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"*Click-fluors*": Triazole-linked saccharide sensors

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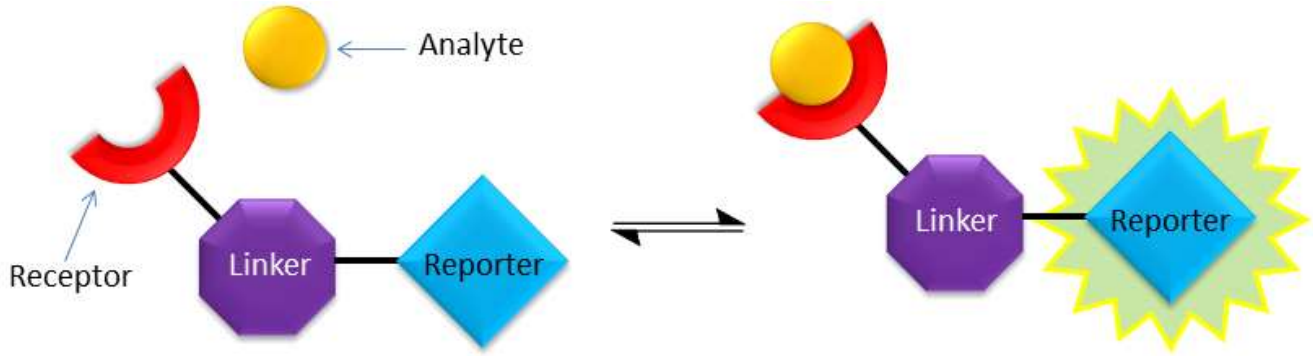
Abstract

A series of boronic acid-containing saccharide receptors was synthesised *via* copper catalysed azide-alkyne cycloaddition (CuAAC) reactions. Their saccharide binding capacity was studied by ^{1}H and ^{11}B NMR spectroscopy titrations and isothermal titration calorimetry (ITC) techniques. Fluorescent sensors were generated by linking a phenylboronic acid (PBA) receptor with fluorophores *via* a triazole-linker. Fluorescence titrations with fructose revealed that the substitution pattern about the PBA influences the fluorescence response to saccharides. Titrations studied by ^{1}H NMR spectroscopy suggested that fructose binding is enhanced when the aromatic ring bearing the boronic acid has the triazole-containing substituent at the *ortho* position. No evidence of either a dative N-B bond or solvent insertion (between B and N) was observed by ^{11}B NMR spectroscopy. These results demonstrate that synthetic accessible triazole receptors may allow rapid sensor synthesis, screening and discovery.

Introduction

Saccharides participate in some of the most essential chemical processes in life, particularly energy metabolism and cell recognition.[1] Accurate detection of saccharides could have an impact on clinical diagnosis of several diseases. For example, certain types of glycoproteins are over-expressed on the surface of cancer cells.[2] As a result, the development of sensing techniques to target these cancer biomarkers are in high demand.[3]

In past decades progress in synthetic molecular probes (chemosensors) has shown significant promise.[4] Synthetic boronic acid-based saccharide detection, which relies on the reversible formation of boronic esters through covalent bonding between diol motifs and boronic acids,[5] is a well-established approach in the glyco-detection area.[6] As illustrated in Scheme 1, a common strategy to assemble fluorescent molecular sensors is to connect a receptor and a fluorophore using a linker motif,[7] numerous molecular chemosensors have been designed and synthesised following this strategy.[8] However, chemosensor synthesis is often non-trivial, requiring multiple synthetic steps and challenging purifications.[9] Strategies for rapid glyco-sensor assembly would be beneficial if chemo-diagnostics could provide new sensors at a relatively comparable rate to biomarker discovery,[10] and therapeutic use.[11]



Scheme 1. Schematic illustration of the process of sensing in a "three-component" chemosensor system.[8b]

In order to establish a simple but effective method for boronic acid-based sensor creation, Scrafton *et al.* (including some co-authors of this report) employed the copper catalyzed azide-alkyne cycloaddition (CuAAC) reaction, commonly referred to as a "*click*" reaction.[12] For such receptors, the term *click-fluor* was coined.[13] The CuAAC reaction has been extensively exploited across the chemical sciences due to well-known merits, such as high yields, operational simplicity, and wide-ranging reaction regimes.[14] In fact, the synthetic advantages of creating molecular chemosensors *via* CuAAC reactions have also been demonstrated in other recent studies,[15] and more boronate ester analogues have also been synthesised as modular building blocks for Suzuki coupling.[16]

In the Scrafton study, compound **1a** (Figure 1) showed fluorescence enhancements upon addition of fructose and mannose. This was assumed to be due to fluorescence emission recovery from the conjugated triazole after the binding of diols, however this assumption has proved to be incorrect and is discussed in this manuscript.

Additionally, an interesting possibility exists with a triazole linker in a boron-based chemosensor. It was previously proposed that one or more of the triazole's nitrogen atoms could interact with an adjacent boronic acid, similar to the *ortho*-aminomethyl phenylboronic acid-based systems reported by James *et al.*[17] and carefully studied by Zhu *et al*.[18] (Figure 1b). This interaction could either involve a direct N-B dative bond, or as Zhu *et al*. found, a solvent insertion. The postulate of a triazole to boronic acid interaction was further investigated in a separate study by Mulla *et al.* with supporting computational analysis (Figure 1c).[19] Their computational study revealed a solvent insertion using the central nitrogen of the triazole.

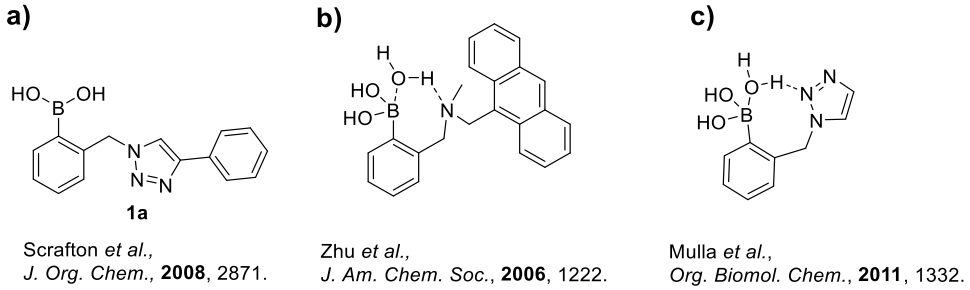


Figure 1. a) Chemical structure of the first "*click-fluor*"; b) the model for amine-based boronic acid interactions in James and Shinkai's system; c) proposed model of triazole nitrogen-boronic acid interaction in aqueous media.

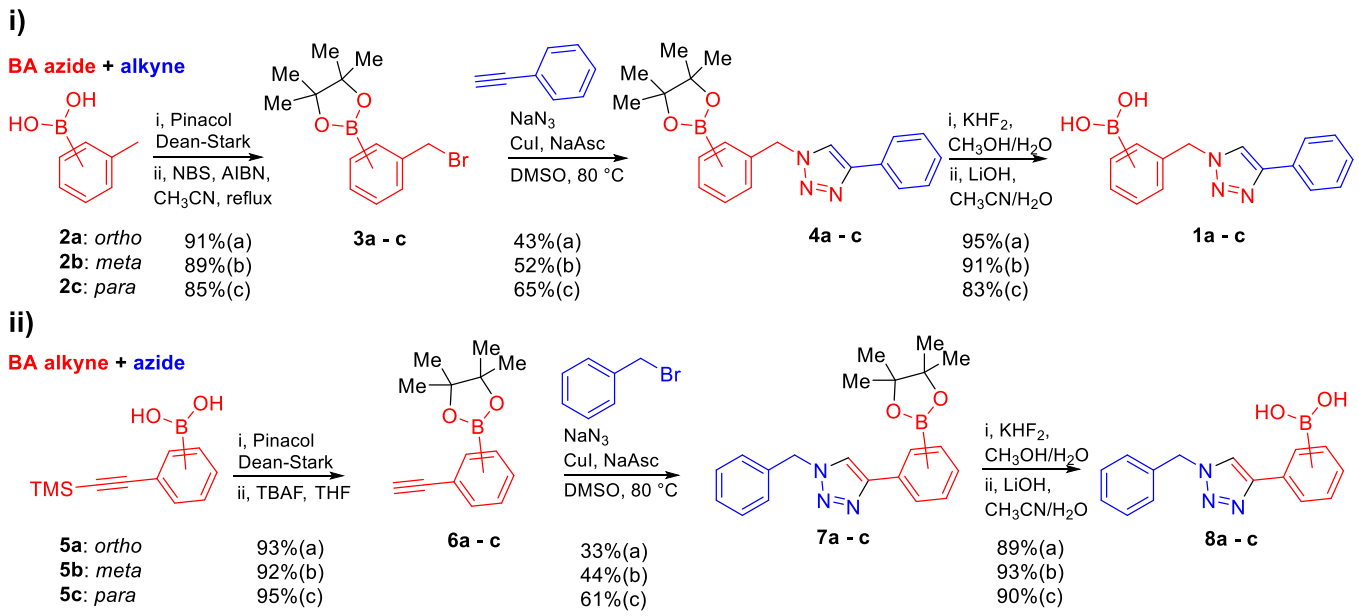
Herein, the so-called *click chemistry* strategy is used to prepare a series of boronic acids with varying regiochemistry of substitution on both the phenylboronic acid and triazole rings, and their performance as saccharide sensors is probed. Importantly, the earlier *click-fluor* observations are revised following investigations into weak background fluorescence witnessed across multiple analyte samples. Further, we set out to investigate whether the computationally proposed structure **1c** would have experimental support, and report that the ^{11}B NMR and fluorescence titrations are best interpreted such that the triazole has no interaction, neither N-B bond nor solvent insertion, with the boronic acid, but rather simply plays the role of a ready synthesised linker. The reasons for this are explained based upon physical organic chemistry principles.

Results and discussion

First generation "click-fluors"

Against a backdrop of our previous report of just a single boronic acid derivative of a triazole,[13] the assembled team wished to study structural effects on boron-saccharide interactions and prepared six regioisomers of compound **1a**

(Scheme 2). Starting from commercially available boronic acid compounds, pinacol protection was performed to minimize side reactions. Moreover, free boronic acids have strong interactions with silica gel, but carrying pinacol esters through the synthetic sequence minimized problems for flash chromatography. Bromomethyl phenyl boronic acid (**3a-c**) and terminal alkyne (**6a-c**) functionalised intermediates were obtained after bromination and TMS deprotection of **2a-c** and **5a-c**, respectively. Azide derivatives for the CuAAC reaction were prepared *in situ*, and triazoles **4a-c** and **7a-c** were formed in 33-65% isolated yield.[20] Deprotection of pinacol esters was carried out by conversion to a potassium trifluoroborate salt, which was subsequently hydrolyzed to deliver the corresponding boronic acids **1a-c** and **8a-c** in good yield.[21]



Scheme 2. Synthetic routes to the six borono-regioisomers of the first generation "*click-fluor*". i) Starting from tolylboronic acids (a: *ortho*, b: *meta*, c: *para*), followed by pinacol protection, bromination, CuAAC reaction and pinacol deprotection to furnish **4a-c**; ii) Starting from [(trimethylsilyl)ethynyl]phenylboronic acids (a: *ortho*, b: *meta*, c: *para*), followed by pinacol protection, TMS deprotection, CuAAC reaction and pinacol deprotection delivering **8a-c**.

Crystals, suitable for single crystal X-ray diffraction structure determination, of compound **8a** were obtained from a methanol solution (Figure 2). The X-ray structure revealed incorporation of one methoxy group (from methanol). An intramolecular O-H···N hydrogen bond with the proximal nitrogen of the triazole ring ((N(33)-H(37) = 1.74(3) Å) was observed in a close to linear arrangement (O(36)-H(37)-N(33) = 161(2)°). It is worth noting that the torsion angle between the boronic acid group and phenyl ring is -19.4(3)° (O(36)-B(1)-C(2)-C(11)), which suggests the boronic acid group was twisted in the system to facilitate the formation of the intramolecular hydrogen bond. This crystal structure shows that in the solid state it is possible for compound **8a** to form a B-OH···N bond interaction, which is akin to solvent insertion prior to sugar binding. It can be further noted that the basicity of the N-atom acting as a hydrogen bond acceptor is enhanced in a triazole ring due to the fact that the nitrogen 2-atoms away in the ring donates electrons via resonance (a fact we will return to in regards to a discussion of Figure 5).

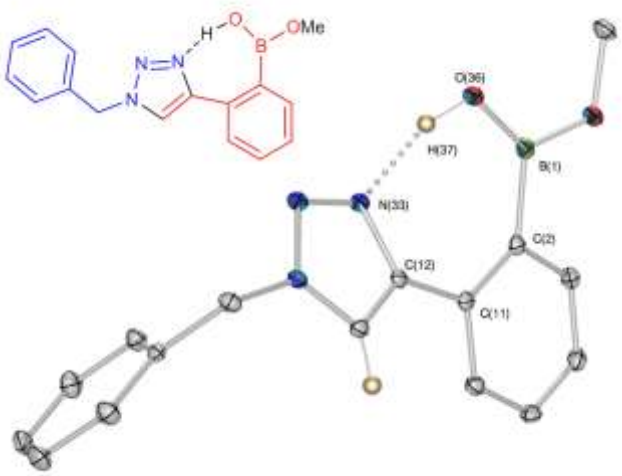


Figure 2. Molecular and X-ray structure of compound **8a** crystallised from methanol. (ORTEP ellipsoids set at 30%, selected bond length (Å), angle (°) and torsions (°): N(33)-H(37) 1.74(3); C(11)-C(2)-B(1) 129.06(14); N(33)-C(12)-C(11)-C(2) 23.20; C(12)-C(11)-C(2)-B(1) 7.65.

Compounds **1a-c** were tested as fluorescent saccharide sensors following previously reported procedures.[13] Solid D-fructose was added to a solution of given concentration boronic acid (**1a-c**) in pH 8.21 methanolic buffer. Whilst the fluorescence responses closely matched that of previously reported,[13] further study of control conditions revealed samples of fructose were contributing to the weak fluorescence observed (see ESI, Figure S1 and S2). In other words, samples of fructose appear to elicit a weak fluorescence response in the absence of any boronic acid. Indeed, in order to verify the origin of the observed signal, the experiments were repeated using different fluorimeters at more than one institution and numerous sources of fructose, including that prepared *in situ* by cleavage of sucrose. Fructose samples were examined by NMR spectroscopy and elemental analysis, and no fluorescent impurity was successfully identified from any analytical technique. Possible origins of the fluorescence include contamination by small amounts of highly fluorescent aromatic species, or n-to-π* transitions of the open-chain form of the sugars[22] (see ESI, Figure S3 and S4, Table S1 for other probed saccharides). Whilst the origin of the weak fluorescent signal of the samples of fructose is not resolved in this study, it remains an intriguing issue warranting further investigation.

Because the fluorescence responses of compounds **1a-c** to saccharides were too weak to be useful (once the background signal from saccharide samples was accounted for), an alternative approach to determine saccharide binding capability was needed. As such, isothermal titration calorimetry (ITC) was employed to determine the saccharide binding constants. ITC is an effective method for studying the binding of small molecules to large biomolecules like proteins and DNA.[23] Furthermore, it has also been used to determine the binding strength between boronic acid derivatives and diol-containing molecules.[24]

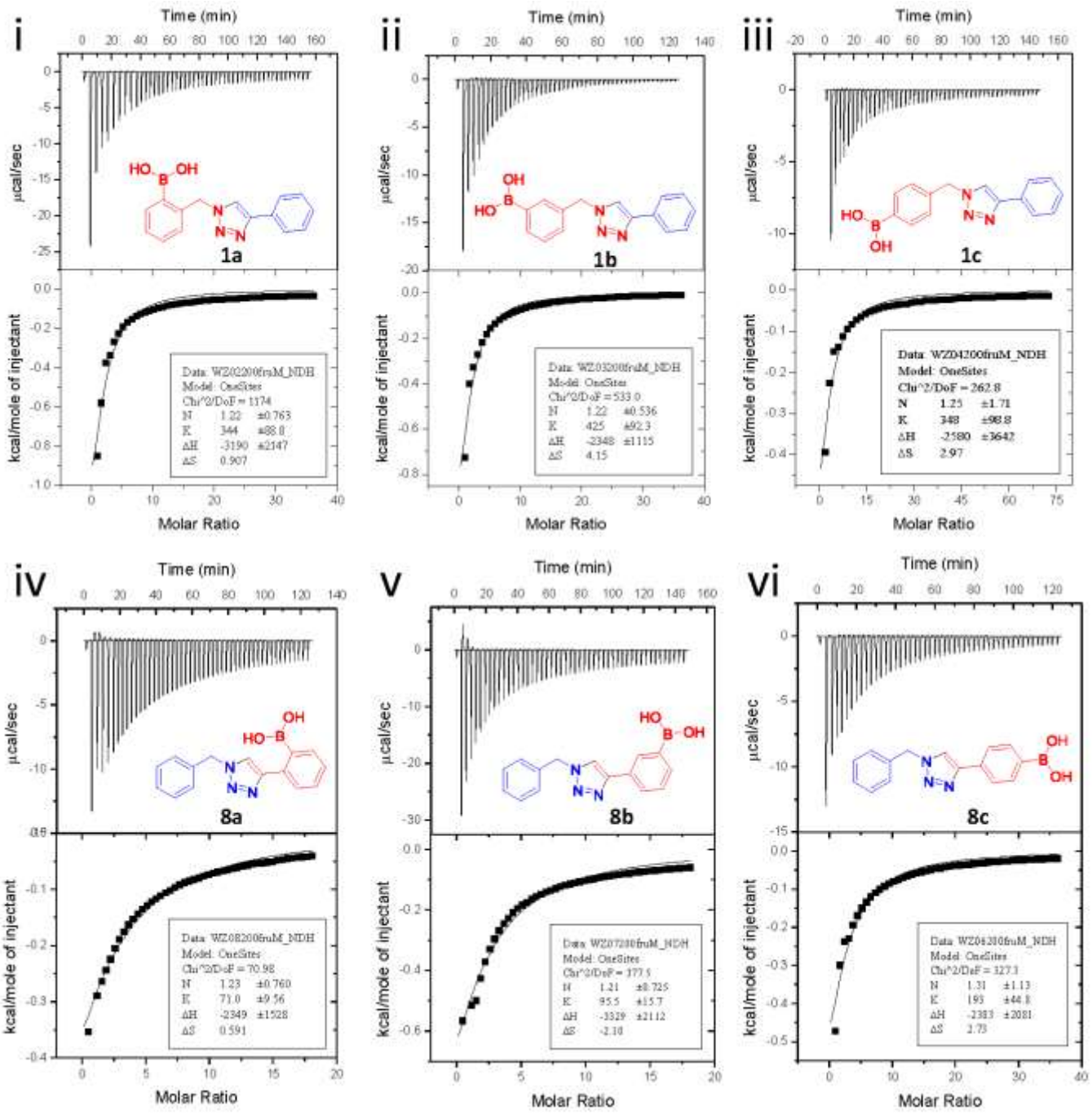
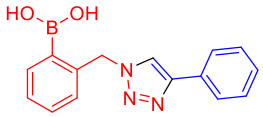
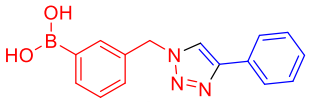
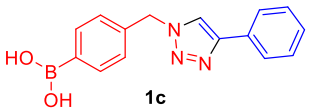
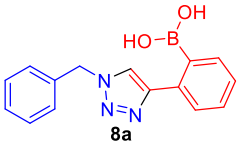
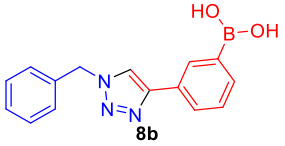
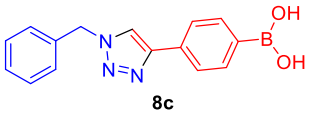


Figure 3. i-iii) ITC results of compounds **1a-c,** respectively, binding with D-fructose in pH 8.21 methanolic buffer; iv-vi) ITC results of compounds **8a-c,** respectively, binding with D-fructose in pH 8.21 methanolic buffer.

Figure 3 shows the results from titrating fructose into solutions of each of the six synthesised boronic acid compounds (**1a-c** and **8a-c**), respectively. The ITC measurements were carried out at pH 8.21 in methanolic buffer. Both the binding site information and binding constants are shown in Table 1. All boronic acid triazole receptors tested in an approximate 1:1 ratio with fructose, which agrees with the widely accepted PBA-fructose binding model.[25]

Table 1. Binding constants of compounds **1a-c** and **8a-c** with fructose determined by ITC

Entry	Compound	Binding site	Binding constants (M^{-1})
1	 **1a**	1.2	3.4×10^2
2	 **1b**	1.2	4.2×10^2
3	 **1c**	1.3	3.5×10^2
4	 **8a**	1.2	7.1×10^1
5	 **8b**	1.2	9.5×10^1
6	 **8c**	1.3	1.9×10^2

A few trends in this data are worth noting. First, in terms of binding constants, boronic acids-triazoles derived azido boronic esters, *ortho* (**1a**), *meta* (**1b**) and *para* (**1c**) (entries 1 to 3, respectively) consistently gave higher binding constants than boronic acids-triazoles derived alkyne boronic esters (entries 4 to 6), by factors between 2 and 4. Apart from that, compound **1a-c** also showed stronger binding strength towards fructose comparing with PBA (binding constant = 1.6×10^2 M^{-1}, see ESI Figure S7). We postulate this is due to an electronic factor, where the triazoles in entries 4-6 deactivate the boron to complexation *via* resonance donation from the triazole, which would reduce the electrophilicity of the boron. Second, there is little to no difference in the binding affinities between the regioisomers **1a-c** in entries 1, 2, and 3. It makes sense that **1b-c** would bind similarly, but until verified we postulated that 1a could possess some form of a N-B interaction, so a different affinity may have been expected. Similarly, there is little difference in entries 4, 5, and 6, although **8c** does bind somewhat more strongly than 8a-b.

Titrations probed by proton NMR spectroscopy have been previously utilised in the study of supramolecular interactions.[26] Herein, titrations of **1a** and **1c** with fructose were studied by ^{1}H NMR spectroscopy, following the protocol of Mulla *et al.*[19] These two compounds were selected in order to compare the effect of a potential triazole-boronic acid interaction on fructose binding. In the studies of Mulla *et al.*, it was proposed that the second triazolyl nitrogen might interact with boron through solvent insertion, which stabilises the boronic acid-diol complex. As such, compounds **1a** and **1c** were dissolved in DMSO-d_6, and a fructose solution (phosphate buffer prepared in D_2O, pD 8.21) was titrated into the boronic acid solutions. As it is shown in Figure 4i, upon addition of fructose solution in D_2O to **1a** (*ortho*), the signal of the exchangeable boronic acid OH protons (Hb) disappear due

to deuterium exchange. However, the signal of the triazole C-H proton (Ha) shifts more than 0.2 ppm downfield, indicating a deshielding effect after fructose binding.

For the experiment with **1c** (*para*) under the same condition, the spectral region of interest is given in Figure 4 ii. The triazole proton of *para* **1c** (Hc) shifts 0.1 ppm to upfield, in contrast to the 0.2 ppm downfield shift noted for *ortho* **1a**. This suggests that binding of fructose has a different influence on the triazole.[27] A downfield shift is indicative of deshielding, such as an inductive effect when the boronic acid binding an electron withdrawing saccharide. The ratio of free boronic acid to fructose bound boronic ester were determined by comparison of the integration of the triazole C-H protons in both cases (Ha *versus* Ha' and Hc *versus* Hc', Figure 4 iv). All the proton NMR spectra of this titration experiment are presented in ESI, see Figure S9 and S10.[28]

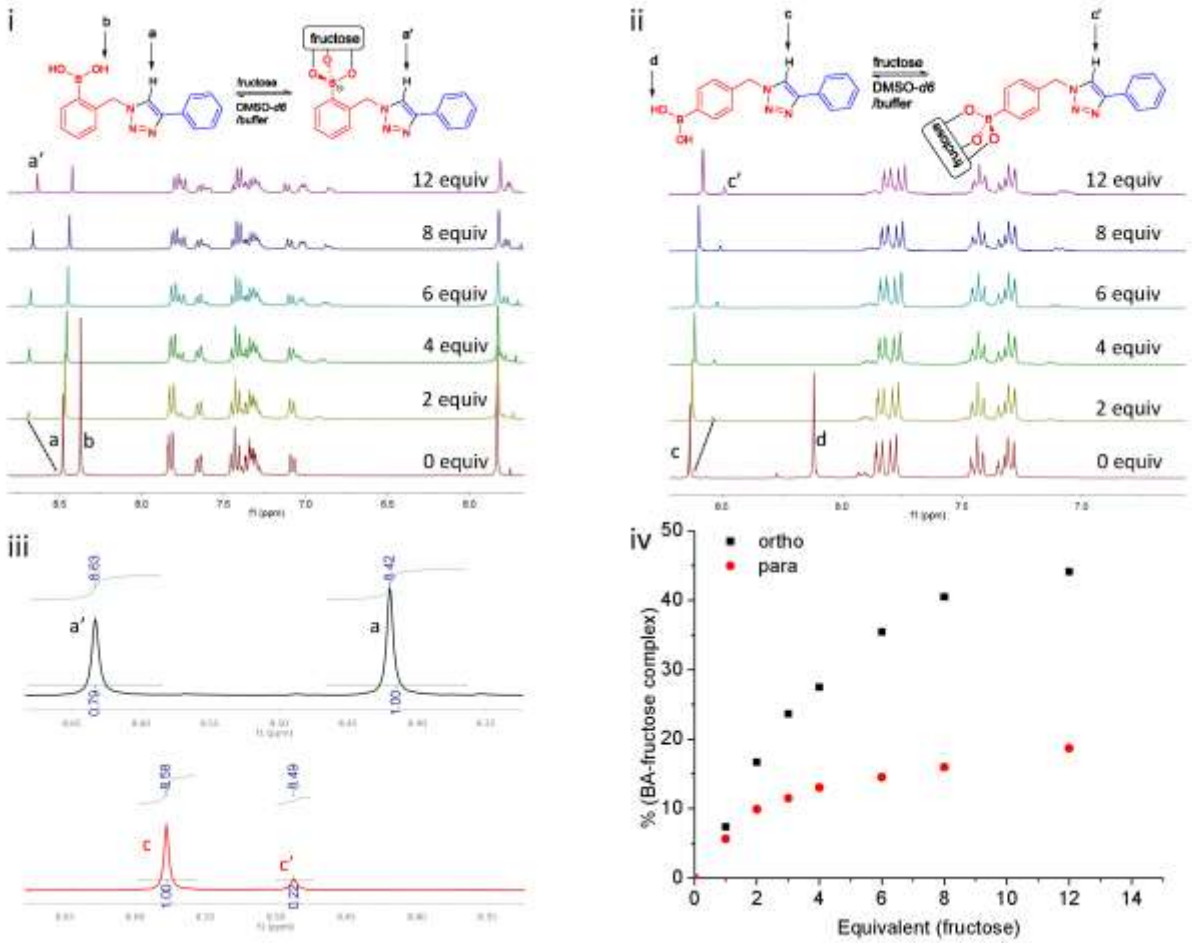


Figure 4. i) ^{1}H NMR titration of **1a** with D-fructose in DMSO-*d6* and phosphate buffer solution (pD 8.21); ii) ^{1}H NMR titration of **1c** with D-fructose in DMSO-*d6* and phosphate buffer solution (pD 8.21). ^{1}H NMR drift slightly (<0.1 ppm) due to the change of solvent composition during titration; iii) integration of triazolyl protons for compound **1a** (up) and **1c** (bottom); iv) the equivalent of fructose plotting against the percentage of BA-fructose complex in the system.

Next, ^{11}B NMR spectroscopy was used to determine whether or not an interaction between a triazole nitrogen atom and the boron atom could be modulating the binding with fructose. If a triazole nitrogen atom were to interact with the boronic acid, it could do so directly, through a N-B bond, or indirectly by inserting a solvent molecule between the nitrogen and boron atoms, or *via* an intramolecular hydrogen bond (as shown in **Figure 1**). In aprotic solvent, such as CD_3CN, the N-B interaction produces a ^{11}B NMR signal at approximately 15 ppm. In protic solvent, such as CD_3OD, a solvent could insert, which would produce a signal at approximately 10 ppm. This same chemical

shift would occur for a sugar that binds *via* three hydroxyl groups, as does fructose.[29] In comparison, a trigonal boron produces a signal at approximately 30 ppm.

The ^{11}B NMR spectra of compounds **1b-c** and **8a-c** in CD_3CN all showed one ^{11}B signal at 28-29 ppm, indicating no N-B dative bonding with the triazole nitrogen atoms takes place and that the boron atom remains trigonal (see ESI, Figure S12. Unfortunately, compound **1a** was not soluble enough in CD_3CN to obtain comparative data). In CD_3OD, all six compounds showed one signal, again at 28-29 ppm, indicating that no solvent insertion takes place between the triazole nitrogen atom and the boron atom as found when more basic nitrogen atoms are placed in the *ortho* position (see ESI, Figure S13). When fructose was added to the solutions in CD_3OD, the signal at 28-29 ppm was replaced by a new signal at 11 ppm, indicative of fructose binding and tetrahedral boron (see ESI, Figure S14). This signal can be attributed to the tridentate binding of fructose to the pyramidalised boron atom.[18] From these experiments, we concluded that there is no N-B interaction, and hence a change in such an interaction cannot be responsible for the change in fluorescence.

These results call into question the assignment of solvent insertion, as found by Mulla (Figure 1c). Yet, this experimental data makes sense based upon pK_a reasoning. The most basic nitrogen of a triazole ring is indicated in Figure 5, indicating where one would expect hydrogen bond acceptance. This site is basic due to resonance, as in imidazole, leading to hydrogen bond acceptance behaviour, as depicted in Figure 2. However, the pK_a of the conjugate acid is still only 1 to 2. Thus, neither of the triazole nitrogens would be expected to make a strong hydrogen bond accepting interaction with an inserted solvent (except in a solid state, Figure 2), nor a reasonably strong dative N-B interaction. Indeed, such interactions are not found with this most basic nitrogen. Hence, using the nitrogen with an even lower basicity seems unlikely.

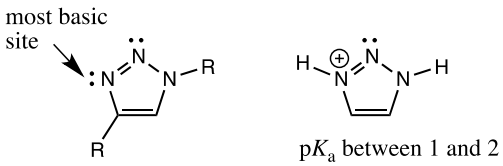


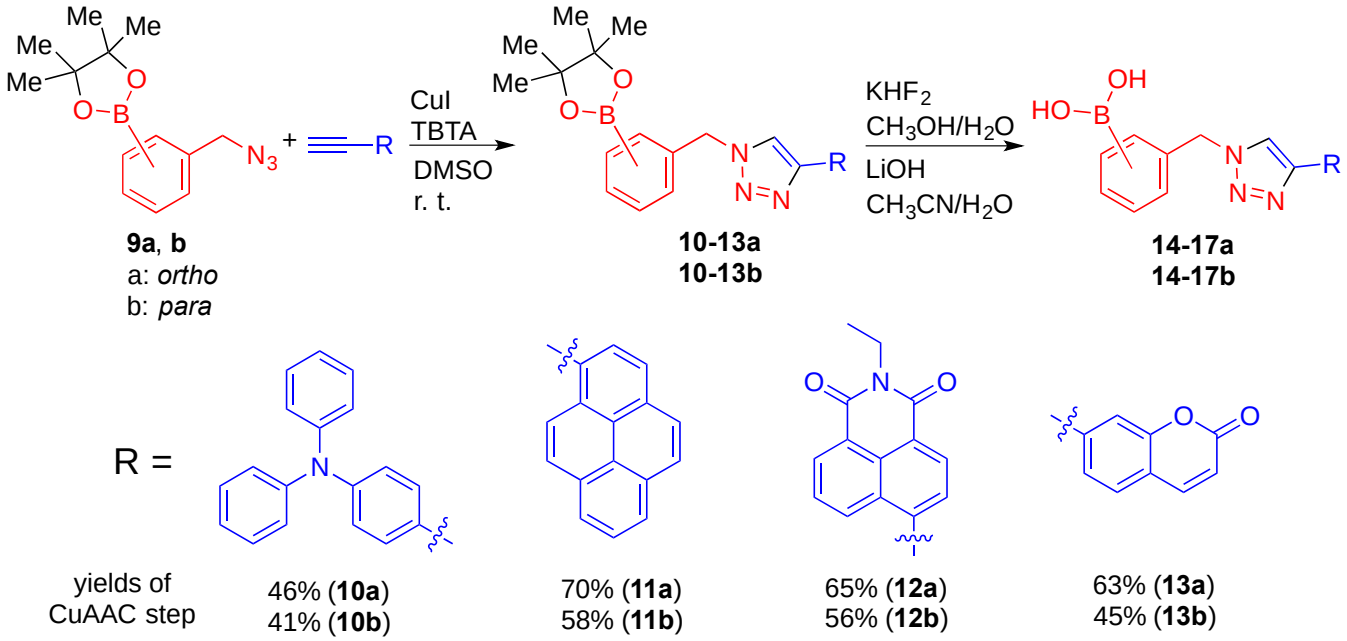
Figure 5. pK_a of the third nitrogen of triazole (left) and imidazole (right).

Second generation "click-fluors"

To rapidly assemble boronic acid receptors suitable for fluorescent sensing *via* triazole formation, with fluorescent responses significantly stronger than any residual background fluorescence from the saccharide samples noted earlier, building blocks that contain fluorophores were used. Considering that boronic acid-triazoles derived from bromomethyl benzenes (**1a-c**) showed greater saccharide binding potential by ITC (Table 1, entries 1 to 3) than the correspond methylacetylene benzenes (Table 1, entries 4 to 6), it was reasoned that superior fluorescent sensors would be derived from azides formed from methyl bromobenzenes. Noting that binding regimes of *ortho* **1a** and *para* **1c** were demonstrated to be divergent (Figure 4), fluorophore-containing versions were selected as suitable structures for further investigation. Terminal alkyne-functionalised fluorophores were readily incorporated into boronic acid-containing triazole deriviatives through CuAAC reactions. The alkyne-functionalised fluorophores were either commercially available or readily prepared *via* Sonogashira cross-coupling reactions.[30] Among them, 4-ethynyl-1,8-naphthalimide and 7-ethynylcoumarin have been reported as fluorescent tags in the area of bioorthogonal chemistry.[31] In this study, they were selected together with diphenylaniline and pyrene based on their electronic properties and scope of fluorescent signaling mechanisms accessible.

The fluorescence responses upon saccharide binding, of both electronically divergent as well as *ortho* and *para*

substituted isomers, could provide further information to understand the role of the triazoles in the boronic acid-based saccharide sensors. Therefore, eight fluorophore-containing boronic acid triazole deriviatives (**14-17**, **a** and **b** respectively) were prepared, and are named "second generation *click-fluors*" in this report.



Scheme 3. Synthetic route of second generation "*click-fluors*" with modified CuAAC reaction condition. Starting from alkyne functionalised fluorophores and (azidomethyl)phenylboronic acid pinacol esters (a: *ortho*, b: *para*), the designed "*click*-fluors" were synthesised by CuAAC reactions and pinacol deprotections in 34 to 63% overall yields.

Copper-mediated side-reactions plagued the preliminary synthetic efforts due to undesired deborylation and the formation of oxidation byproducts. Alternative synthetic methods have been developed to avoid the undesired outcomes of side reactions.[16, 32] For example, Wang *et al.* reported a method to protect the boronic acid group by adding fluoride.[33] Maison *et al.* also suggested the addition of tris(benzyltriazolylmethyl)amine (TBTA) as a ligand for copper.[34] In these studies, boronic esters with pyrene and coumarin fluorophores were synthesised with modest yields.[15b, 35] In the present work, optimisation of the reaction conditions revealed that isolation of boronic acid azide derivatives (rather than *in situ* preparation) offered significant improvements in formation of the desired products. Copper-mediated deborylation and oxidation were overcome by reducing the copper catalyst loading and adding TBTA to accelerate the CuAAC reactions.[36] As a result, complete conversion of the alkynes was achieved and yields of the CuAAC step were improved from 32 to 70% (**11a**), 34 to 65% (**12a**) and 29 to 63% (**13a**). Further work is currently underway to understand how to improve the isolated yields of this class of click reaction products.[32] Material suitable for single crystal X-ray diffraction structure determination of key intermediate **13a** was obtained (**Figure 6**) by crystallisation from a mixture of hexane and ethyl acetate. No interaction between triazole ring nitrogen atoms the and boronic ester was observed in the material crystalised from aprotic solvent. However, the distance between O(3) and H(26) is measured as 2.655(1) Å, which could be indicative of a weak hydrogen bond with triazole C-H being the hydrogen bond donor.[37]

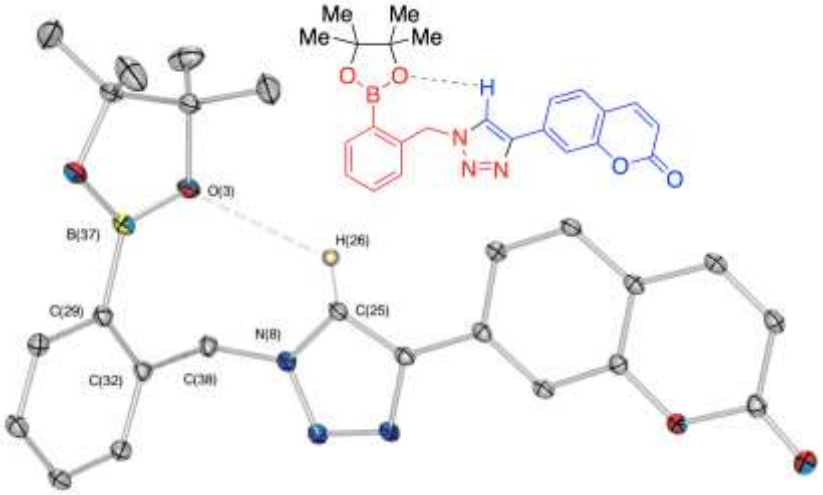


Figure 6. Chemical and X-ray crystal structure of compound **13a**. ORTEP, ellipsoids plot at 30%, selected angle: O(3)-H(26) 2.655(1) Å; selected torsion: O(3)-B(37)-C(29)-C(32) 6.833°.

Ortho- and *para*-regioisomers of **14**-**17** (**a** and **b** respectively) were synthesised from each of the four selected alkyne-appended fluorophores. The synthesised compounds were tested as fluorescent probes for fructose binding using the same protocol as described by Scrafton *et al*. The *ortho* and *para* congeners (**14a** and **14b**) gave strikingly different fluorescence responses to fructose, with *ortho* **14a** showing a stronger fluorescence recovery. A similar trend of fluorescence responses was observed with compound **17a** and **17b** under the same experimental conditions. These results agree with the ^{1}H NMR titration study, which showed divergent responses between *ortho* and *para* triazole isomers of **4** to fructose. Because there is no evidence of a hypothetical N-B interaction facilitated by saccharide binding from the ^{11}B NMR spectroscopic studies earlier, it is possible that the stronger binding strength is caused by the different pK_a between *ortho* and *para* boronic acids. With the triazole group at the *ortho* position, the electron-withdrawing capacity through induction is greater than that of the *para* isomers. As a result, the *ortho* boronic acids could have a lower pK_a, which means stronger binding strength under the experimental pH (8.21). The same fluorescence recovery was not recorded in the cases of compounds **15a**,**b** and **16a,b** (see ESI, Figure S5). This result suggests that the properties of the fluorophore also have an impact on fluorescence modulation, possibly due to the overall effect on the pK_a of the boronic acids. Differences in fluorescence quantum yields can be difficult to decipher, and thus the reason for greater recovery with the *ortho*-derivatives is unclear. However, the most common deactivation process for fluorescence is internal conversion from loose rotors, and with a triazole in the *ortho* position hydrogen bonds as observed Figure 2 and 6 with a bound tridentate fructose would restrict bond rotations within the complexes possibly leading to higher extents of emission turn-on than for other regioisomers. Clearly, more direct evidence is needed in future studies.

Furthermore, fluorescence titrations were carried out with the synthesised compounds and glucose. The anticipated low binding affinity for mono-boronic acids with glucose[5a] was also apparent in the present system as only weak responses were recorded (see ESI, Figure S6).

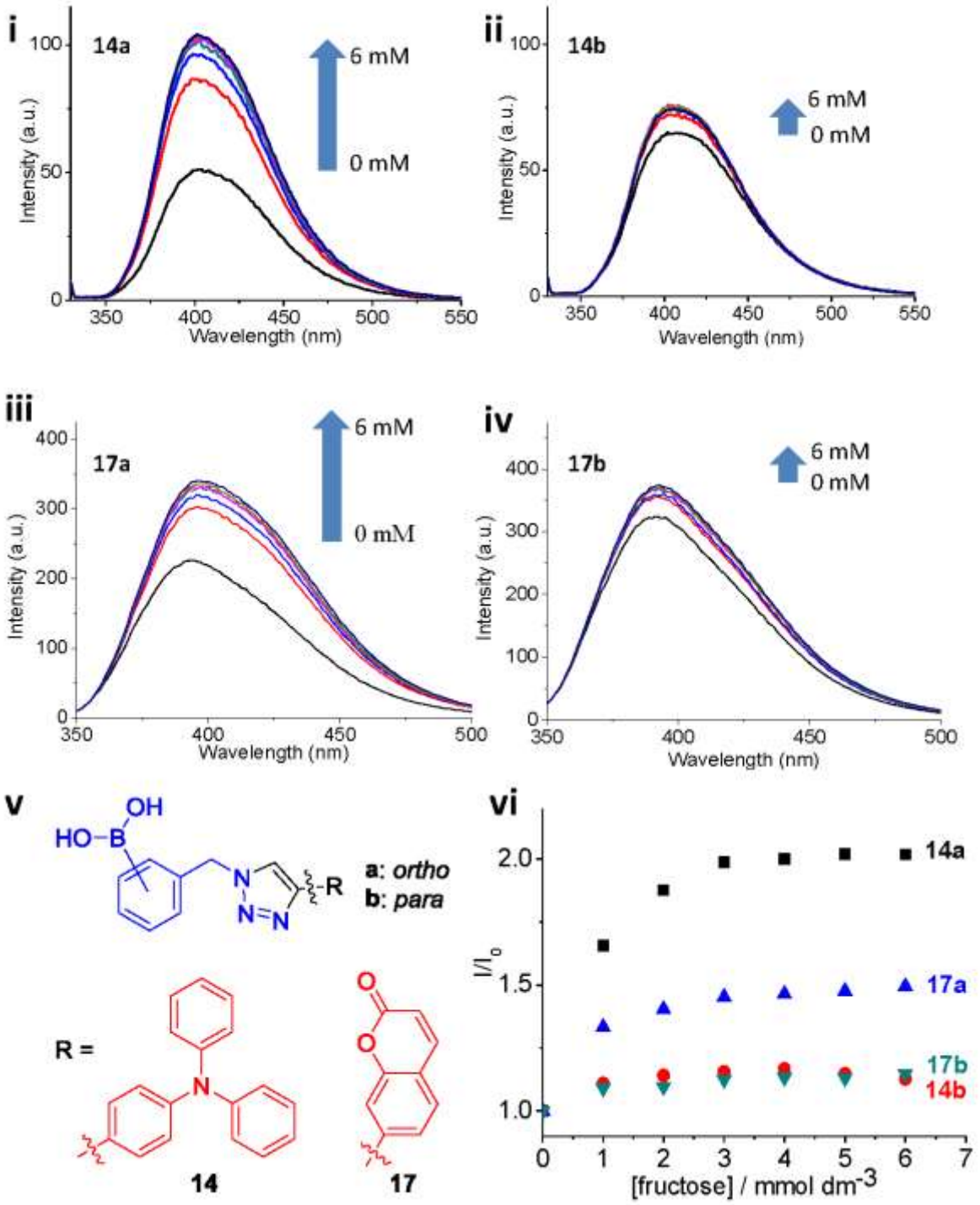


Figure 7. i) Fluorescence spectra of **14a** in the presence of increasing concentration of D-fructose (0-6 mM); ii) Fluorescence spectra of **14b** in the presence of increasing concentration of D-fructose (0-6 mM); iii) Fluorescence spectra of **17a** in the presence of increasing concentration of D-fructose (0-6 mM); iv) Fluorescence spectra of **17b** in the presence of increasing concentration of D-fructose (0-6 mM); v) chemical structures of the tested compounds; vi) plotting fluorescence enhancement of the tested compounds against the concentrations of D-fructose. Excitation wavelength: 290 nm (**14a,b**); 330 nm (**17a,b**).

Conclusion

In summary, six regioisomers of previously reported **1a** (including **1a**) were synthesised as "*click-fluors*" in this

work. ^{1}H, ^{11}B NMR titrations and ITC were employed to study the effect of triazole-boronic acid distance upon saccharide binding capacity. The result of ^{1}H NMR spectroscopy titrations suggests that the *ortho*-triazole compound has stronger fructose binding strength than the *para*-triazole isomer under the experimental conditions. However, no indication of direct N-B interaction was observed by comparing ^{11}B NMR signals of the synthesised "*click-fluors*" before and after binding with fructose. As a result, a possible hypothesis of different pK_a of the boronic acids as a result of differing triazole substitution was suggested. In the case of *ortho*-triazole compound **1a**, the adjacent triazole ring could have a stronger inductive electron-withdrawing effect comparing with the *para*-triazole isomer **1c**.

A fluorescence study was conducted following the procedure of Scrafton *et al*. For the first generation "*click-fluors*," the result suggested that the fluorescence of these molecules needed to be improved for saccharide sensing. Therefore, four alkyne-functionalised fluorophores with different electronic properties were selected for the synthesis of second generation "*click-fluors.*" Specifically, triphenylamine, coumarin, 1,8-naphthalimide and pyrene were attached on different positions of PBA through CuAAC reaction. By comparing the fluorescence response of *ortho* and *para* regioisomers upon addition of fructose, it is found that *ortho*-triazole sensors generate stronger fluorescence enhancement after binding with fructose, which could be due to the difference between the pK_a of *ortho* (**14a**, **17a**) and *para* (**14b**, **17b**) boronic acids and difference in internal conversion.

Considering the wide range and easy access of alkyne-functionalised fluorophores, it is reasonable to expect more sensor molecules with larger fluorescence responses could be created using the same strategy. Moreover, the improvement on the efficiency of CuAAC reaction with boron moieties could be beneficial to develop boronic acid functionalised materials *via* click chemistry.[38] Ongoing efforts are focused on synthesising and studying multi-boronic acid compounds using the same strategy.

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Contributions

WZ participated in all aspects, performed the synthesis, fluorescence studies and ITC experiments and co-wrote the manuscript. JSF conceived the project, oversaw all aspects and co-wrote the manuscript. BMC designed and carried out the NMR titration experiments with EVA, who both contributed to writing aspects of the manuscript. SAH synthesised one compound of the report prior to the main work being carried out. AY carried out XRD experiments, Louise Male is thanked for assistance with XRD experiments. JSF, EVA and TDJ discussed the manuscript and contributed to the interpretation of the results. All co-authors commented on the manuscript.

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