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Ortho-substituted aryl monoboronic acids have improved selectivity for D-glucose relative to D-fructose and L-lactate

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ABSTRACT

Ortho-substituted aryl monoboronic acids have been found to have improved selectivity for D-glucose compared to D-fructose and L-lactate. These findings are supported by computational studies on the B3LYP/6-31G(d) level using Gaussian. This finding is of interest for development of boronate based D-glucose sensors.

1. Introduction

Aryl boronic acids are often used in carbohydrate recognition[1–6] because they are small and flexible, compared to other classes of carbohydrate binders, such as lectins[7] and artificial macrocycles.[8,9] These features make aryl boronic acids easy to incorporate as recognition motifs in spectral probes or peptides, without dramatically changing the physical properties of the larger structures. The recognition event appears since boronic acids and boronates react with 1,2-*cis*-diols or 1,3-diols by reversible formation of the corresponding boronate esters. It is commonly believed that optimal binding affinity is achieved when pH is above pK_a for a specific boronic acid. Earlier literature[10,11] reports that the binding strength depends on pK_a of both the diol and the boronic acid. The event of carbohydrate binding is however complicated, since carbohydrates are polyols, which can exist in different anomeric configurations of six membered and five membered heterocyclic rings.

Glucose monitoring is of great importance in the treatment of *diabetes mellitus*. Boronic acids with displacement constants around 15–16 mM for D-glucose are desired,[12] because blood glucose is fluctuating between 2 and 30 mM in diabetes patients. The maximum sensitivity is achieved when K_d is in the middle of the binding curve.

Aryl boronic acids with selective recognition of D-glucose over other polyol species are therefore of great interest, since D-glucose is the major carbohydrate present in human blood ($\approx$5 mM)[13] compared to D-fructose (<0.1 mM, even after a fructose-rich meal).[14,15] However D-fructose generally shows stronger binding affinity to most aryl monoboronates compared to D-glucose. An explanation might be, that under physiological conditions, D-fructose mainly exists in the furanose form, which can bind to boronates in tridentate configuration, while D-glucose mainly exists in the glucopyranose form, and binds in a bidentate configuration.[16,17] Notably L-lactate is also present in human blood as a metabolite after anaerobic biological processes, and α-hydroxy acids are also capable of forming boronate esters with aryl boronic acids.[18,19] Diboronates[20–22] can show selectivity towards D-glucose, but they are synthetically challenging, compared with the synthesis of aryl monoboronates, and usually their solubility is low in aqueous media. Carbohydrate affinities of some boronates can be measured by exploiting their UV/vis or fluorescence properties, or in cases were such spectroscopic properties are missing, by competitive binding to spectroscopic probes.[23–29] Another commonly used technique is the pH-depression method,[30] where the drop in pH can be correlated with the binding constant between these two species. However this method suffers from the fact that it requires a high amount of boronic acid in solution. Furthermore this method assumes that the boronic acid diester is fully converted to the tetrahedral anionic form, as a consequence of the lowered pK_a value.

In order to overcome the lack of spectroscopic properties of the given aryl boronic acids, we have adopted the method of UV/vis-titration experiments with the colored compound alizarin red sodium (ARS) in a three component competitive binding assay. This assay has successfully been employed by Wang and co-workers.[11,31,32] As shown in Fig. 1, ARS binds reversibly to the aryl boronate, forming the corresponding aryl boronate ester, which displaces the absorption spectrum, and changes the color of the aqueous solution from clear red towards yellow.

Upon addition of the polyol to the ARS-boronate solution, ARS is released competitively, changing the color of the solution back towards red.

We used the ARS assay to screen a series of aryl boronic acids performing the measurements in a physiological environment at

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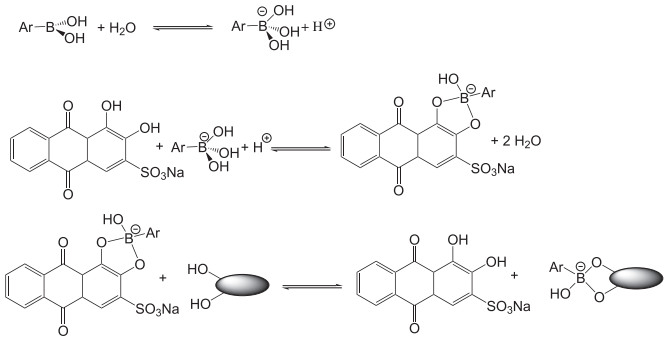

Fig. 1. Competitive binding of ARS and polyol to aryl monoboronate in a three component system.

neutral pH. Comparing our data with data obtained using a non-physiological phosphate buffer, we report small changes in the respective binding affinities. Generally decreased selectivity towards D-fructose is achieved, due to the presence of *ortho*-substituents at the aryl monoboronic acids.

We have discovered that *ortho*-substituted aryl monoboronic acids bind D-fructose with a reduced strength compared to aryl monoboronic acids with no *ortho*-substituents. This is a valuable discovery, because a lot of synthetic effort can be avoided in the preparation of suitable boronic acid dyes, since aryl monoboronic acids generally are easier to access, and more soluble.

2. Results and discussion

Screening studies of a series of aryl boronic acids were performed in order to obtain selectivity towards binding of D-glucose. The measurements were performed in a physiological saline buffer, containing 10 mM phosphate, 2.7 mM KCl, and 137 mM NaCl in water, pH 7.4. The structures are shown in Fig. 2, and calculated K_d-values are shown in Table 1. Compound **1** and **2** are very similar in structure at the binding site, since they both contain a methyl group attached in the *ortho*-position to the boronic acid functionality. Our studies indicate that binding of D-fructose is disturbed, since the displacement constant K_d is about 10 mM for both boronic acids. Normally K_d is about 0.5–1 mM for D-fructose.[10] K_d for D-glucose is about 60 mM for **1** and 30 mM for **2**. The sigmoidal curves for binding of **1** and **2** with D-glucose, D-fructose, and L-lactate are shown in Fig. 6.

The aryl boronic acids **3** and **4** were used as reference compounds to determine the influence of an *ortho*-positioned methyl group. D-Fructose is bound remarkably better (one order of magnitude) by **3** and **4** than it is bound with **1** and **2**, probably because the more stable tridentate D-fructose-boronate complex is favored, when no *ortho*-substituents are present, see Fig. 3.[17] The complexes formed between D-glucose and **3** and **4**, respectively, are also stronger, probably due to the decreased pK_a of **3** and **4** compared to **1** and **2**.

The difference in binding strength of D-glucose to **1** and **2**, might be explained by the difference in acidity. This is also the case for **3** and **4**. Pyrrolidine is more electron-donating than dimethylamine, thus decreasing the acidity of **1** and **3** compared to **2** and **4**.[33] Our results suggest that the binding strength towards D-fructose depend less on the acidity of the boronic acid than the binding strength towards D-glucose. The proposed steric clash with an *ortho*-substituent, which disfavors tridentate binding of D-fructose is shown in Fig. 4.

It is commonly believed that an α-hydroxy acid reacts with the trigonal form (boronic acid),[34,35] but there are also implications that α-hydroxy acids can react with the tetrahedral boronate at a slower rate.[36] Charge repulsion between the negatively charged L-lactate and the aryl boronate presumably occurs. Therefore the

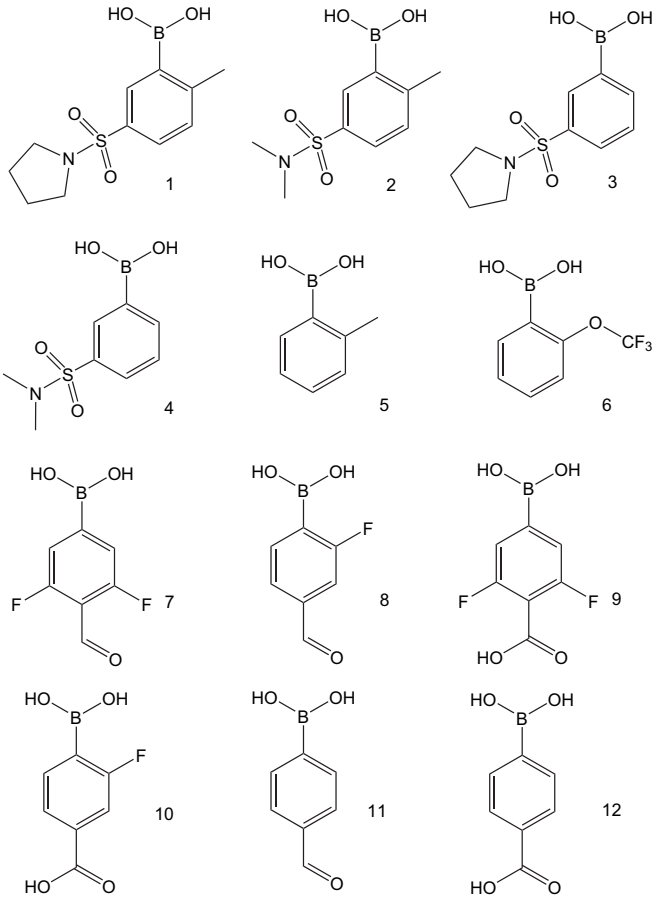

Fig. 2. Aryl boronic acids tested for binding strength to D-glucose, D-fructose, and L-lactate.

first statement is in good agreement with observation of larger K_d (42 mM) for binding of L-lactate to **2**, compared to **1** (K_d=29 mM), because more of **2** is converted to the corresponding boronate at physiological pH.

However the complexes formed between L-lactate and **3** and **4** are remarkably stronger compared to the complexes formed with **1** and **2**, which is the opposite of what one would expect, since pK_a of **3** and **4** are lower than pK_a for **1** and **2**. This suggests an effect of the *ortho*-substituent too since decreased binding of L-lactate finds place.

Compound **5** was used as a reference compound to state the influence of pK_a for the binding affinity. Our results showed a little stronger binding affinity of **5** to D-fructose compared to the binding affinity of **1** and **2**. Poor binding affinity of **5** to D-glucose was observed, and L-lactate was bound significantly better in the presence of **5** compared to **1** and **2**, respectively. This can be explained by the much higher pK_a value of **5**.

The binding affinity of **6** to D-glucose is slightly less compared to **2**, and D-fructose is bound one order of magnitude more strongly by **6**. Decreased intramolecular hydrogen bonding from the boronate OH to oxygen in the trifluoromethoxy group could explain why the binding of D-fructose is not disturbed. The smaller size of O versus CH_3 can also explain the increased binding affinity to D-fructose. L-lactate though is tightly bound.

The reduced binding affinity of D-fructose to the tested *ortho*-fluoro aryl monoboronic acids (**8** and **10**), compared to **11** and **12** can be explained by a favorable intramolecular hydrogen bond, see Fig. 5, which generally favors bidentate complex formation, thus disfavoring the tridentate D-fructose–boronate complex. However the binding affinity of D-fructose to **8** are increased compared to **1** and **2**.

Table 1
Displacement constants (K_d) reported in mM, shown for the series of boronic acids in physiological saline buffer. n is the number of measurements performed for each determination. Standard deviations σ are shown to the right in bold

	D-Glucose $K_d(n)$	D-Fructose $K_d(n)$	L-Lactate $K_d(n)$
1	60 (6) (**6.1**)	13 (7) (**2.5**)	29 (7) (**2.8**)
2	30 (3) (**2.6**)	10 (5) (**1.6**)	42 (4) (**3.5**)
3	9.1 (3) (**0.3**)	1.5 (3) (**0.5**)	1.9 (3) (**0.2**)
4	5.9 (3) (**0.2**)	0.9 (3) (**0.1**)	4.9 (3) (**0.1**)
5	— (3) —	5.7 (3) (**0.1**)	5.9 (3) (**0.2**)
6	63 (3) (**6.6**)	1.6 (3) (**0.3**)	7.5 (3) (**0.1**)
7	25 (3) (**1.7**)	3.2 (3) (**0.5**)	47 (3) (**2.0**)
8	26 (9) (**3.1**)	2.5 (9) (**0.6**)	22 (3) (**1.0**)
9	28 (6) (**2.1**)	3.4 (5) (**0.3**)	49 (3) (**3.2**)
10	40 (5) (**5.2**)	11 (5) (**2.7**)	13 (5) (**2.6**)
11	10 (3) (**2.0**)	1.0 (3) (**0.2**)	4.8 (3) (**0.4**)
12	414 (3) (**37.3**)	0.9 (3) (**0.3**)	5.6 (3) (**0.7**)

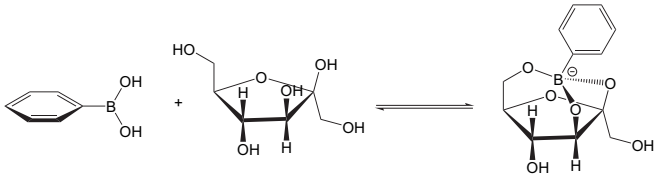

Fig. 3. Favorable tridentate binding of D-fructose to an aryl monoboronic acid with no *ortho*-substituents. The tridentate structure has earlier been elucidated by Norrild and Eggert.[17]

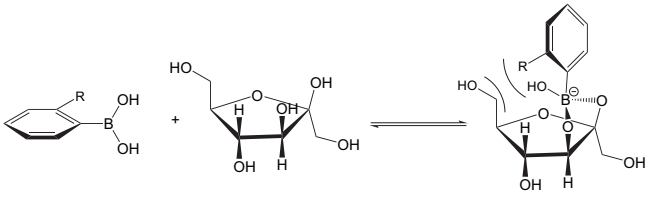

Fig. 4. Proposed steric clash for D-fructose induced by an *ortho*-positioned substituent in an aryl monoboronic acid.

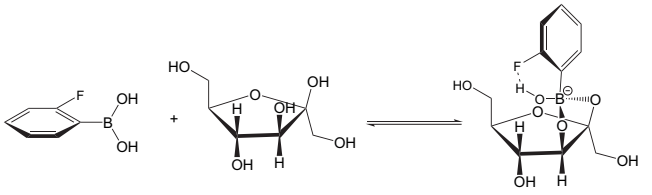

Fig. 5. Suggested intramolecular hydrogen bonding from boronate OH to fluorine, preventing D-fructose from binding tridentately.

Compounds **7** and **9** are very similar in structure. However both boronic acids are likely to be converted to either the boronate (**7**) and/or carboxylate (**9**) at pH 7.4. This is consistent with the observed low binding affinity to L-lactate. In both cases, the binding affinity to D-glucose is around 20 mM D-fructose is bound with a similar strength (K_d=3.2 mM and K_d=3.4 mM) in both species. L-lactate is bound more strongly to **8** and **10**, compared to **7** and **9**. This is consistent with our expectations since the additional electron-withdrawing fluorine substituent increase acidity of **7** and **9** compared to **8** and **10**. D-Glucose is bound with a similar strength to **7**, **8**, and **9** with a K_d, close to 15–16 mM, which is desired. D-Fructose however is bound more efficiently to **8** than to **10**. Since **8** and **10** are similar at the recognition site, the diminished selectivity in the latter case cannot be explained by competitive intramolecular hydrogen bonding as suggested, see Fig. 5. In accordance with our measurements, **10** is the boronate where the selectivity towards D-fructose is decreased most among **7**–**10**, whereas

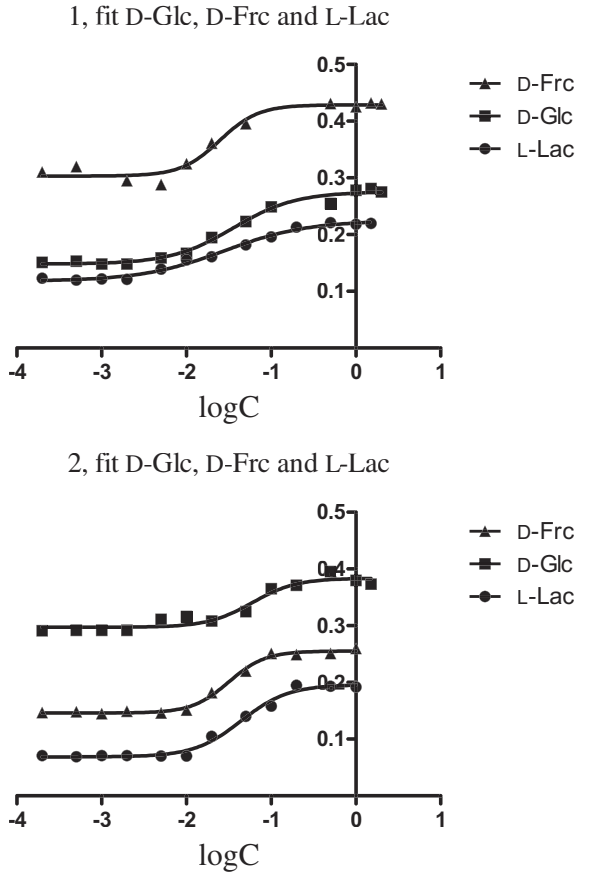

Fig. 6. Curve fit using GraphPad Prism 5.0. The measured absorbance at 350 nm, was plotted against logarithm to concentration data, using sigmoidal dose response (variable slope). [**1**, **2**]=100 μM and [ARS]=50 μM, here in the physiological saline buffer.

boronate **7** and **9** display the most decreased selectivity towards L-lactate.

The aryl boronic acids **11** and **12** were used as reference compounds to determine the influence of the *ortho*-positioned fluorine. The K_d-values indicate formation of a stronger D-fructose complex between D-fructose and **11** and **12**, compared to **8** and **10**. L-lactate is bound stronger as well, which can be explained by the higher pK_a values of **11** (7.6) and **12** (8.0) compared to **8** and **10**.[11] D-glucose is poorly bound to boronate **12** compared to **8**, **10**, and **11**, which cannot be explained by pK_a arguments.

The ratios of K_d for D-glucose/D-fructose K_d(D-Glc)/K_d(D-Frc), and D-glucose/L-lactate K_d(D-Glc)/K_d(L-Lac) is given in Table 2. It is clearly seen that boronate **1**, **2**, and **10** displays the greatest reduced selectivity towards D-fructose, while boronate **2**, **7**, and **9** displays

Table 2
Ratios of K_d for D-glucose/D-fructose and K_d D-glucose/L-lactate for the series of boronic acids in a physiological saline buffer

	K_d(D-Glc)/K_d(D-Frc)	K_d(D-Glc)/K_d(L-Lac)
1	4.6	2.1
2	3.0	0.7
3	6.1	4.8
4	6.6	1.2
5	—	—
6	39.4	8.4
7	7.8	0.5
8	10.4	1.2
9	8.2	0.6
10	3.6	3.1
11	10.0	2.1
12	460.0	73.9

the greatest reduced selectivity towards L-lactate. Boronate **12** however shows a remarkable preference of forming the D-fructose-boronate complex.

Compounds with larger *ortho*-substituents, such as *o*-ethoxymethyl, *o*-aminomethyl, and *o*-phenyl- have been tested, but they have not shown any binding affinity to D-glucose. Furthermore no significant binding affinity to D-fructose and L-lactate has been achieved.

Binding constants were also determined in a phosphate buffer, with a similar strength as a physiological buffer (100 mM phosphate, pH 7.4), and in a methanolic phosphate buffer (53.2 w/w% MeOH, 52 mM phosphate, 40 mM NaCl). Measurements showed diminished binding of D-glucose, improved binding of D-fructose and L-lactate in the 100 mM phosphate buffer with **2**, compared to the physiological saline buffer containing 10 mM phosphate, 2.7 mM KCl, and 137 mM NaCl in water, pH 7.4. This suggests that the ionic strength of the solution influences binding affinity. Binding of D-glucose was remarkably diminished in the methanolic buffer, binding of D-fructose unchanged, and binding of L-lactate improved, compared to the physiological buffer. This indicates that the acidity of **2** is decreased in the methanolic buffer compared to the physiological buffer solution. The values of K_d are shown in Table 3.

Table 3
Displacement constants (K_d) reported in mM shown for **2**. *n* is the number of measurements performed for each determination. Standard deviations are shown to the right in bold

	(**2**) phosphate (K_d)(*n*):	(**2**) phosphate/MeOH.(K_d)(*n*):
D-Glucose:	62 (5) (**3.2**)	160 (6) (**24.4**)
D-Fructose:	4.7 (5)(**0.3**)	8.4 (6) (**0.5**)
L-Lactate:	23 (6) (**3.6**)	3.2 (5) (**0.1**)

The ratios K_d (D-Glc)/K_d(D-Frc) and K_d (D-Glc)/K_d(L-Lac) are calculated in Table 4, showing that the best D-glucose selectivity is obtained in the phosphate buffer, compared to the methanolic phosphate buffer.

Table 4
Ratios K_d(D-Glc)/K_d(D-Frc) and K_d(D-Glc)/K_d(L-Lac) for **2** in the phosphate buffer and in the methanolic phosphate buffer, respectively

(**2**) Phosphate K_d (D-Glc)/K_d(D-Frc):	13.2
(**2**) Phosphate K_d (D-Glc)/K_d(L-Lac):	2.7
(**2**) Phosphate/MeOH K_d (D-Glc)/K_d(D-Frc):	19.0
(**2**) Phosphate/MeOH K_d (D-Glc)/K_d(L-Lac):	50.0

We performed pK_a-titrations of **2** in a 53.2 w/w% methanolic solution, but realized that the pK_a for the boronic acid cannot be determined under these conditions, because **2** partially form the methylboronate. The pK_a of the methylboronate and the pK_a of the boronic acid cannot be distinguished in this experiment.

To support our experimental investigations, computational calculations were performed on B3LYP/6-31G(d) level with Gaussian.

The energy optimized structures of the tridentate complexes formed between D-fructose and *p*-methyl phenyl monoboronate, and *o*-methyl phenyl monoboronate, respectively, were compared, see Fig. 7. The tridentate complex is in accordance with previous structural elucidation performed by Norrild and Eggert.[17] The latter complex is 4.6 kJ/mol less stable, indicating that an *o*-methyl substituent reduces tridentate complex formation.

The optimized structure of the bidentate complex formed between D-fructose and *o*-methyl phenyl monoboronate shown in Fig. 8, shows that free rotation around the C–B bond can stabilize the bidentate complex. Thus according to our calculations, the bidentate D-fructose–boronate complex is not disturbed due to the presence of an *ortho*-methyl substituent.

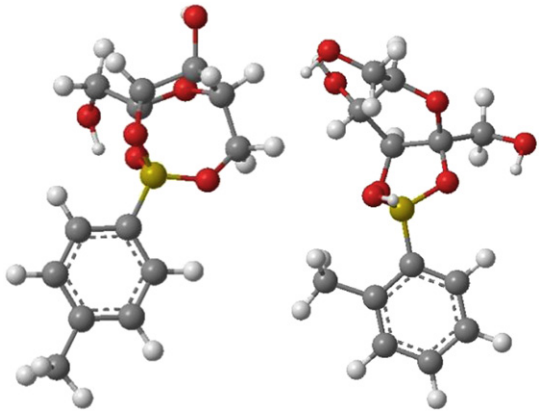

Fig. 7. Two energy optimized structures of tridentate complexes formed between D-fructose and *p*-methyl phenyl monoboronate (left), and *o*-methyl phenyl monoboronate (right). The left complex is 4.6 kJ/mol more stable than the right. Grey=C, white=H, red=O and yellow=B.

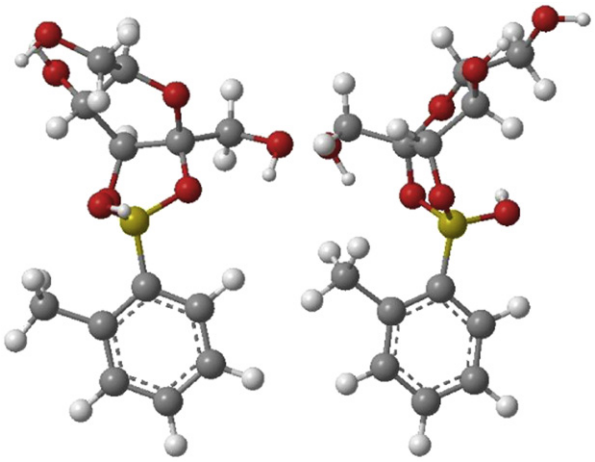

Fig. 8. The energy optimized structure of the bidentate complex formed between D-fructose and *o*-methyl phenyl monoboronate (left). The left complex is 2.1 kJ/mol more stable than the right, and the stabilization corresponds to free rotation around the C–B bond. Grey=C, white=H, red=O and yellow=B.

In good agreement with our assumptions, the optimized structure of the bidentate complex formed between *o*-fluoro phenyl monoboronate and D-fructose shows a preference for a favorable H–F hydrogen bond (3.3 kJ/mol more stable than the bidentate complex with no H–F bond formation), see Fig. 9. This is in good agreement with calculations performed by Razgulin and Mecozzi.[37]

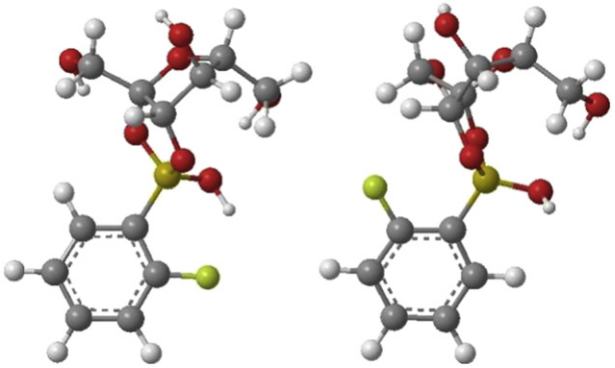

Fig. 9. The energy optimized structure of a complex formed between D-fructose and *o*-fluoro phenyl monoboronate (left). The left complex is 3.3 kJ more stable than the right. Grey=C, white=H, green=F, red=O and yellow=B.

Thus the *ortho*-fluoro substituent favors the bidentate D-fructose–boronate complex, which decreases the D-fructose selectivity.

The optimized tridentate complexes formed between D-fructose and *o*-fluoro phenyl monoboronate, and *p*-fluoro phenyl monoboronate, respectively, were compared, and showed a significant energy difference. The latter complex was calculated to be 7.0 kJ/mol more stable than the former. This suggests electrostatic effects, such as lone pair–lone pair repulsion, which disfavors the tridentate D-fructose *o*-fluoro phenyl monoboronate complex. However steric repulsion might also contribute, since the covalent radius of fluorine approximately is twice the covalent radius of hydrogen; the radii are 60 pm and 30 pm, respectively.[38,39] The two tridentate complexes are shown in Fig. 10.

pK_a values of the monoboronic acids have been determined by titration of the corresponding boronates, and are shown in Table 5. The titration curve for **5** is shown in Fig. 11. The relative values are in good agreement with our results for K_d. Besides the Hammett linear free energy relationship has been used to calculate pK_a values for selected aryl monoboronic acids (**7** and **8**), see Table 5. The calculations involves the equation employed by Wang and Springsteen,[11] and the found pK_a values are in good agreement with our arguments. The much higher pK_a values of **1** and **2** compared to **3** and **4**, might be explained sterically by an unfavorable boronate *ortho*-methyl interaction. The high D-glucose affinity for **1** and **2** might then be explained by a favorable lipophilic D-glucose–boronate interaction.

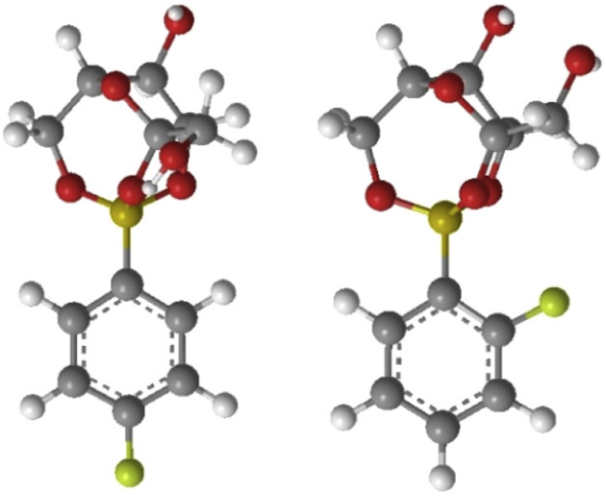

Fig. 10. The energy optimized structure of a tridentate complex formed between D-fructose and *p*-fluoro phenyl monoboronate (left), and *o*-fluoro phenyl monoboronate. The left complex is 7.0 kJ/mol more stable than the right. Grey=C, white=H, green=F, red=O and yellow=B.

Table 5
pK_a values of selected aryl monoboronic acids, determined by titration of the corresponding base. For **7** and **8**, the calculated Hammett-values are shown in the brackets to the right

Aryl monoboronic acid	pK_a (titration)
1	8.4
2	8.2
3	7.6
4	7.2
5	9.3
6	8.3
7	6.3 (6.2)
8	6.8 (6.7)

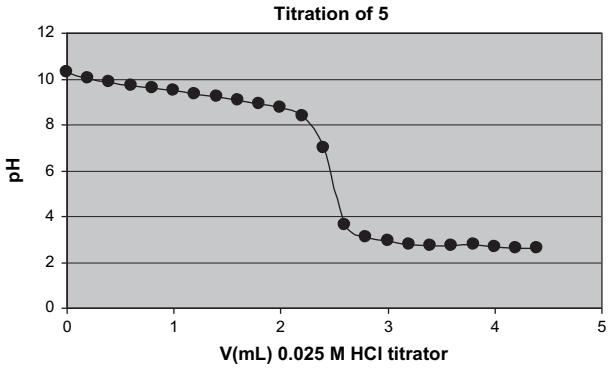

Fig. 11. Titration curve for the corresponding base of **5**, where pK_a is determined to 9.3 for the aryl monoboronic acid.

3. Conclusion

The experimental outcome shows that *ortho*-substituted aryl monoboronic acids are capable of binding D-fructose with a decreased selectivity relative to D-glucose in a physiological buffer, presumably due to a steric effect of the *ortho*-positioned methyl substituent, disfavoring the tridentate boronate-D-fructose complex. This is supported by computational calculations on B3LYP/6-31G(d) level with Gaussian.

Competitive intramolecular hydrogen bonding, in the case of *ortho*-positioned fluorine favors bidentate D-fructose–boronate complex formation, thus D-fructose binding is decreased. Computational calculations have also supported these theories. However calculations have further shown that the disfavored tridentate D-fructose *o*-fluoro phenyl monoboronate complex contributes even more to the decreased D-fructose selectivity, possibly due to electrostatic effects, such as lone pair–lone pair repulsion. Steric effects might also contribute since the covalent fluorine radius is about twice the covalent hydrogen radius. Our experimental results show that other effects do impact the decreased binding of D-fructose, as seen when **7** and **8** are compared. **7** does not contain any *ortho*-substituents, but two *meta*-fluoro substituents, while **8** contain an *ortho*-fluoro substituent.

Furthermore our measurements indicate that L-lactate generally is weakly bound when the boronate is the dominant configuration, presumably because of unfavorable coulomb interactions. The glucose displacement constant for **2**, **7**, **8**, and **9**, are closest to 15–16 mM, which is desired, since blood glucose is fluctuating between 2 and 30 mM in diabetes patients, and the maximum sensitivity is achieved when K_d matches the middle of the binding curve. Compounds with larger *ortho*-substituents, such as *o*-ethoxymethyl, *o*-aminomethyl, and *o*-phenyl- has been tested, but they have not shown any binding affinity to D-glucose, and only insignificant binding affinity to D-fructose and L-lactate. This further outlines how steric repulsion can disturb boronate ester-formation.

Our results are promising for developing the D-glucose monitoring dyes based on aryl monoboronic acids, which are attractive compared to more synthetically challenging, notoriously larger and thus usually less soluble aryl diboronic acid dyes.

4. Experimental

4.1. General

The computational calculations were performed on B3LYP/6-31G(d) level with Gaussian.

The aryl boronic acids **1**–**6**, **11**, and **12** were purchased from Combi Blocks and used as received. Compounds **7** and **8** were

purchased from Sigma–Aldrich and used as received. **9** and **10** were prepared by oxidation of the corresponding aldehydes with $KMnO_4$.[40] Alizarin red sodium, D-glucose, D-fructose, L-lactate, P4417 (phosphate buffered saline pellets), and P7994 (phosphate buffer pellets) were purchased from Sigma–Aldrich and used as received. Deionized water was used for the binding studies.

All data were fitted in GraphPad Prism 5.0, where the measured absorbances at 340 nm, 350 nm, and 360 nm were plotted against the logarithm to concentration data, using sigmoidal dose response fit (variable slope). K_d=100 μM (boronate) and [Ligand]=50 μM (ARS), or [Ligand]=52 μM (ARS). UV/vis-measurements were all performed on a Perkin–Elmer apparatus.

4.2. pK_a determination of aryl monoboronic acids via acid–base titration

Aryl monoboronic acid (**1**–**8**) (0.125 mmol) was dissolved in 5 mL of 0.025 M NaOH and 5 mL of deionized water, creating the corresponding base (1.25×10^{-2} M), which was titrated with 0.025 M HCl.

4.3. UV/vis-titration with ARS in saline buffer

(1a). Buffer containing ARS and aryl boronic acid: 1.0 L buffer containing 20 mM phosphate, 5.4 mM potassium chloride, and 274 mM sodium chloride was prepared. Afterwards 36 mg ARS (0.1 mmol) was dissolved with stirring for 5 h while slightly heating (40 °C–50 °C), [ARS]=100 μM. 0.05 mmol aryl boronic acid was dissolved in 250 mL of the prepared ARS buffer solution, [boron]=200 μM.

(2a). Polyol solution: A polyol solution was prepared by mixing 20.0 mmol (D-fructose or D-glucose, 3.61 g) and 5 mL ARS buffer containing aryl boronic acid. pH was adjusted with NaOH (7.4), and addition of water to a total volume of 10 mL. This gave a buffered solution (10 mM phosphate, 2.7 mM KCl, 137 mM NaCl), [ARS]=50 μM, [boron]=100 μM, and [polyol]=2 M. The L-lactate solution was prepared by mixing 20.0 mmol of L-lactate (2.24 g) and 5 mL ARS buffer containing aryl boronic acid. Addition of deionized water to a total volume of 10 mL was performed, while adjusting pH to 7.4, by addition of 50 μL 0.01 M HCl. This gave buffer (10 mM phosphate, 2.7 mM KCl, 137 mM NaCl), [ARS]=50 μM, [boron]=100 μM, and [L-lactate]=2 M.

(3a). Physiological buffer containing ARS and aryl boronic acid: 6 mL ARS buffer containing aryl boronic acid was diluted with 6 mL of deionized water, to obtain [ARS]=50 μM and [boron]=100 μM in a physiological buffer (10 mM phosphate, 2.7 mM KCl, 137 mM NaCl, pH=7.4).

(4a). Sample preparation: ARS-boronate-polyol solution mixed with ARS-boronate-solution, gave the following 14 polyol concentrations: [polyol]=200 μM, 500 μM, 1 mM, 2 mM, 5 mM, 10 mM, 20 mM, 50 mM, 100 mM, 200 mM, 500 mM, 1.0 M, 1.5 M, and 2.0 M. Sample consument (**2a**) about 2.7 mL. Sample consument (**3a**) about 11.3 mL Samples with L-lactate was prepared as the polyol solutions.

4.4. UV/vis-titration with ARS in phosphate buffer

(1b). Buffer containing ARS and boronic acid: 250 mL 200 mM phosphate buffer was prepared. Afterwards 9 mg ARS (0.025 mmol) was dissolved with stirring for 5 h while slightly heating (40 °C–50 °C), [ARS]=100 μM. 0.05 mmol aryl boronic acid was dissolved in the prepared ARS buffer solution, [boron]=200 μM.

(2b). Polyol solution: preparation was performed as in the previous experiment with D-glucose and D-fructose, resulting in 100 mM phosphate, [ARS]=50 μM, [boron]=100 μM, and [polyol]=2 M. The L-lactate solution was prepared as in the previous experiment, resulting in 100 mM phosphate, [ARS]=50 μM, [boron]=100 μM, and [L-lactate]=2 M.

(3b). Phosphate buffer containing ARS and aryl boronic acid: 6 mL ARS buffer containing aryl boronic acid was diluted with 6 mL of deionized water, to obtain [ARS]=50 μM and [boron]=100 μM in a 100 mM phosphate buffer (pH=7.4).

(4b). Sample preparation: sample preparation was carried out as described in previous experiment.

4.5. UV/vis-titration with ARS in 53.2 w/w% MeOH, 52 mM phosphate, and 40 mM NaCl buffer

(1c). Phosphate/MeOH buffer containing ARS and aryl boronic acid: 480 mL 104 mM methanolic phosphate buffer containing 53.2 w/w % MeOH was prepared by mixing 200 mL deionized water and 300 mL MeOH, and 8.5 g phosphate buffer pellets. Afterwards 18 mg ARS (0.05 mmol) was dissolved in 480 mL buffer solution, stirring for 4 h at room temperature, which gave [ARS]=104 μM. 0.048 mmol boronic acid was dissolved in 240 mL buffer solution, which gave [boron]=200 μM.

(2c). MeOH (53.2 w/w%): 53.2 g MeOH (67.3 mL) and 46.8 g deionized water (46.8 mL) was mixed, to obtain 53.2 w/w% MeOH.

(3c). Polyol solutions: D-glucose: 10.0 mmol D-glucose (1.803 g) was dissolved in 5 mL of the solution prepared under procedure (**1c**). Afterwards 0.1 mL 4 M HCl was added, and the total volume was adjusted with 53.2 w/w% MeOH (prepared under **2c**) to 10 mL. This gave [D-glucose]=1 M, [phosphate]=52 mM, and [NaCl]=40 mM, pH=7.4. D-Fructose: 20.0 mmol D-fructose (3.608 g) was dissolved in 5 mL of the solution prepared under (**1c**). Afterwards 0.1 mL 4 M HCl was added, and the total volume was adjusted with 53.2 w/w% MeOH (prepared under **2c**) to 10 mL. This gave [D-fructose]=2 M, [phosphate]=52 mM, and [NaCl]=40 mM, pH=7.4. L-lactate: 20.0 mmol L-lactate was dissolved in 5 mL of the solution prepared under procedure (**1c**). Afterwards the volume of the solution was adjusted with 53.2 w/w% MeOH, while addition of 4 M HCl dropwise until pH=7.4. The total volume was 10 mL. This gave [L-lactate]=2 M, [phosphate]=52 mM, and [NaCl]=40 mM.

(4c). Buffer used in sample preparation: 6 mL of solution prepared under procedure (**1c**), was diluted with 5.9 mL 53.2 w/w% MeOH, and pH was adjusted with 0.12 mL 4 M HCl to 7.4. This gave [boron]=100 μM, [ARS]=52 μM, and [NaCl]=40 mM. Total volume 12 mL.

(5c). Sample preparation. D-Glucose: solutions with 12 different D-glucose concentrations were prepared by mixing the ARS-boronate-D-glucose solution with the ARS-boronate-solution: [D-glucose]=200 μM, 500 μM, 1 mM, 2 mM, 5 mM, 10 mM, 20 mM, 50 mM, 100 mM, 200 mM, 500 mM, and 1.0 M. Sample consument (**3c**) about 1.9 mL. Sample consument (**4c**) about 10.1 mL D-fructose and L-lactate: ARS-boronate-D-fructose or ARS-boronate-L-lactate solution mixed with ARS-boronate-solution, gave the following 14 polyol concentrations: [polyol]=200 μM, 500 μM, 1 mM, 2 mM, 5 mM, 10 mM, 20 mM, 50 mM, 100 mM, 200 mM, 500 mM, 1.0 M, 1.5 M, and 2.0 M.

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