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Highly sensitive detection of saccharides under physiological conditions with click synthesized boronic acid-oligomer fluorophores†

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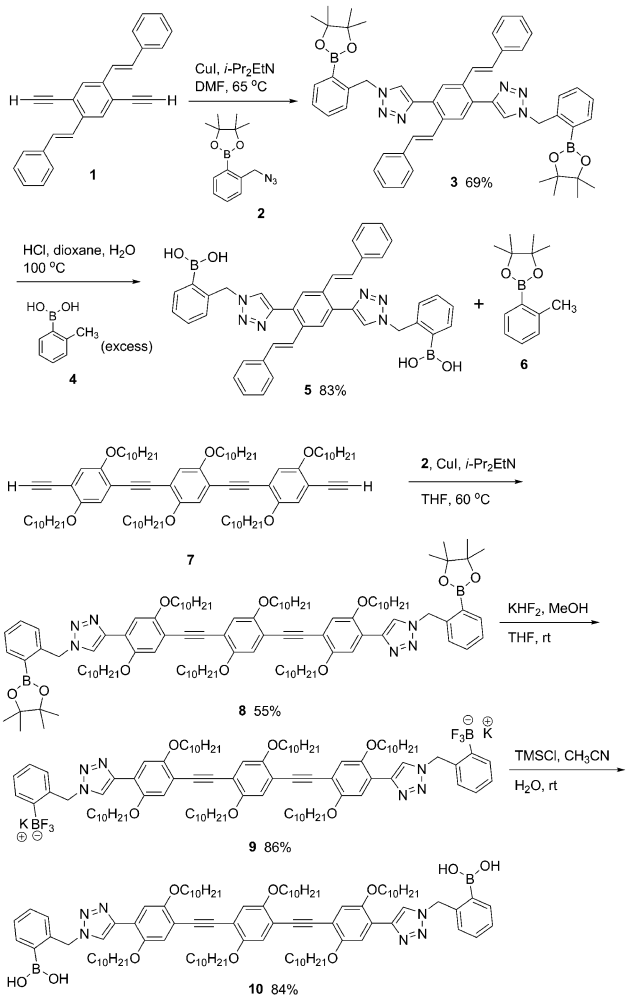
Two phenylboronic acid based saccharide sensors bearing conjugated oligomer fluorophores with linear and cruciform π-frameworks were synthesized in a modular approach utilizing a Cu-catalyzed alkyne azide cycloaddition (click) reaction. The cruciform fluorophore showed excellent saccharide sensing function under physiological conditions in the mM range, whereas the linear fluorophore gave very limited sensing functions. The different fluorescent sensing behaviours highlight the important role of oligomer fluorophore in the development of effective saccharide sensors.

Detection and recognition of saccharides under physiological conditions and monitoring the concentration of saccharides *in vivo* are challenging tasks that require sustained efforts to be dedicated to development and refinement of new sensor systems. At present, boronic acid receptors have been extensively used in conjunction with various fluorogenic groups to form functional fluorescent sensors for saccharides.[1] In this context, the sensor design has been recently focused on tuning the receptor, donor/ligand, and linkage groups in order to strengthen boronic acid-saccharide binding at neutral pH, to enhance photoinduced electron/energy transfer for effective signaling, and to introduce cooperativity for binding with specific saccharides.[2] On the other hand, the fluorophore component acting primarily as the "read-out" unit of the sensor can significantly influence solvation, steric hindrance of the binding site, and polarity matching with saccharides. Hence, the fluorophore effect also has key impact on the efficiency and selectivity of fluorescent sensors.[2g]

In the current literature, the fluorophores utilized for constructing saccharide sensors are mostly polyaromatic-based fluorogenic groups such as napthalene, anthracene, and diazobenzene.[1,2] Conjugated oligomers and polymers, owing to the excellent controllability and tunability of their optoelectronic properties, have found wide-ranging applications in fluorescent sensory devices.[3] However, fluorescent saccharide sensors based on π-conjugated oligomer fluorophores have not been investigated, despite their excellent photophysical properties. To address this issue, we have recently designed and synthesized two boronic acid attached oligomers (**5** and **10**, Scheme 1) as models for a qualitative understanding of the basic structure-property relationships for oligomer-based fluorescent saccharide sensors. Note that the π-frameworks of the two oligomer fluorophores are designed in two distinct motifs, cruciform and linear. As such, the effect of



Scheme 1 Click synthesis of boronic acid appended cruciform and linear conjugated oligomers.

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oligomer fluorophore on sensor properties can be readily probed by way of comparative studies.

The synthesis of the two fluorophores **5** and **10** (henceforth referred to as cruciform and linear respectively in the following discussions) was carried out *via* a modular strategy using a popular click reaction, namely Cu-catalyzed alkyne azide cycloaddition (CuAAC),[4] as the key synthetic step.

As shown in Scheme 1, the synthesis of cruciform **5** began with a CuAAC reaction between an azido-pendant phenylboronate **2**[5] and an alkynylated phenylene vinylene oligomer **1**[6] in the presence of CuI and *i*-Pr_2EtN in DMF at 65 °C. Compared with numerous high-yielding CuAAC reactions reported in the literature, the yield of this click reaction was only at a moderate level (69%). The reduced efficiency of reaction is likely due to some side reactions involving the insertion of Cu into the C–B bonds.[7] The boronate groups of intermediate **3** was then hydrolyzed *via* a transesterification reaction between boronate **3** and excess *o*-tolylboronic acid (**4**) in dioxane/H_2O to give boronic acid-cruciform **5** along with a tolylboronate byproduct **6**. It should be noted that compound **6** serves a useful precursor for the preparation of azido-phenylboronate **2**. From a practical standpoint, this hydrolysis approach can be viewed as synthetically economical.

In a similar manner, linear oligomer **7** was coupled with azide **2** through a click reaction, yielding boronate intermediate **8** in 55% yield. For the hydrolysis of **8**, however, the transesterification method was problematic due to the formation of some intractable impurities. To circumvent the purification difficulty, a stepwise hydrolysis route was then used, in which the boronate was first converted into trifluoroborate with KHF_2 and then hydrolyzed into boronic acid in the presence of trimethylsilyl chloride (TMSCl).[8] This method led to the desired product **10** in a very good yield and satisfactory purity.

The modular synthesis of **5** and **10** clearly demonstrates the power of click chemistry for rapid generation and screening of boron-based organic functional fluorophores. It is noteworthy that there has been a surging interest in using click synthesis to prepare fluorogenic compounds for optical sensing and imaging purposes in recent years.[5,9] Particularly worth mentioning is that Fossey and James recently devised a "click-fluor",[5,10] the structure of which comprised of a phenyl group and an *o*-methylphenylboronic acid moiety connected through a 1,2,3-triazolyl linker through click chemistry. The "click-fluor"[5] has shown reasonable fluorescent sensing functions towards saccharides in an alkaline aqueous medium (pH 8.21) requiring the presence of an organic co-solvent (52% wt MeOH in H_2O), while the concentrations of saccharides to be detected were in the range of *ca.* 10^{-2} to 1 M.

In our work, we tested the fluorescent sensing functions of cruciform **5** and linear **10** for four selected saccharides, D-fructose, D-ribose, D-galactose, and D-glucose, under physiological conditions. Despite the limited solubility of fluorophores **5** and **10** in water, dissolution of them in a neutral (pH 7.41) aqueous potassium phosphate buffer solution with concentration (10^{-5} M) suited for fluorescence spectroscopic measurements was readily attained with the aid of a minimal amount of DMSO (H_2O/DMSO > 99 : 1, v/v). The fluorescence quantum yields (Φ) of **5** and **10** determined in aqueous phosphate buffer solutions are 7.5% and 6.3% respectively.

Table 1 Stability constants (K) for complexation of cruciform **5** with various saccharides determined by global spectral fitting

Saccharide	log K_1 (M^{-1})	log K_1K_2 (M^{-2})
D-fructose	4.02 ± 0.5	7.27 ± 0.5
D-ribose	3.24 ± 0.2	6.09 ± 0.1
D-galactose	3.87 ± 0.2	6.26 ± 0.1
D-glucose	2.53 ± 0.3	4.21 ± 0.4

For the cruciform sensor **5**, significant fluorescence enhancement (turn-on sensing) was observed during the titrations with saccharides as shown in Fig. 1A–D. Titration of the four saccharides resulted in similar trends of spectral changes with increasing titration. The emission spectrum of cruciform **5** is composed of a peak at 430 nm and a shoulder at 403 nm. The two bands become more distinctive as the titration progresses, and the emission profile at the ending point of titration shows vibronic structures resembling those of the emission spectrum of cruciform boronate **3**, which is indicative of complexation of boronic acid with saccharides. The affinities of **5** for saccharides vary to a large extent as manifested by the plots in Fig. 1E, which show the correlation between fluorescence enhancement (F/F_0) at 430 nm and saccharide concentration. It can be clearly seen that the selectivity of cruciform **5** for different saccharides is in the order of: D-fructose > D-ribose ~ D-galactose > D-glucose. Also of remark in Fig. 1E is the high sensitivity of **5** for D-fructose and D-ribose in the range of 10^{-3} to 10^{-4} M. Such a performance renders cruciform **5** at least comparable if not superior to those water-soluble fluorescent sensor systems reported in the recent literature with high saccharide sensitivity under physiological condition.[11]

To gain a quantitative understanding of the mechanisms for complexation between sensor **5** and various saccharides, global spectral fitting analysis was undertaken using the modified Marquardt-Levenberg algorithm implemented in the *SPECFIT* software package.[12] Table 1 lists the stability constants (K) determined by global spectral fitting.

From Table 1 it can be seen that all the four saccharides bind to cruciform **5** in a two-step mechanism. The spatial arrangement of the two boronic acid receptors in **5** does not facilitate any "pincer-like" binding mode with saccharides. D-Fructose shows the strongest binding strength with **5** which is about two orders of magnitude greater than that of D-glucose. The binding constants for D-ribose and D-galactose are similar and stay in the middle range among the four saccharides. The quantitative measurements are in agreement with the trend of saccharide sensitivity revealed in Fig. 1E.

The complexation of phenylboronic acid with saccharides (polyols) is reversible and pH dependent,[1a,b] while the formation of stable phenylboronic acid and saccharide complex at neutral pH usually requires stabilization effect by some ligand groups such as in the case of the widely known "Wulff-type" receptor (*o*-dialkylaminomethylphenylboronic acid).[13] From the fluorescence titration experiments, it is evident that the boronic acid groups in cruciform **5** are effectively bound to saccharides under physiological conditions. This result suggests that the 1,2,3-triazolyl moiety resulting from the click reaction not only acts as a linker group, but might play an important role in saccharide binding. To gain a deeper insight into this aspect, ^{1}H NMR titration of **5** with D-fructose was then performed. For solubility reason, the

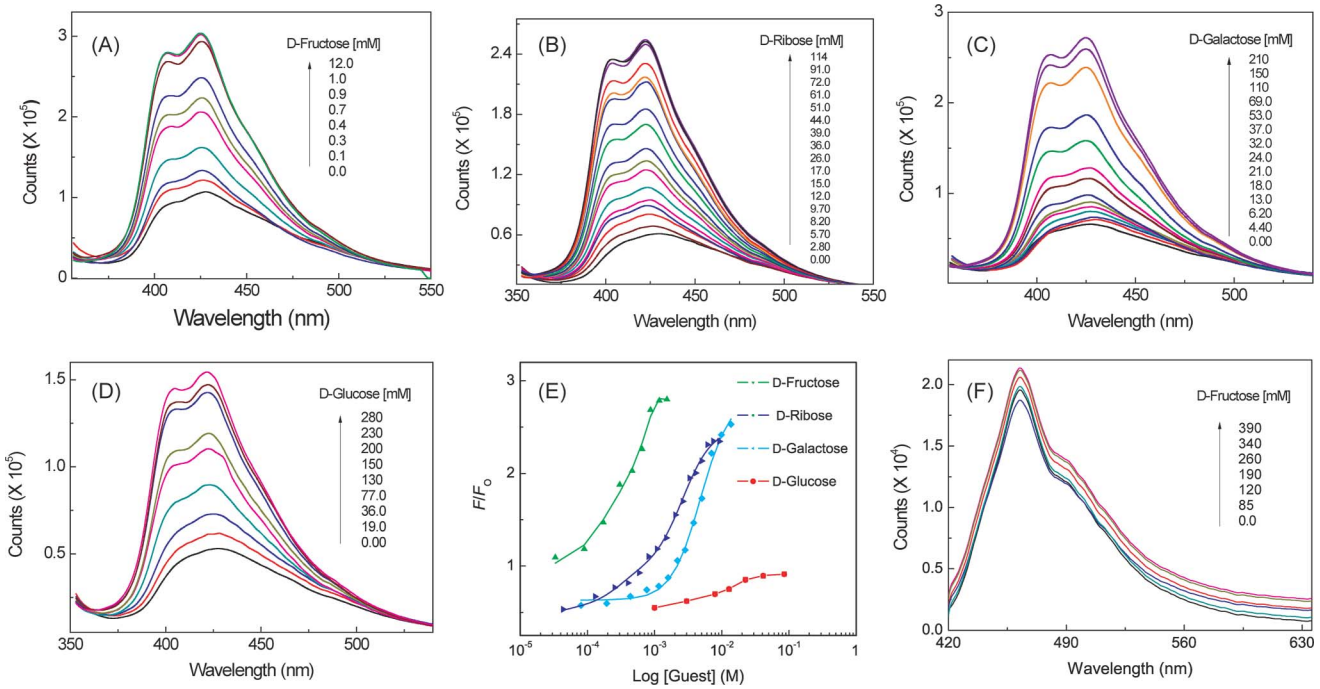

Fig. 1 (A) Fluorescence titration of **5** (11.8 μM) with D-fructose in an aqueous buffer solution (pH 7.41) at 298 ± 3 K (λ_{ex} = 340 nm). (B) Fluorescence titration of **5** (13.8 μM) with D-ribose in an aqueous buffer solution (pH 7.41) at 298 ± 3 K (λ_{ex} = 340 nm). (C) Fluorescence titration of **5** (13.8 μM) with D-galactose in an aqueous buffer solution (pH 7.41) at 298 ± 3 K (λ_{ex} = 340 nm). (D) Fluorescence titration of **5** (12.0 μM) with D-glucose in an aqueous buffer solution (pH 7.41) at 298 ± 3 K (λ_{ex} = 340 nm). (E) Plots of fluorescence enhancement (F/F_o, λ = 430 nm) against saccharide concentrations with fittings extracted from *SPECFIT*. (F) Fluorescence titration of **10** (5.8 μM) with D-fructose in a basic aqueous buffer solution (pH 8.21) at 298 ± 3 K (λ_{ex} = 340 nm).

experiments were conducted by adding aliquots of D-fructose dissolved in a phosphate/D_2O buffer solution at pD 7.41 to a DMSO-d_6 solution of **5**. Detailed NMR titration results are delineated in Fig. 2A.

Although the change of solvent system from water to DMSO may lead to considerable variation of binding constants, the binding motif however should not be changed as much. As shown in Fig. 2A, the signal of boronic acid protons (Hb) vanishes gradually with increasing titration of D-fructose, while the signals of methylene and aromatic protons (Hc and Hd) show a slight upfield shift by less than 0.1 ppm. These observations also corroborate the transformation of boronic acid to boronate ester in binding to saccharides. Of particular note is that the triazolyl proton (Ha) dramatically shifts to downfield by more than 0.2 ppm. This result indicates that the triazolyl group experiences a pronounced change of chemical environment in binding with saccharides, likely induced by solvation or hydrogen bonding effect. A water insertion binding motif is therefore proposed based on density functional theory (DFT) calculations. According to the model system illustrated in Fig. 3, in binding with a 1,2-diol the triazolyl unit adjacent to the phenylboronic acid group acts as a ligand (hydrogen bond acceptor) to coordinate with a molecule of water. This water insertion model is analogous to the binding motif of the "Wulff-type" boronic acid receptor in aqueous media. Energetically, it provides stabilization to the boron-diol complex by $\Delta H = -11.2$ kcal mol^{-1}, which well accounts for the significant binding of cruciform **5** with various saccharide in neutral aqueous solutions.

Fluorescence titrations of linear sensor **10** with various saccharides in neutral aqueous solutions were also carried out using the same protocol as that for cruciform **5**. In a sharp contrast, the emission spectrum of **10** did show any changes during the titrations. Increasing the basicity of solution did not improve the sensing function significantly. For instance, addition of D-fructose to **10** in a basic buffer solution at pH 8.21 resulted in only a slight drifting of spectral baseline (Fig. 1F). Given that boronic acid tends to strongly complex with saccharides under basic conditions, the poor saccharide sensing function displayed by sensor **10** can be reasonably ascribed to an inert response of the linear OPE fluorophore to saccharide binding events. The marked difference between cruciform **5** and linear **10** in fluorescent sensing for saccharides thus underscores that *the nature of fluorophore is a determinative factor controlling sensory performance*. For the remarkable fluorescence transduction properties exhibited by cruciform **5**, neither photoinduced electron transfer (PeT) nor intramolecular charge transfer (ICT) mechanism offers meaningful interpretation. Instead, the sensing mechanism can be rationalized from a standpoint of aggregation-modulated emission. Recent studies have disclosed that the aggregation state of OPVs in solution can exert very significant impact on their excited-state dynamics and emission yields.[14] To shed light on this point, we further analyzed the aggregation behaviour of cruciform **5** without and with binding to saccharides. Aliquots taken from the solution prepared for fluorescence titration of **5** with D-fructose were spin-cast on freshly cleaved mica surfaces for atomic force microscopic (AFM) imaging. The aggregates

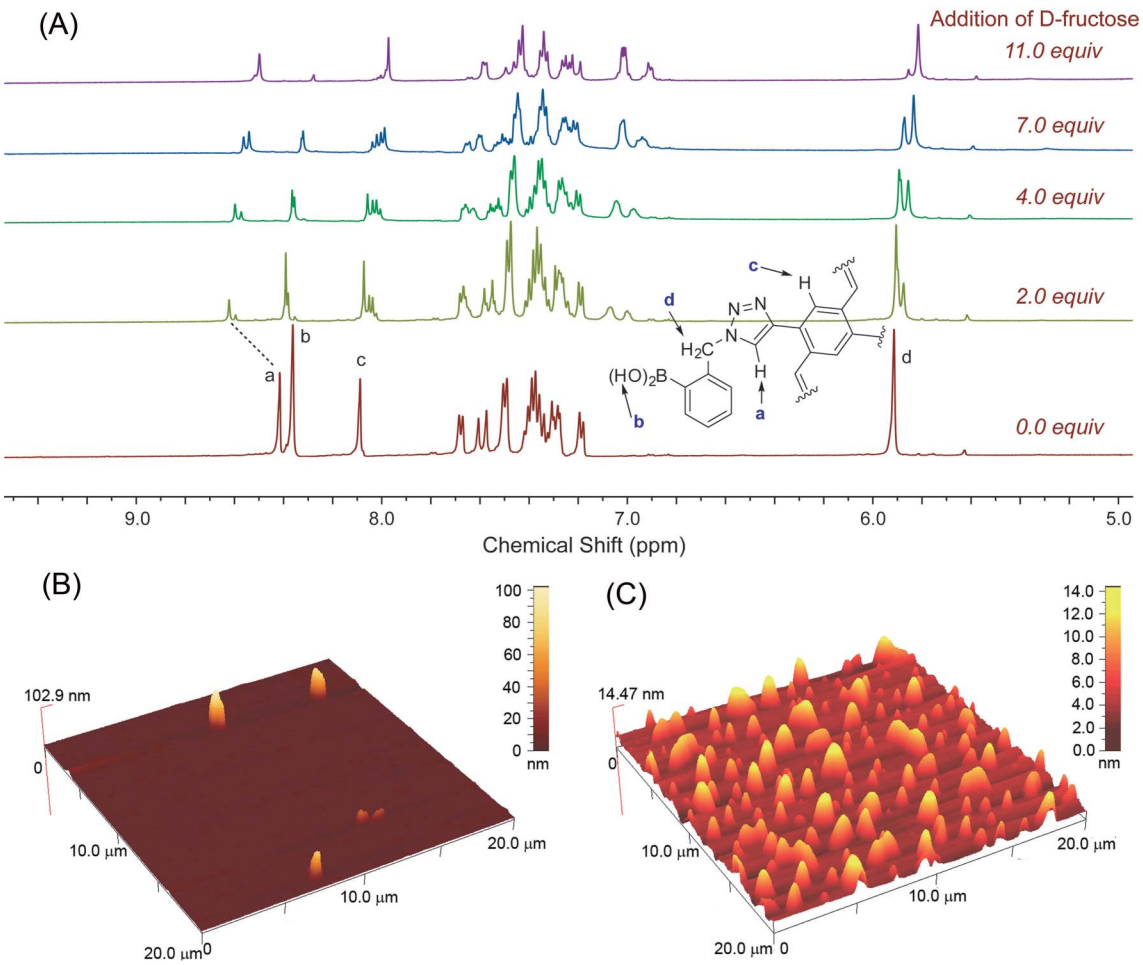

Fig. 2 (A) ^{1}H NMR titration of **5** with D-fructose in DMSO-d_6 and phosphate buffer solution (pD 7.41) at 298 ± 3 K. (B) AFM image (tapping mode) of aggregates of **5** on mica. (C) AFM image (tapping mode) of aggregates of **5** and D-fructose on mica.

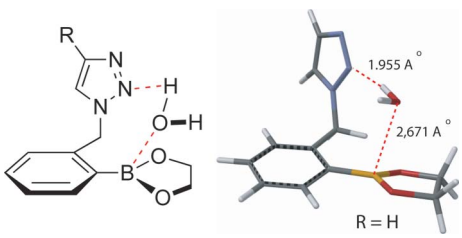

Fig. 3 DFT (B3LYP/6-31G*) optimized structure for the complex of *o*-triazolylmethylphenylboronic acid with ethylene glycol in the presence of water.

of cruciform **5** prior to complexation with D-fructose are in a size range of 60 to 100 nm (see Fig. 2B). After complexation with D-fructose, much smaller (6–14 nm) aggregates are formed (Fig. 2C). Clearly, the binding of cruciform **5** with saccharides results in considerably reduced aggregate sizes in solution, and it is reasonable to believe that the aggregation effect plays a crucial role in the fluorescence enhancement observed in the titrations of cruciform **5** with various saccharides. Two types of rationalization can be conceived at this juncture. First, the complexation with saccharides leads to increased de-aggregation/solvation of cruciform **5** to suppress fluorescence self-quenching.[15] On the other hand, the possibility that saccharide binding induces more planar OPV orientation in the aggregates of **5** may also offer a sound explanation for the observed fluorescence enhancement. The latter argument is in line with the aggregation-induced emission (AIE) for some π-conjugated systems.[16] Further investigations to debunk the detailed photophysical mechanism using dynamic light scattering (DLS) and time-resolved fluorescence spectroscopic techniques are underway, and the results will be disclosed in due course.

In summary, we have prepared conjugated oligomer-boronic acid hybrids as fluorescent saccharide sensors using an efficient and modular synthetic strategy. The cruciform sensor **5** detects saccharides with high sensitivity under physiological conditions, which arises from two major factors: (i) the triazole linker acts as a hydrogen bond donor to stabilize boron-saccharide complex at neutral pH, and (ii) the OPV fluorophore shows sensitive fluorescence responses to the aggregation state in solution which is modulated by saccharide binding. Our study highlights the importance of tuning and manipulation of the "fluorophore parameter" to improve the performances of oligomer-based fluorescent chemo- and bio-sensors.

Acknowledgements

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