.8, a ~1:500 **3**/D-fructose ratio was required to form ~50% of the **3**-D-fructose boronate ester complex; at pH 7.0 this was reduced to ~1:50, and at pH 8.0, ~1:10



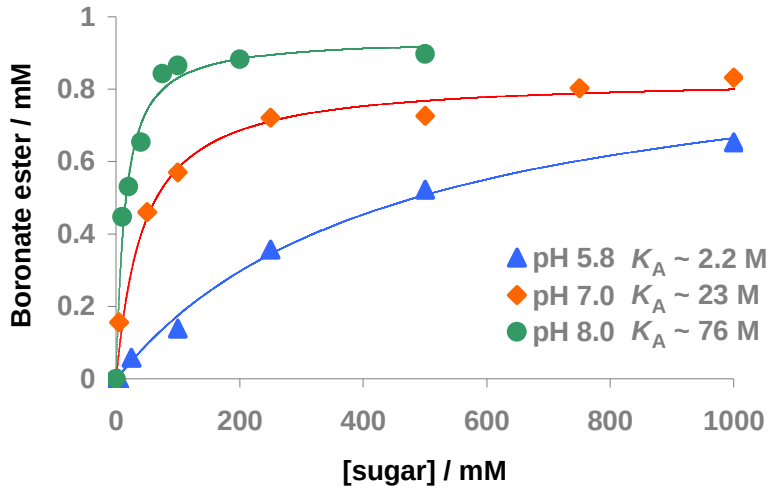
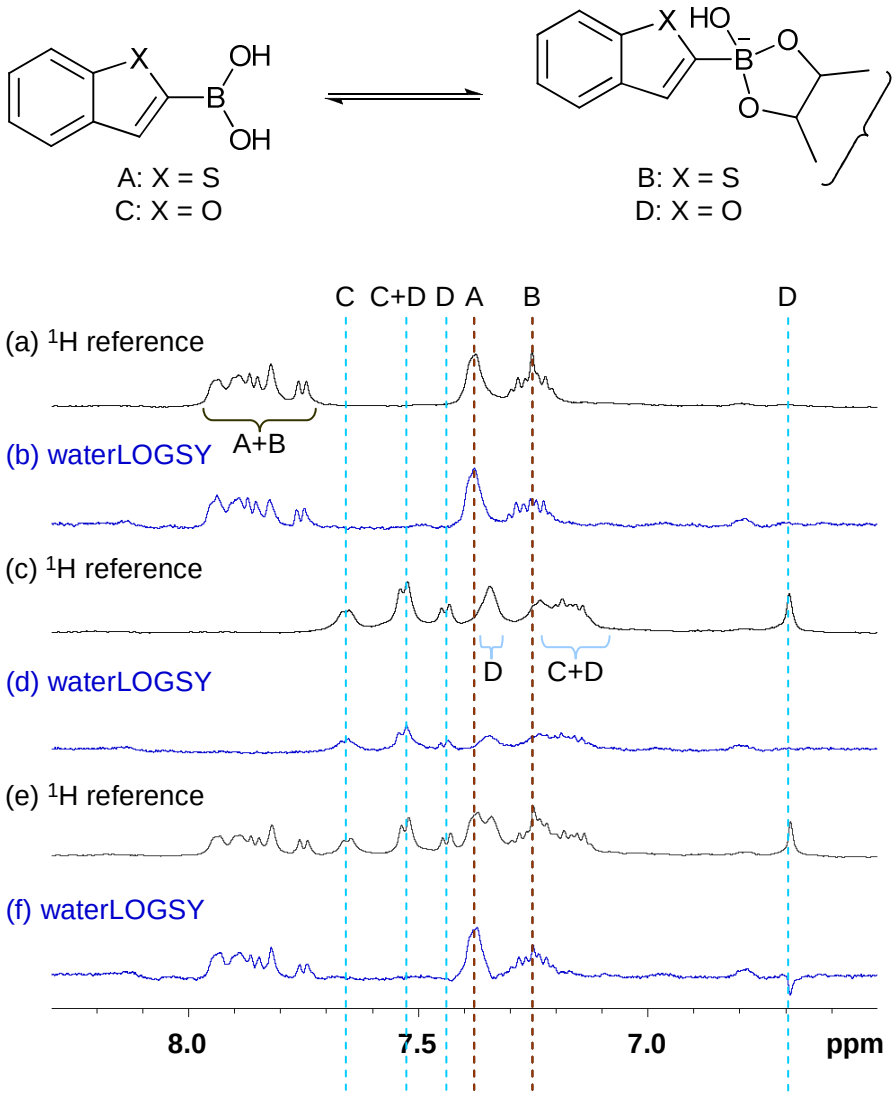


Figure S9: ^{1}H waterLOGSY analyses of αCT and D-fructose with **1** and/or **2**. (a) and (b): In the absence of **2**, both **1** and **1**-D-fructose bind to αCT; (c) and (d): In the absence of **1**, both **2** and **2**-D-fructose bind to αCT; (e) and (f): In a mixture of **1** and **2**, **1** and **1**-D-fructose are preferentially bound by αCT. Conditions for the experiments: 200 μM αCT; 5 mM **1**; 5 mM **2**; 15 mM fructose.



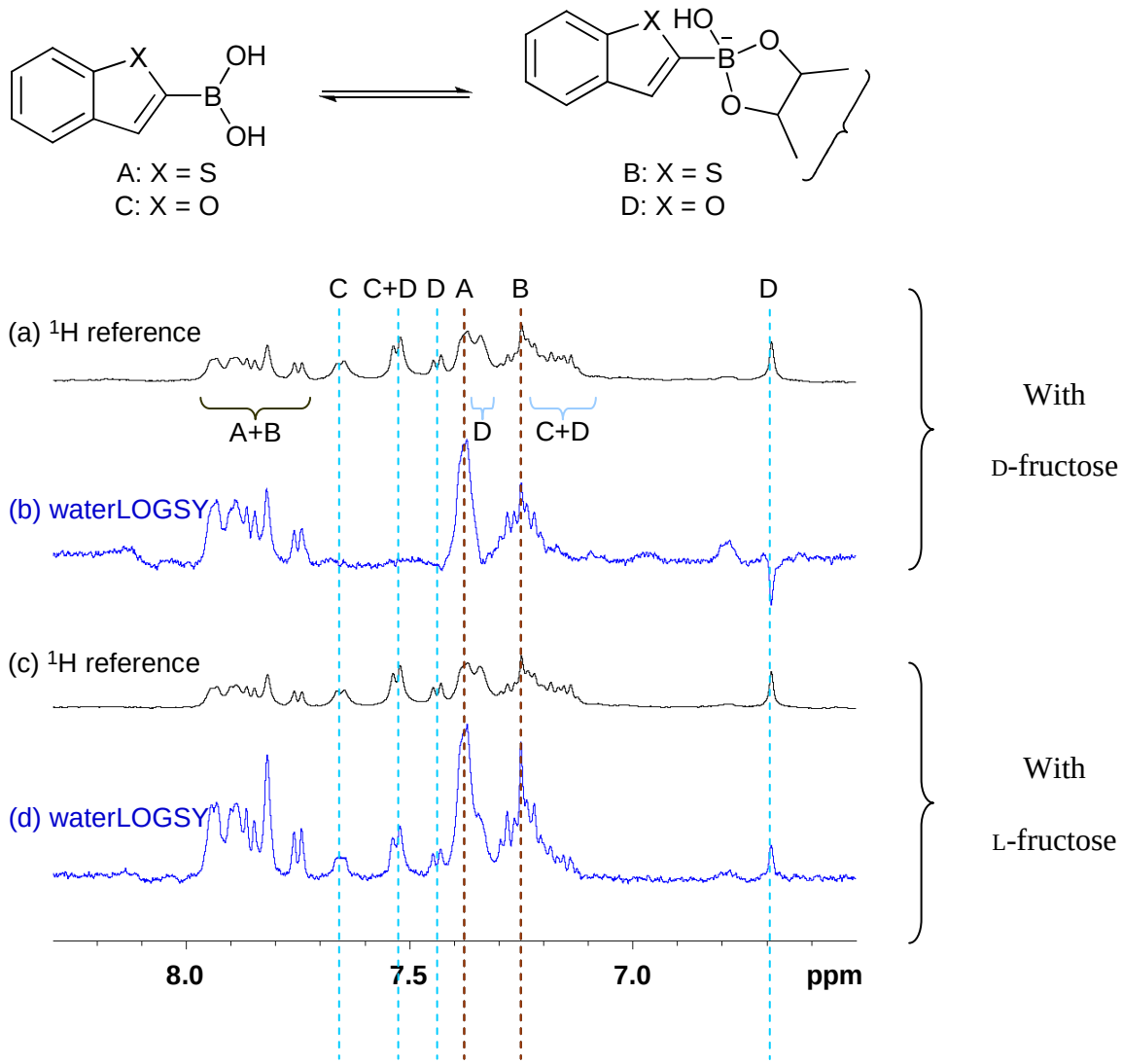
In the waterLOGSY experiment, bulk water magnetization is transferred via the solvated protein-ligand complex to the free ligand. Differences in rotational correlation

time between the solvated protein-ligand complex and the solvated free ligand result in binders and non-binders displaying waterLOGSY signals with opposite signs.[2]

The observed waterLOGSY responses for the boronic acid and boronate ester may originate from direct binding of each species to the enzyme, or indirectly from the binding of either species, followed by their interconversion in solution, provided the interconversion is fast with respect to the relaxation of the binding species.

The signals observed in waterLOGSY experiments rely on, among other factors, the exchange kinetics of the reversibly forming protein-ligand complex; in situations of very strong binding the bound residence time may become too long, meaning longitudinal relaxation dominates before the ligand dissociates, leading to a nil response (a “false negative”).

Figure S10: waterLOGSY spectrum showing αCT preferentially binds to **1** and **1**-D-fructose boronate ester, but no selectivity is observed in the presence of L-fructose. Conditions for the experiments: 200 μM αCT; 5 mM **1**; 5 mM **2**; 15 mM fructose.



Supplemental Reference:

1. P. K. Glasoe and F. A. Long, *J. Phys. Chem.* 1960, **64**, 188–190.

2. C. Dalvit, P. Pevarello, M. Tatò, M. Veronesi, A. Vulpetti and M. Sundström, *J. Biomol. NMR* 2000, **18**, 65–68.