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Synthesis and carbohydrate binding studies of fluorescent α-amidoboronic acids and the corresponding bisboronic acids

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ABSTRACT

Fluorescent boronic acids are very useful for the design and synthesis of carbohydrate sensors. In an earlier communication, we first described the effort of developing water soluble fluorescent α-amidoboronic acids, which change fluorescence upon sugar binding. In this report, we describe a general method of functionalizing such boronic acids and their applications in the preparation of bis-α-amidoboronic acids with significantly enhanced binding for oligosaccharides as compared to their monoboronic acid counterparts. The advantages of good water solubility, easy modification to generate diversity, and modularity in synthesis will make α-amidoboronic acids very useful building blocks for future synthesis of boronic acid-based fluorescent sensors.

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1. Introduction

It is well-known that boronic acids are able to bind with compounds containing diols, hydroxyls, and other nucleophiles.[1–5] Such properties have been widely explored for the design and synthesis of boronic acid-based sensors for hydroxyl group-containing compounds[4–17] and aptamers for glycoprotein recognition.[18,19] Recently, the Schultz lab has developed a way to express boronic acid-modified proteins for carbohydrate recognition.[20] We have coined a term, boronolectin, to refer to all these boronic acid-based lectin mimics.[3] Most previous efforts in the boronic acid-based sensing area have been focused on arylboronic acids for stability and availability reasons (arylboronic acids are widely available because of their applications in important synthetic reactions such as the Suzuki coupling reaction).[21,22] However, arylboronic acids generally lack water solubility and are hard to further functionalize. Their stability is also often a problem.[23–25] α-Amidoboronic acids[26,27] form an important class of boronic acids with proven stability in vivo,[25,28] high water solubility, and known affinity for diols and hydroxyl groups. In our previous studies, α-amidoboronic acids (**D-1** and **L-1**, Fig. 1) with an appropriate fluorophore appended were shown to change fluorescent properties upon sugar binding and were water soluble.[29] In addition, amidoboronic acids can be easily functionalized at the acyl position thus allowing for preparation of bis- or multi-boronic acids using readily established chemistry. Specifically, these boronic acids can be easily functionalized for click coupling under very mild conditions.[30–32] Herein we describe the synthesis and evaluation of an enantiomeric pair of such bisboronic acids. The work is to demonstrate the feasibility of the chemistry and the unique (and unexpected) binding properties of the bisboronic acid products.

2. Results and discussion

For the synthesis of the bisboronic acids **2**, we were interested in using a click reaction for tethering two boronic acid units. Therefore, we were in need of one boronic acid functionalized with an azido group and another with an alkynyl group. As shown in Scheme 1, the synthesis of the α-amidoboronic acids **1** and its corresponding bisboronic acids **2** started with 1-(chloromethyl) naphthalene **3**. The stereochemistry for the subsequent steps was controlled by using different isomers of pinanediol as the protecting group.[33–36] Compound **5** was obtained by following literature procedures.[33] Reaction of **D-5** with lithium hexamethyldisilazane following literature procedures gave, presumably, intermediate **D-6**, which was directly acylated without purification by treating with an appropriate acid anhydride to give (−)-pinanediol amido boronate **D-7b** and **D-7c**. At this step, the introduction of different functional groups such as an azido or alkynyl group is very easy. The acid anhydrides were prepared from the corresponding carboxylic acids by treating with *N*-dicyclohexylcarbodiimide (DCC) and 4-dimethylamino pyridine (DMAP) at room temperature for 6 h.

The bisboronate **D-8** was prepared by reacting alkynylboronic acid **D-7b** and azidoboronic acids **D-7c** in the presence of Cu(I). At last, the deprotection of the pinanediol group with boron trichloride gave the final bisboronic acid **D-2**. The same synthetic

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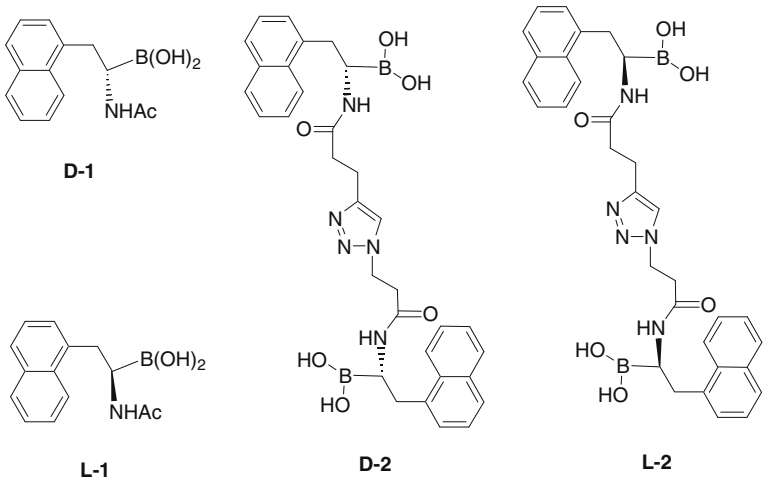
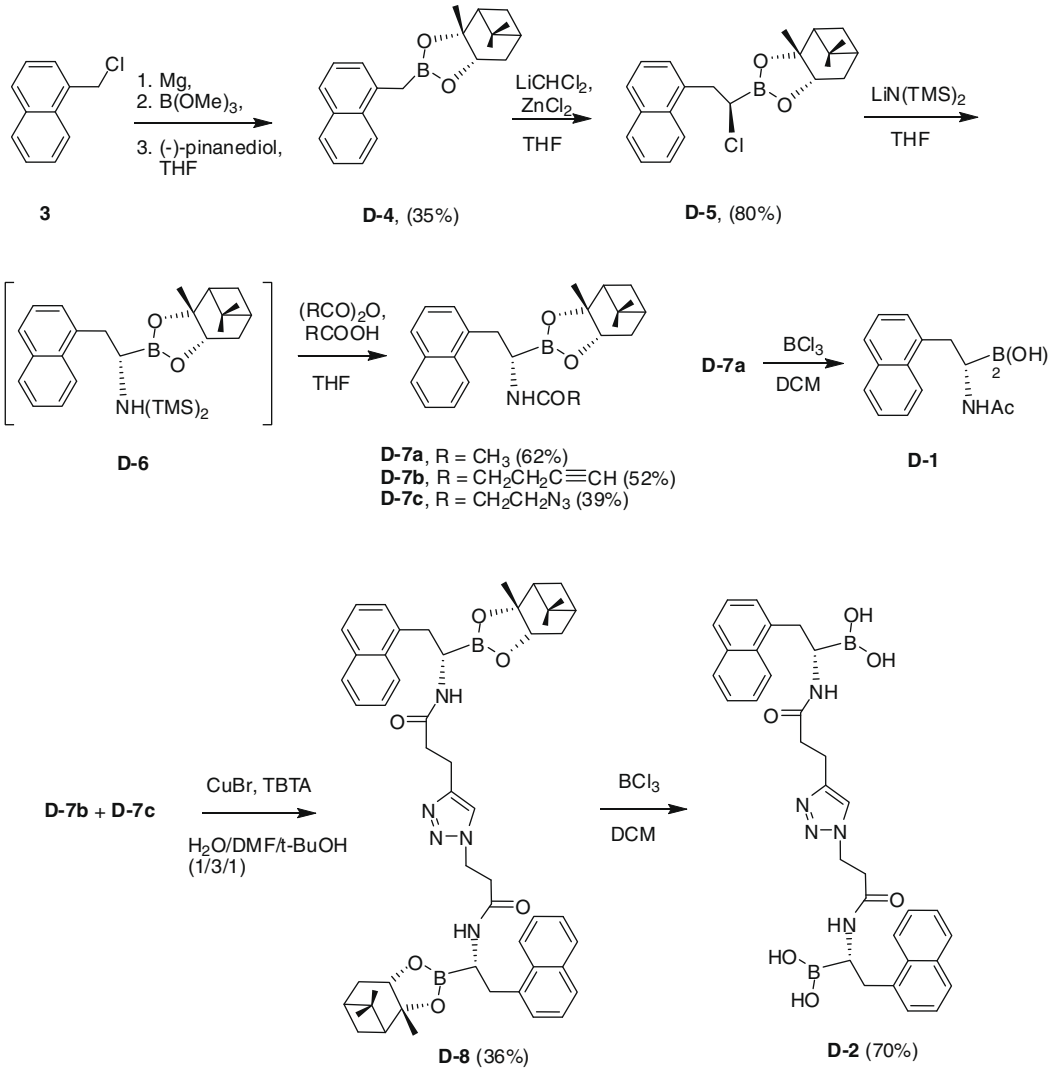


Figure 1. Enantiomeric pairs of α-amidoboronic acids **1** and the corresponding bisboronic acids **2**.



Scheme 1. Synthesis route of **D-1** and **D-2**.

route using (+)-pinanediol as the protecting group yielded **L-2**. Both isomers were characterized by NMR and MS. The enantiomeric purity was established by determining the optical rotation of each isomer and chiral HPLC.

In our previous studies, monomers of the naphthalene-based amidoboronic acid showed good water solubility in the concentration range of 1×10^{-4}–1×10^{-6} M.[29] Hereby we also examined the water solubility of the synthesized bisboronic acids using UV

Table 1
Apparent association constants (K_a) of amidoboronic acids with different sugars

K_a (M^{-1})	D-Fructose	D-Glucose	D-Sorbitol	D-Galactose	D-Mannose	D-Mannitol	D-Maltose	α-D-Lactose	β-D-Lactose
L-1	46 ± 2[a]	2 ± 0[a]	102 ± 1[a]	4 ± 0	4 ± 0	65 ± 11	1 ± 0	1 ± 0	1 ± 0
D-1	55 ± 8[a]	2 ± 0[a]	100 ± 6[a]	3 ± 0	4 ± 0	55 ± 6	1 ± 0	1 ± 0	1 ± 0
L-2	497 ± 49	25 ± 7	819 ± 57	86 ± 12	52 ± 5	254 ± 26	20 ± 4	36 ± 7	25 ± 3
D-2	372 ± 57	13 ± 3	754 ± 87	48 ± 7	39 ± 5	286 ± 68	29 ± 12	46 ± 3	35 ± 3

Binding studies were conducted in phosphate buffer (0.1 M) at pH 7.4 ([boronic acid] = 5×10^{-6} M).
[a] Data are from Ref. 30.

before any binding studies. The UV absorbance of both enantiomers showed good linearity in the concentration range of 1×10^{-4}–1×10^{-6} M. The concentration of amidoboronic acids we used for binding studies was 5×10^{-6} M, well within the range of solubility. Addition of monosaccharides to bisboronic acids induced similar fluorescent intensity changes as the monomers (**1**) in aqueous phosphate buffer. In our previous studies of monoboronic acid **D-1**, the fluorescent intensity decreased by 80%, 34%, and 33% upon binding with D-fructose, D-glucose and D-sorbitol with apparent binding constants being 46 M^{-1}, 1.5 M^{-1} and 102 M^{-1}, respectively (Table 1).[29] In this study, for bisboronic acid **D-2**, the fluorescent intensity decreased by a maximum of 50% upon binding with D-fructose (0.5–20 mM), and the binding constant was 372 M^{-1}, which was sevenfold higher than that of the monomer **D-1** (Fig. 2 and Table 1). This increase in binding constants from monoboronic acids to bisboronic acids was observed for all the carbohydrates we studied (Table 1). For example, the binding constants between the bisboronic acid and glucose, mannose, galactose, and sorbitol were all at least eightfold higher than that of the monoamidoboronic acids. It needs to be noted that the order of the binding constants of α-amidoboronic acids **1** and bisboronic acids **2** with different monosaccharides was the same as that of arylboronic acids (Table 1).[4–6,37] The observed increase in binding of bisboronic acids for monosaccharides is intriguing. In order to see whether the spatial arrangements of the boronic acids would accommodate bidentate binding with fructose and other monosaccharides, the conformational features of the two bisboronic acids were examined using computational chemistry. Specifically, the structures of bisboronic acids were optimized by DFT method B3LYP and the 6−31+G(d,p) base set along with the PCM solvation model implemented in the GAUSSIAN 03 program, as described in our previous studies.[16,17] Modelling results are depicted in Figure 3. The distance between the boronic acid pair for both the bisboronic acids is about ~10 Å, which is possible for bidentate binding with any of the monosaccharides. However, we did not conduct further computational work because of a lack of experimental evidence as to the binding positions. It should be noted that there is no experimental evidence to prove bidentate binding. Therefore, both mono and bidentate binding modes are distinct possibilities.

Another very interesting property of the bisboronic acids is their ability to bind disaccharides maltose and lactose with K_a in the range of 35–45 M^{-1}. There have been some other boronic acid-based receptors that show strong binding to either maltose or lactose.[38,39] To the best of our knowledge, these bisboronic acids represent the first examples of significant binding to both of these two disaccharides by any bisboronic acid-based fluorescent sensors.

With the improved affinity for mono- and di-saccharides in mind, we were also interested in studying the binding of these two bisboronic acids with oligosaccharides. Therefore, the binding affinity of the synthesized bisboronic acids with several tetrasaccharides (sodium salt of neocarratetraose-41-*O*-sulfate (**T1**), *N*, *N*′, *N*″, *N*‴ tetraacetyl chitotetraose (**T2**), and lacto-*N*-tetraose (**T3**), Fig. 4) was also determined. As shown in the Table 2, the apparent association constants (K_a) of **L-2** with **T1**, **T2**, and **T3** were 2422, 12611, and 18107 M^{-1}, respectively. **D-2** has slightly different binding constants with the three tetrasaccharides from that of

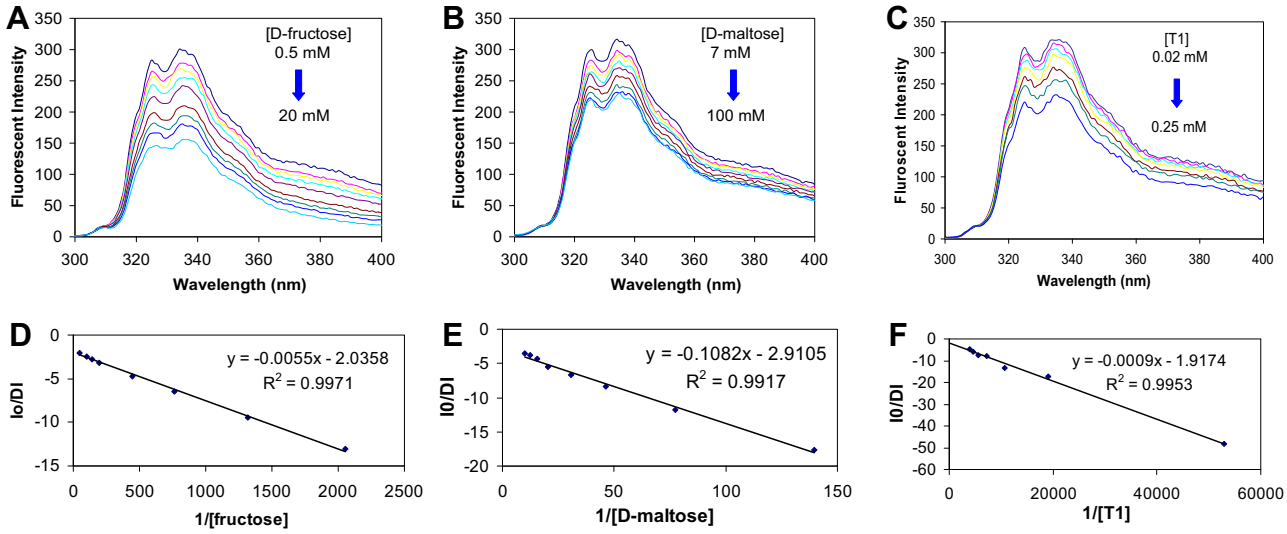


Figure 2. (A) Fluorescent spectral changes of boronic acid **D-2** (5×10^{-6} M) upon addition of D-fructose (0.5–20 mM) in phosphate buffer (0.1 M) at pH 7.4: λ_{ex} = 280 nm, λ_{em} = 334 nm; (B) fluorescent spectral changes of **D-2** (5×10^{-6} M) upon addition of D-maltose (7–100 mM) in phosphate buffer (0.1 M) at pH 7.4: λ_{ex} = 280 nm, λ_{em} = 334 nm; (C) fluorescent spectral changes of **D-2** (5×10^{-6} M) upon addition of neocarratetraose-41-*O*-sulfate (Na^+) (**T1**) (0.02–0.25 mM) in phosphate buffer (0.1 M) at pH 7.4: λ_{ex} = 280 nm, λ_{em} = 334 nm; (D) binding curve of **D-2** upon addition of D-fructose; (E) binding curve of **D-2** upon addition of D-maltose; (F) binding curve of **D-2** upon addition of **T1**.

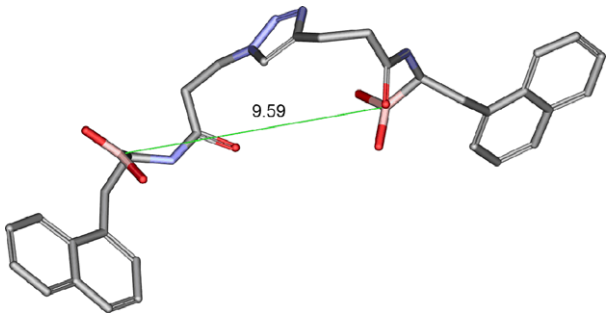


Figure 3. The DFT optimized structures for **L-2**. Hydrogen atoms are not shown for clear depiction. The distances between the boronic acid pairs are labelled as angstrom (Å).

L-2, showing some very small stereo-discrimination. As a control, we also studied the binding of these three tetrasaccharides with the mono-α-amidoboronic acids (**D-1** and **L-1**). Interestingly, no fluorescent property change was observed of the monoboronic acids (**D-1** and **L-1**) with the addition of 5 mM of these three tetrasaccharides. Such results mean that the binding constants between the monoamidoboronic acids and the tetrasaccharides were far less than 200 M^{-1} and much smaller than the corresponding binding constants with the bisboronic acids (Table 2). The significantly enhanced binding affinities of the bisboronic acids for the tetrasaccharides, compared with the monoboronic acids (Table 1), suggest that single-pair functional interactions were not enough for tight

Table 2
Apparent association constants (K_a) of bisboronic acids **D-2** and **L-2** with three tetrasaccharides

K_a (M^{-1})	**T1**: Neocarratetraose-41-*O*-sulfate (Na^+)	**T2**: *N*, *N*′, *N*″, *N*‴ Tetra-acetyl chitotetraose	**T3**: Lacto-*N*-tetraose
D-2	2422 ± 292	12611 ± 2160	18107 ± 1260
L-2	3154 ± 282	12568 ± 1150	19148 ± 2747

Binding studies were conducted in phosphate buffer (0.1 M) at pH 7.4 ([boronic acid] = 5×10^{-6} M).

binding of the bisboronic acids with these tetrasaccharides. When compared against the binding with mono- and di-saccharides, the binding constants of these bisboronic acids with the three tetrasaccharides were also much higher. This is especially true when comparing with the binding to disaccharides. However, at this time we have no structural information, which explains why these bisboronic acids showed improved binding for the three tetrasaccharides studied. It should be noted that the ability for a bisboronic acid to bind to an oligosaccharide containing only pyranose sugars is very significant since most biologically important glycans on cell surface are composed of pyranose sugars.[15]

It is interesting to note that no noticeable difference was observed between the D and L isomers in their binding with carbohydrates and in their spectral changes even for the bisboronic acids′ binding with tetrasaccharides.[29] The binding constants for the L- and D-enantiomers with D-sugars were very similar (Table 1). For example, the apparent association constants (K_a) of **L-2** with D-sorbitol, D-maltose, and lacto-*N*-tetraose (**T3**) were 819, 20,

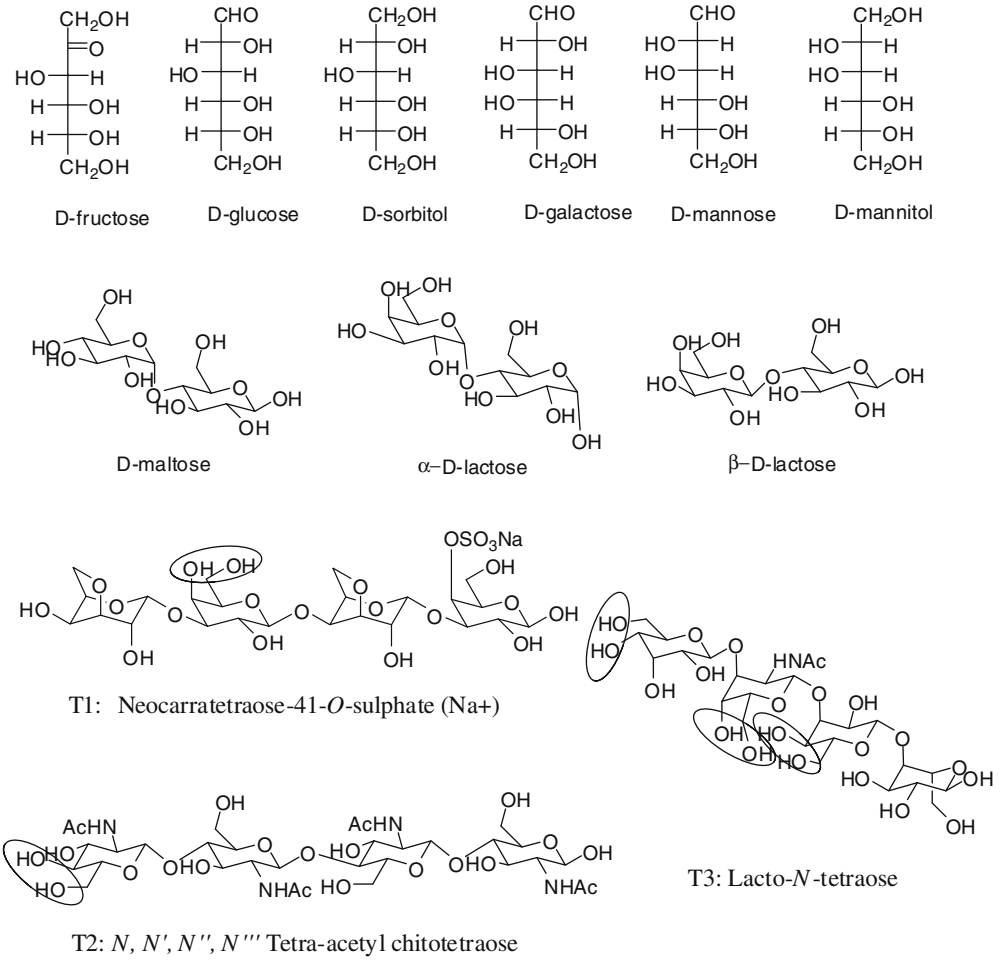


Figure 4. Structures of the saccharides that were used for binding studies with α-amidoboronic acids **1** and the corresponding bisboronic acids **2**.

and 18107 M^{-1}, respectively. On the other hand, the apparent association constants (K_a) of **D-2** with D-sorbitol, D-maltose, and lacto-*N*-tetraose (**T3**) were 754, 29, and 19148 M^{-1}, respectively. Such results were surprising to us because the boronic acids have at least one chiral center and are enantiomerically pure. One would have expected to see some discrimination between enantiomers (**L-1** vs **D-1** and **L-2** vs **D-2**) in binding with a chiral molecule (sugar) since the end complexes are diastereomers in both cases. The similarity in binding constants between enantiomers suggests that the only functional group that was involved in binding was the boronic acid moiety and both the amido group and the naphthalene group were not involved in a substantial way in binding. Therefore, in order to take advantage of the chiral nature of amidoboronic acid for enantiomeric discrimination, as well as to achieve better selectivity for specific carbohydrates, additional interaction points and rigid linkers are needed.

Among the three tetrasaccharides, the binding affinity of **T1** is substantially smaller than that of **T2** and **T3**. In search for possible reasons for this difference, one can examine several factors: (1) presence of diol groups that bind to the boronic acid unit, (2) the number of such diol groups, and (3) the relative distance and orientation of these diol groups. If one examines diol 'sites' on each carbohydrate that could engage in cyclic boronate formation, one could only find 4,6-diols that are known to form such complexes.[5,40,41] **T1** and **T2** have one such 'site' each, and **T3** has three. Because **T2** and **T3** have similar binding constants with **D-2** and **L-2**, the sheer number of such binding 'sites' is obviously not the reason contributing to the different affinities. As described earlier, these three tetrasaccharides do not induce fluorescent property changes in the monoboronic acids (**L-1** and **D-1**). This means that mono-functional group interactions would not be enough for the observed binding between the bisboronic acids with the tetrasaccharides. Since **T1** and **T2** only have one diol pair each, the second interaction point is most likely a single hydroxyl group, which is known to be an important contributing factor for tight interactions with a boronic acid,[5] while for **T3**, binding could involve two pairs of diols. However, a detailed understanding of why **D-2** and **L-2** would bind **T2** and **T3** more tightly than **T1** has not been achieved. We have attempted computational chemistry work to answer this question. However, since the binding sites are not known, there are too many possible permutations to allow for high-level calculations in order to achieve reliable answers.

3. Conclusions

We have demonstrated the synthesis and binding evaluation of the first pair of bisamidoboronic acids. First, these α-amidoboronic acids showed good water-solubility and significant fluorescent intensity changes upon binding with different carbohydrates. Second, the bisboronic acids showed greater binding affinities for monosaccharides such as glucose, fructose, and sorbitol than monoamidoboronic acids. Third, these two bisboronic acids represent the first examples of bisboronic acid fluorescent sensors that show significant binding to both maltose and lactose. Their ability to bind saccharides containing only pyranose sugars is very significant. Fourth, in binding with 3 tetrasaccharides, the bisboronic acids have much higher affinity than the corresponding monoboronic acids (almost no binding), indicating (but not proving) bidentate binding. Fifth, the ability for the bisboronic acids to bind to **T2** implies that the postulated bidentate binding may involve interactions between a boronic acid and a single hydroxyl group. Sixth, one unexpected finding of the study is the lack of stereochemical discrimination in these chiral amidoboronic acids. One reason could be that the side-chain functional groups were not engage in binding at all and the other reason could be that no bidentate binding was involved. Though we have demonstrated the promising potential of bisamidoboronic acids, it is clear that much more needs to be done to fully take advantage of the utility of amidoboronic acids for sensor design and synthesis.

4. Experimental

4.1. General methods and materials

^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer in deuterated chloroform ($CDCl_3$) or MeOD with either tetramethylsilane (TMS) (0.00 ppm) or the NMR solvent as the internal reference unless otherwise specified. Fluorescence spectra were recorded on a Shimadzu RF-5301 PC spectrofluorometer. Absorption spectra were recorded on a Shimadzu UV-1700 UV–vis spectrophotometer. Quartz cuvettes were used in all fluorescent and UV studies. All pH values were determined by a UB-10 Ultra Basic Benchtop pH meter (Denver Instrument). Analytical thin-layer chromatography (TLC) was performed on Merck Silica Gel 60 plates (0.25 mm thickness with F-254 indicator). Sugars, buffer ingredients and chemicals were bought from Aldrich or Acros and were used as received. Water used for the binding studies was doubly distilled and further purified with a Milli-Q filtration system. Solvents for extraction and chromatography were used as received. Dry solvents (DMF, DMSO) were purchased from Acros.

4.2. Synthesis

4.2.1. (−)-Pinanediol [(1*S*)-1-pent-4-ynoylamino-2-(1-naphthyl) ethyl] boronate (D-7b)

Lithium hexamethyldisilazane was prepared at −78 °C from hexamethyldisilazane (0.16 mL, 0.78 mmol, 1.2 equiv) and *n*-BuLi (0.3 mL, 0.75 mmol, 1.16 equiv) in THF (1 mL) under protection of nitrogen. (−)-Pinanediol [(1*R*)-1-chloro-2-(1-naphthyl)ethyl]-boronate (240 mg, 0.65 mmol, 1.0 equiv) in THF (1 mL) was then added at −78 °C to the solution of lithium hexamethyldisilazane with stirring. The solution was allowed to warm to room temperature slowly and then stirred for additional 8 h. This solution was designed as solution A 4-pentynoic acid (479 mg, 4.88 mmol, 7.5 equiv), DCC (402 mg, 1.95 mmol, 3.0 equiv), and DMAP (8.6 mg, 0.07 mmol, 0.1 equiv) were mixed and dissolved in dry THF (3 mL) at room temperature. The mixture was stirred at room temperature for 6 h to form solution B. Then solution B was added into the solution A at −78 °C under the protection of nitrogen. The solution of the mixture was allowed to warm to room temperature slowly and then stirred overnight. Then dH2O (5 mL) was added and organic solvent was evaporated under vacuum. The water solution was extracted by ethyl acetate (20 mL × 3). The combined ethyl acetate layers was washed by 5% $NaHCO_3$ solution (10 mL), dH_2O (10 mL) and brine (10 mL), and dried over $MgSO_4$. Then solvent was evaporated under vacuum. Column chromatography (silica gel, hexanes–ethyl acetate, 2:1) gave **D-7b** (130 mg, 52%) as yellow solid. TLC (hexanes–ethyl acetate, 4:1, R_f = 0.50); chiral HPLC (Chiralcel OD NP HPLC 250 × 10 mm column, 280 nm), elution condition: isocratic 5% isopropanol in hexane, flow rate = 1.0 mL/min, HPLC rt = 11.7 min; $[\alpha]_D^{20} = +44.9$ (*c* 1.00, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.10 (d, *J* = 8.8 Hz, 1H), 7.98 (q, *J* = 9.2 Hz, 1H), 7.77 (d, *J* = 8.4 Hz, 1H), 7.51–7.56 (m, 2H), 7.42 (t, *J* = 7.6 Hz, 1H), 7.36 (d, *J* = 6.8 Hz, 1H), 6.36 (s, 1H), 4.35 (t, *J* = 8.8 Hz, 1H), 3.53 (d, *J* = 10.4 Hz, 1H), 3.21–3.23 (m, 2H), 2.41–2.50 (m, 5H), 2.26 (d, *J* = 10.4 Hz, 1H), 2.08 (d, *J* = 10.8 Hz, 1H), 1.92–1.96 (m, 2H), 1.87 (s, 1H), 1.53 (d, *J* = 10.4 Hz, 1H), 1.45 (s, 3H), 1.33 (s, 3H), 0.92 (s, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 174.3, 136.5, 134.1, 132.0, 128.8, 127.2, 126.7, 126.1, 125.8, 125.5,

124.0, 84.3, 81.9, 70.3, 52.2, 40.0, 38.2, 36.4, 34.3, 32.1, 29.0, 27.4, 26.7, 24.2, 14.4; MS (ESI-), *m*/*e* (relative intensity), 428.2 $(M-1)^-$.

4.2.2. (+)-Pinanediol [(1*R*)-1-pent-4-ynylamino-2-(1-naphthyl) ethyl] boronate (L-7b)

L-7b was prepared using the same procedures as above. (51%); chiral HPLC (Chiralcel OD NP HPLC 250 × 10 mm column, 280 nm), elution condition: isocratic 5% isopropanol in hexane, flow rate = 1.0 mL/min, HPLC rt = 8.8 min; $[\alpha]_D^{20} = -54.6$ (*c* 1.00, MeOH); MS (ESI-), *m*/*e* (relative intensity), 428.1 $(M-1)^-$.

4.2.3. (−)-Pinanediol [(1*S*)-1-(4-azido)propionylamino-2-(1- naphthyl)ethyl] boronate (D-7c)

Lithium hexamethyldisilazane was prepared at −78 °C from hexamethyldisilazane (0.19 mL, 0.91 mmol, 1.2 equiv) and *n*-BuLi (0.35 mL, 0.88 mmol, 1.16 equiv) in THF (1 mL) under the protection of nitrogen. (−)-Pinanediol [(1*R*)-1-chloro-2-(1-naphthyl)ethyl] boronate (280 mg, 0.76 mmol, 1.0 equiv) in THF (1 mL) were then added at −78 °C to the solution of lithium hexamethyldisilazane with stirring. This solution was allowed to warm to room temperature slowly and stirred for additional 8 h. This was designated A 3-azido-propionic acid (656 mg, 5.7 mmol, 7.5 equiv), DCC (475 mg, 2.3 mmol, 3.0 equiv), and DMAP (9.8 mg, 0.08 mmol, 0.1 equiv) were mixed and dissolved in dry THF (3 mL) at room temperature. The mixture was stirred at room temperature for 6 h to form solution B. Then solution B was added into solution A at −78 °C under the protection of nitrogen. The solution of the mixture was allowed to warm to room temperature slowly and then stirred overnight. Then dH_2O (5 mL) was added and organic solvent was evaporated under vacuum. The water solution was extracted by ethyl acetate (20 mL × 3). The combined ethyl acetate layers was washed by 5 % $NaHCO_3$ solution (10 mL), dH_2O (10 mL) and brine (10 mL), and dried over MgSO4. Then the solvent was evaporated under vacuum. Column chromatography (silica gel, hexanes–ethyl acetate, 2:1) gave **D-7c** (131 mg, 39%) as white solid. TLC (hexanes–ethyl acetate, 2:1, R_f = 0.50); chiral HPLC (Chiralcel OD NP HPLC 250 × 10 mm column, 280 nm), elution condition: isocratic 5% isopropanol in hexane, flow rate = 1.0 mL/min, HPLC rt = 12.2 min; $[\alpha]_D^{20} = +40.8$ (*c* 1.00, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.09 (d, *J* = 7.2 Hz, 1H), 7.89 (d, *J* = 7.2 Hz, 1H), 7.78 (d, *J* = 8.4 Hz, 1H), 7.52–7.56 (m, 2H), 7.42–7.46 (m, 1H), 7.36 (d, *J* = 7.2 Hz, 1H), 6.08 (s, 1H), 4.36 (t, *J* = 8.4 Hz, 1H), 3.51–3.60 (m, 3H), 3.22–3.31 (m, 2H), 2.39–2.42 (m, 3H), 2.23–2.25 (m, 1H), 2.07 (s, 1H), 1.94 (d, *J* = 8.8 Hz, 2H), 1.41–1.48 (m, 4H), 1.33 (s, 3H), 0.91 (s, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 172.3, 136.2, 134.1, 132.0, 128.8, 127.3, 126.9, 126.1, 125.8, 125.4, 123.9, 84.9, 51.9, 46.8, 39.9, 38.2, 36.1, 34.0, 33.5, 28.8, 27.3, 26.6, 24.2; MS (ESI-), *m*/*e* (relative intensity), 477.3 $(M+CH_3OH-H)^-$, 445.3 $(M-1)^-$.

4.2.4. (+)-Pinanediol [(1*R*)-1-(4-azido)propionylamino-2-(1-naphthyl)ethyl] boronate (L-7c)

L-7c was prepared using similar procedures as described above. (36%); chiral HPLC (Chiralcel OD NP HPLC 250 × 10 mm column, 280 nm), elution condition: isocratic 5% isopropanol in hexane, flow rate = 1.0 mL/min, HPLC rt = 10.3 min; $[\alpha]_D^{20} = -45.1$ (*c* 1.00, MeOH); MS (ESI-), *m*/*e* (relative intensity), 463.2 $(M+H_2O-H)^-$, 445.1 $(M-1)^-$.

4.2.5. *N*-[(1*S*)-1-((−)-Pinanedioxaborol-2-yl)-2-naphthalen-1-ylethyl]-3-(1-{2-[(1*S*)-1-((−)-pinanedioxaborol-2-yl)-2-naphthalen-1-yl-ethylcarbamoyl]-ethyl}-1*H*-[1,2,3]triazol-4-yl)-propionamide (D-8)

To the mixture of compounds **D-7b** (52.1 mg, 0.12 mmol, 1.0 equiv), **D-7c** (54.2 mg, 0.12 mmol, 1.0 equiv), tris[(1benzyl-1*H*-1,2,3-triazol-4-yl)methyl] amine (TBTA) (9.5 mg, 0.018 mmol, 0.15 equiv) and copper(I) bromide (5.2 mg, 0.036 mmol, 0.3 equiv), a mixed solvent of H_2O/DMF/*t*-BuOH (0.5 mL/1.5 mL/0.5 mL) was added. The solution was stirred at room temperature for 5 h. Then methanol (60 mL) was added and solid was filtered out. The solvent was evaporated under vacuum. The crude product was purified by HPLC (C18 RP column, 280 nm). Elution condition: 50% CH_3CN/MeOH (flow rate = 2.0 mL/min). rt = 15 min. This gave **D-8** (38 mg, 36 %) as a white powder. $[\alpha]_D^{20} = +70.3$ (*c* 1.00, MeOH); ^{1}H NMR (MeOD, 400 MHz) δ 8.00 (t, *J* = 8.8 Hz, 2H), 7.87 (d, *J* = 7.2 Hz, 2H), 7.82 (s, 1H), 7.75 (d, *J* = 8.0 Hz, 2H), 7.45–7.52 (m, 4H), 7.37–7.42 (m, 2H), 7.28–7.36 (m, 2H), 4.70 (t, *J* = 6.4 Hz, 2H), 4.22–4.26 (m, 2H), 3.35–3.42 (m, 2H), 2.96–3.08 (m, 8H), 2.76 (d, *J* = 7.2 Hz, 2H), 2.37 (t, *J* = 9.2 Hz, 2H), 2.11–2.14 (m, 2H), 1.97 (s, 1H), 1.85–1.88 (m, 4H), 1.39–1.45 (m, 8H), 1.31 (s, 6H), 0.90 (s, 6H); ^{13}C NMR (MeOD, 400 MHz) δ 177.6, 175.0, 145.2, 136.3, 136.1, 134.3, 134.2, 131.8, 128.5, 126.7, 126.6, 126.5, 126.5, 125.5, 125.5, 125.2, 125.2, 123.3, 123.2, 123.0, 83.0, 82.6, 76.1, 75.9, 52.4, 52.2, 45.4, 44.7, 40.2, 40.1, 40.0, 37.9, 37.8, 36.4, 36.2, 34.5, 34.3, 31.5, 31.3, 29.8, 28.6, 28.5, 26.5, 26.1, 23.3, 20.6; MS (ESI-), *m*/*e* (relative intensity), 874.6 $(M-1)^-$.

4.2.6. *N*-[(1*R*)-1-((+)-Pinanedioxaborol-2-yl)-2-naphthalen-1-yl-ethyl]-3-(1-{2-[(1*R*)-1-((+)-pinanedioxaborol-2-yl)-2-naphthalen-1-yl-ethylcarbamoyl]-ethyl}-1*H*-[1,2,3]triazol-4-yl)-propionamide (L-8)

L-8 was prepared using a procedure similar to the preparation of **D-8**. (41%); $[\alpha]_D^{20} = -60.7$ (*c* 0.80, MeOH); MS (ESI-), *m*/*e* (relative intensity), 874.4 $(M-1)^-$.

4.2.7. *N*-[(1*S*)-1-Boroxo-2-naphthalen-1-yl-ethyl]-3-(1-{2-[(1*S*)-1-boroxo-2-naphthalen-1-yl-ethylcarbamoyl]-ethyl}-1*H*-[1,2,3]triazol-4-yl)-propionamide (D-2)

To a solution of boron trichloride (0.21 mL of 1 M solution in dichloromethane, 0.27 mmol, 5.0 equiv) in dichloromethane (1 mL) at −78 °C under the protection of nitrogen, compound **D-8** (37 mg, 0.04 mmol, 1.0 equiv) in dichloromethane (1 mL) solution was added. The mixture was stirred at −78 °C for 1 h. Then solvent was evaporated under vacuum and condensed in a salt ice trap. The solid residue was washed with water (5 mL × 2), dichloromethane (5 mL × 2), ethyl acetate (5 mL × 2), and hexanes (5 mL × 2). This gave **D-2** (17 mg, 70 %) as a white powder. $[\alpha]_D^{20} = +83.9$ (*c* 0.08, MeOH); ^{1}H NMR (MeOD, 400 MHz) δ 8.03 (m, 2H), 7.87 (d, *J* = 7.6 Hz, 3H), 7.75 (d, *J* = 8.4 Hz, 2H), 7.49–7.53 (m, 4H), 7.39–7.49 (m, 2H), 7.27–7.31 (m, 2H), 4.76 (t, *J* = 6.4 Hz, 2H), 3.37 (s, 2H), 3.06–3.12 (m, 4H), 2.91–3.03 (m, 4H), 2.80 (d, *J* = 4.0 Hz, 2H); ^{13}C NMR (MeOD, 400 MHz) δ 179.2, 176.8, 137.9, 137.7, 135.7, 135.6, 133.3, 129.9, 128.1, 127.8, 127.0, 126.7, 126.6, 125.0, 124.7, 124.6, 77.8, 47.2, 35.0, 32.0, 30.7, 21.7, 19.7; MS (ESI-), *m*/*e* (relative intensity), 588.2 $(M-H_2O-H)^-$, 606.2 $(M-1)^-$; HRMS, calcd for $C_{32}H_{32}{}^{11}B_2N_5O_5$: 588.2590, found: 588.2598.

4.2.8. *N*-[(1*R*)-1-Boroxo-2-naphthalen-1-yl-ethyl]-3-(1-{2-[(1*R*)-1-boroxo-2-naphthalen-1-yl-ethylcarbamoyl]-ethyl}-1*H*-[1,2,3] triazol-4-yl)-propionamide (L-2)

L-2 was prepared in a similar fashion as **D-2** (62 %); $[\alpha]_D^{20} = -125.9$ (*c* 1.00, MeOH); MS (ESI-), *m*/*e* (relative intensity), 588.4 $(M-H_2O-H)^-$, 606.4 $(M-1)^-$; HRMS, calcd for $C_{32}H_{32}{}^{11}B_2N_5O_5$: 588.2590, found: 588.2590.

4.3. Procedures for the binding studies (D-2 binding with T1 as an example)

Solutions of bisboronic acid **D-2** (5×10^{-6} M) and **D-2** (5×10^{-6} M) with Neocarratetraose-41-*O*-sulphate (Na^+) (**T1**, 2.5×10^{-4} M) were prepared in 0.1 M phosphate buffer at pH 7.40, respectively. Then, these two solutions were mixed into a 1 cm cuvette. In

the solution, the ratio of boronic acid + sugar was increased gradually ([**T1**] is from 2×10^{-5} M to 2.5×10^{-4} M). After shaking for 2 min, the solution was used to test the fluorescence intensity or UV absorbance immediately. Six to eight points were collected for the calculation of apparent binding constant K_a. The binding of sugar with the boronic acid compound gave concentration dependent fluorescent intensity changes (Fig. 2). Binding constants K_a were calculated using Eq. (1), where I_0 is initial fluorescent intensity, b is the path length of absorption and ΔI_{max} is the maximal fluorescent intensity change:

$$([\mathbf{D\text{-}2}]b)/\Delta I = I_0/(\Delta I_{max}[\mathbf{T1}]K_a) + I_0/(\Delta I_{max}) \quad (1)$$

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.01.017.

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