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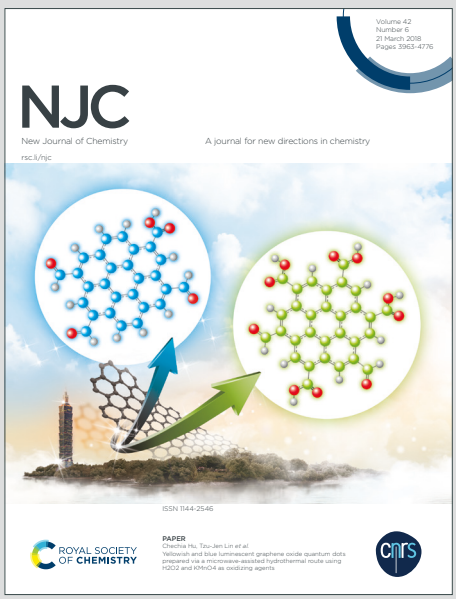

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Water-soluble diboronic acid-based fluorescent sensors recognizing D-sorbitol

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Herein, several boronic acid-based sensors were reported selectively recognizing D-sorbitol in aqueous solution. The fluorescence of sensor **15c** was enhanced by 1.5-folds when combined with a low concentration of D-sorbitol (8.88 μM), and the fluorescence enhanced by 0.6-folds after binding to D-fructose, while not changed obviously when combined with other carbohydrates, including D-arabinose, D-galactose, D-mannose, D-ribose, D-maltose, D-xylose, D-glucose and D-glucosamine. Fluorescence tests were carried out in DMSO/PBS (pH 9, 0.1M) solution (1:99, v/v), at room temperature, which indicated sensors are water-soluble. In addition, competition studies showed that sensor **15c** works well as a D-sorbitol-specific fluorescence sensor in both the absence and presence of carbohydrates interferent. And sensor **15c** has a high binding constant (10922±776 M^{-1}) and a low limit of detection (6.91×10^{-7} M) in pH 9 among investigated sensors toward D-sorbitol. Furthermore, sensor **15c** was applied to the analysis in real samples (D-sorbitol for oral administration). These studies indicated that sensor **15c** may serve as a potential tool to detect the content of D-sorbitol in drug and food products.

Introduction

D-sorbitol was first discovered by Joseph Boussingault and isolated from the fresh juice of the mountain ash berries in 1872.[1] Subsequently, D-sorbitol has been widely applied as thickeners and sweeteners in food industry, such as sugar-free food products intended for diabetics, starting material for Vitamin C synthesis, and so on.[2] Owing to poor absorption, D-sorbitol is degraded by colonic bacteria to low molecular-weight acids that increase stool acidity and osmolarity, stimulating colonic peristalsis.[3-6] Therefore, D-sorbitol is often used in hospitals as one of the osmotic laxatives. Furthermore, D-sorbitol has the characteristics of reducing ocular hypertension and high intracranial pressure.[7] Some D-sorbitol prescription drugs are often utilized, such as D-sorbitol eye drops for the treatment of glaucoma,[8, 9] D-sorbitol injections for reducing intracranial pressure,[10-12] *etc*. Considering widely used context, high concentration of D-sorbitol *in vivo* is considered to be one biomarker of diabetic complications.[2, 13-15] Several traditional methods including enzymatic or chromatographic (GC-MS, HPLC, *etc.*) have been reported previously for the assay of D-sorbitol in the diagnosis of diabetes complications.[2, 14-16] However, these methods still have some limitations such as bulky expensive and elaborate instrumentation, tedious and time-consuming, *etc*. Hence, simple, low-cost and relatively accurate D-sorbitol sensors are urgently needed. Owing to boronic acids having the unique ability to bind 1,2- or 1,3-diols in aqueous media, boronic acid-based fluorescent sensors may provide some advantages for detecting D-sorbitol, including high sensitivity, quick response, *etc*. Although some boronic acid-based sensors for sugar alcohol determination have been developed over the past decade,[2, 17-20] there are still not meeting the requirement to detect D-sorbitol in daily life.

Herein, we reported the synthesis and fluorescent spectral studies of water-soluble boronic acid-based sensors for recognizing D-sorbitol. Owing to simple synthesis, good sensitivity and selectivity, sensor **15c** may serve as a potential tool to detect the content of D-sorbitol in drug, even for the early diagnosis of diabetic complications *via* detecting content of D-sorbitol in body fluid.

Owing to the large aromatic conjugated system in structure, the 2-aryl-quinoline-4-carboxy derivatives have good fluorescence properties.[21-23] The synthesis method of 2-(4-boronophenyl)quinoline-4-carboxylic acid and its diboronic acid derivatives have been reported by our research group.[24, 25] Due to the previous synthetic route of diboronic acid compounds that used irritating $SOCl_2$ as acid chloride agent and not easily removed DMF as solvent, more green and simple synthetic methods need to continues to explore. Compared to using DCC and HOBT to activate the carboxyl group, we found that the use of DMT-MM as a

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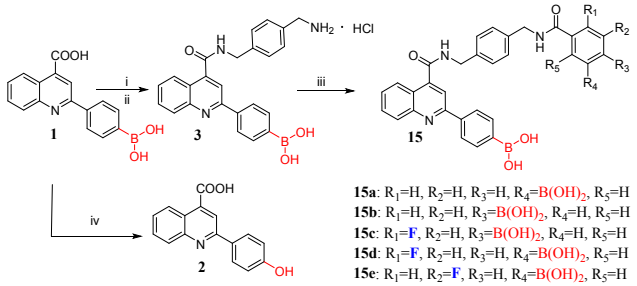
condensing agent achieved fewer by-products. Owing to the use of DMT-MM in a two-step amide condensation reaction carried out in scheme 1, the improved route completely avoided the use of $SOCl_2$. Moreover, MeOH was used instead of DMF as the solvent, which was easily removed by concentration under reduced pressure after the reaction was completed. The reaction was carried out under mild conditions (at room temperature), therefore, the synthesis method was more environmentally friendly than the previous synthesis method.[26]



Scheme **1** Synthetic route of sensor **2** and **15**. (i) 1-(N-Boc-aminomethyl)-4-(aminomethyl)benzene, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium, rt, 16 h, 66%. (ii) HCl, rt, 4 h. (iii) Benzeneboronic acid, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium, triethylamine, rt, 16 h, 40-60%. (iv) 30% H_2O_2, rt, 4 h, 74%.

Results and discussion

A sensor stock solution (1×10^{-3} M) was prepared in DMSO and 100 µL of the stock solution was diluted to 10 mL using 0.1 M phosphate buffer (pH 9), obtaining blank sensor solution (1×10^{-5} M). Then a carbohydrate-sensor stock solution (1.5×10^{-4} M) was prepared by the blank sensor solution. And 1 mL of the sensor (1×10^{-5} M) with different carbohydrates concentration (0 to 1.3×10^{-4} M) was prepared by adding different volumes of the carbohydrate-sensor stock solution. Record the fluorescence spectrum. The UV–vis absorption spectra of the sensors were recorded in DMSO/H_2O (1:99, v/v), as shown in the Fig. S1. The excitation wavelength of boronic acid sensors was set at 337 nm (slit: 5 nm/5 nm) and diboronic acid sensors have a maximum fluorescence emission intensity at around 418 nm (Fig. S10). The excitation wavelength of sensor **2** was set at 345 nm and sensor **2** have a maximum fluorescence emission intensity at around 450 nm (Fig. S8).

Fluorescence properties

To investigate the fluorescent binding affinities of compounds **15a-e** to various carbohydrates, we carried out a series of fluorescence activity studies, and found that the sensors have a significant fluorescence response to D-sorbitol (Fig. S2-7). Interestingly, sensor **15c** has a high binding constant (10922±776 M^{-1}) and a low limit of detection (LOD) in pH 9 (6.91×10^{-7} M) among tested sensors toward D-sorbitol, as shown in Fig. S5. However, it was found that sensor **15a**, **15b**, and **15e** have similar binding constants and LOD compared to sensor **15c**. However, the binding constant and LOD of sensor **15d** was close to that of monosensor **1** (Table 1). It may be ascribed to the *para* electron withdrawing induction effect of the fluorine atom on the phenylboronic acid ring, resulting in that the binding ability of the boronic acid functional group to the *ortho*-dihydroxy group of D-sorbitol is weakened. Therefore, the overall binding ability is slightly stronger than that of monosensor **1**, but it is significantly lower than other diboronic acid sensors without *para* electron withdrawing induction effect of the fluorine atom on the phenylboronic acid ring.

Table 1 Key information of sensors combining with of D-sorbitol.

Sensors	$(I-I_0)/I_0$	LOD[a] (M)	K_{eq}[b] (M^{-1})
1	8.26	1.53×10^{-6}	4598±776
2	0.15	-	-
15a	10.21	8.14×10^{-7}	8886±395
15b	9.89	7.64×10^{-7}	9396±556
15c	10.52	6.91×10^{-7}	10922±776
15d	8.34	1.22×10^{-6}	5148±725
15e	9.76	5.48×10^{-7}	8828±1145

[a] The value was calculated by $3\delta/S$ (R^2>0.99) (Fig. S2B-7B); [b] The value was calculated by Benesi–Hildebrand equation based on three times of measurement (R^2>0.99) (Fig. S2C-7C).

When the low concentration of different carbohydrates (8.88 µM) were added, respectively, the amplitude changes of the fluorescence enhancement of sensors **1**, **2** and **15c** were as shown in the following Fig. 1. D-sorbitol caused the most significant change in the various carbohydrate-sensor mixtures, and followed by D-fructose. The fluorescence of sensor **15c** was enhanced by 1.5-folds when combined with a low concentration of D-sorbitol (8.88 µM), and the fluorescence enhanced by 0.6-folds after binding to D-fructose, while not changed obviously when combined with other carbohydrates, including D-arabinose, D-galactose, D-mannose, D-ribose, D-maltose, D-xylose, D-glucose and D-glucosamine. The sensor **2** without boronic acid group had almost no fluorescence respond to the test carbohydrates (Fig. S8). The monoboronic acid sensor **1** was enhanced by 1.1-folds after binding to D-sorbitol. Furthermore, the fluorescence enhancement of monoboronic acid sensor **1** caused by D-sorbitol was 2-folds of D-fructose, while diboronic acid sensor **15c** increased to 2.2-folds. These studies indicate that boronic acid groups play a key role in recognizing carbohydrates. Sensor **1** and **15c** can selectively recognize D-sorbitol in low concentrations of various carbohydrates under pH 9 phosphate buffer solution. Although the influence of D-fructose can't be avoided, the recognition ability toward D-sorbitol can be improved by increasing the recognition site of boronic acids.

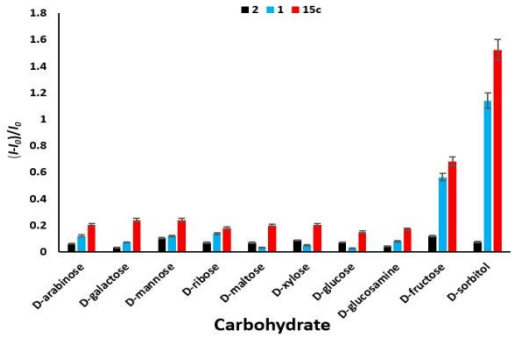


Fig. 1 Relative fluorescence intensity of sensor **1**, **2** and **15c** to a low concentration of carbohydrates (8.88 µM) in phosphate buffer at pH 9.

In order to further investigate the binding ability of sensor **15c** toward D-sorbitol, fluorescence titrations were carried out. Upon

the addition of increasing concentrations of D-sorbitol from 0 to 13×10^{-5} M, the fluorescence of sensor **15c** increased by 11-folds (Fig. 2A). Other sensor-carbohydrate fluorescence titrations were shown in Fig. S9-10. Moreover, as shown in Fig. 2B, it was found a good linear relationship between the fluorescence intensity of sensor **15c** and the concentration of D-sorbitol in the range of from 8.88×10^{-6} to 104.2×10^{-6} M with a correlation coefficient of R^2 = 0.98496. Therefore, the linear regression equation was determined to be I=17508000c+394.88005, where c is the concentration of D-sorbitol, I is the maximum emission fluorescence at 418 nm. The LOD was then calculated to 6.79×10^{-7} M with the following equation:[25, 27, 28]

$$\text{LOD} = 3\delta/S$$

where δ is the standard deviation of the 8 times blank signal of the sensor, and S is the slope of the calibration curve.

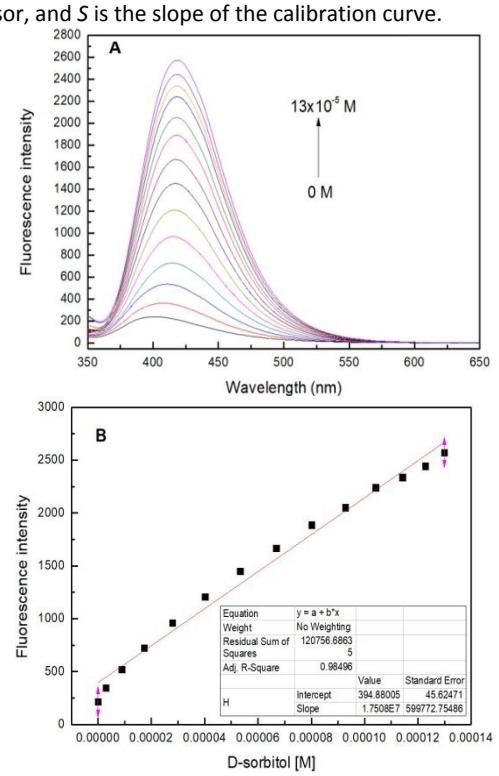


Fig. 2 A) Fluorescence spectra of sensor **15c** (1×10^{-5} M) in the presence of different concentrations of D-sorbitol in DMSO/PBS (pH 9, 0.1M) solution (1:99, v/v), at room temperature; B) The photograph of sensor **15c** linear relationship.

In addition, it was found that the reciprocal of the enhanced fluorescence amplitude of sensor **15c** showed a good linear relationship with the reciprocal of the D-sorbitol concentration (R^2 = 0.99674), as shown in Fig. 3. Therefore, fluorescence data were processed using the Benesi–Hildebrand equation and the binding constant (K_a) was calculated to 10922±776 M^{-1} based on three times of measurement with the following equation: [24, 25, 29]

$$\frac{1}{I-I_0} = \frac{1}{I_1-I_0} + \frac{1}{(I_1-I_0)K_a[C]_{D\_sorbitol}}$$

where I_1 is the intensity of the guest-host complex, I_0 is the starting fluorescence intensity of free guest, I is the observed fluorescence intensity of guest and guest-host mixture. $[C]_{D-sorbitol}$ is the concentration of D-sorbitol. Using the curve of $(I_1\text{-}I_0)/(I\text{-}I_0)$ *versus* 1/ $[C]_{D-sorbitol}$, the K_a that is the ground state binding constant for the 1:1 complex formation can be calculated from the intercept/slope. According to the value of K_a calculated, the sensor **15c** has a high binding affinity with D-sorbitol. In addition, due to the plot processed by the Benesi-Hildebrand equation exhibited a good linear relationship (R^2 = 0.99674), the binding ratio of 1:1 is determined.[29]

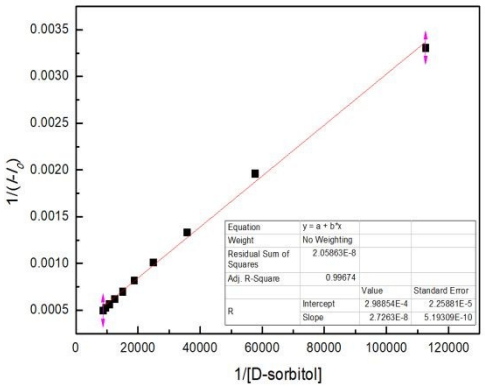


Fig. 3 Benesi-Hildebrand plot of sensor **15c** $1/(I - I_0)$ *versus* 1/[D-sorbitol]

Response time

As an important aspect of sensors to practical applications, it is very necessary to investigate the sensitivity of sensor **15c**. Therefore, the response time experiments of the reaction system were carried out. The fluorescence of sensor **15c** (1×10^{-5} M) is increased at 418 nm immediately and reached the maximum fluorescence emission intensity after the addition of 13×10^{-5} M of D-sorbitol. A fluorescent scan to record required 0.5 min to prepared. The response time of investigation was from 0 to 25 min, and the recorded time was set at 0 min, 1 min, 2 min, 4 min, 8 min, 12 min, 16 min, 20 min, 25 min, respectively. It was found that the fluorescence of sensor **15c** reached the maximum fluorescence emission intensity followed by very weak fluorescence decay over time (Fig. 4). Besides, the response time test was carried out at room temperature (such as 25 °C). These results indicated that sensor **15c** had a remarkably fast response time toward D-sorbitol.

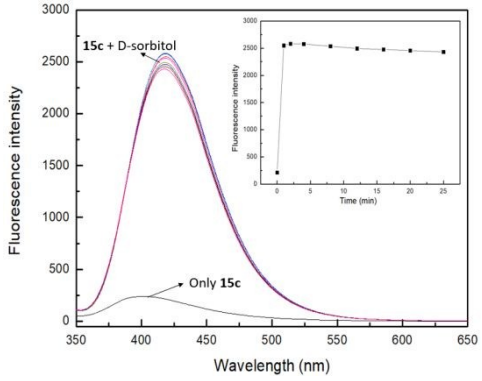


Fig. 4 Fluorescence spectra of sensor **15c** (1×10^{-5} M) upon addition of 13×10^{-5} M of D-sorbitol from 0 to 25 min in DMSO/PBS (pH 9, 0.1M) solution (1:99, v/v), at room temperature. **Inset**: Plot of the fluorescence intensities at 418 nm over 25 min.

pH titration

Owing to boronic acids showing different manifestations in acidic and alkaline environments,[30-33] it is necessarily to investigated suitable pH conditions for the interesting process of sensor **15c**

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recognizing D-sorbitol. Phosphate buffer (0.1 M) was used as a buffer solvent, and hydrochloric acid and sodium hydroxide solutions were used to adjust the pH. The pH of the sensor to study was set at 6, 7, 8, 9, 10, 11, 12, respectively. Different concentrations of D-sorbitol were used for each pH to perform fluorescence titration. The fluorescence intensity of sensor **15c** was recorded as the change of pH after adding different concentrations of D-sorbitol. It was found that sensor **15c** has a large fluorescence response in the range of pH 9 to 12 (Fig. 5). Considering needing a large fluorescence response and avoiding the trouble of detecting the alkaline solution of buffer solution, pH 9 is a suitable value for the detection of D-sorbitol.

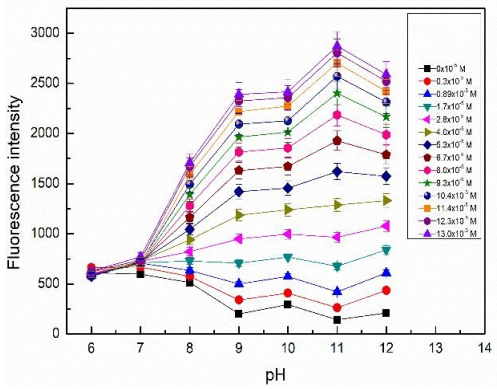

Fig. 5 Fluorescence responses of sensor **15c** (1×10^{-5} M) to D-sorbitol in DMSO/H_2O (1:99, v/v, phosphate buffer, 0.1 M) at different pH values. Concentrations of D-sorbitol are given in the plot.

Competition experiments

In order to further evaluate the selectivity of sensor **15c** for D-sorbitol over other carbohydrates, interference experiments in buffer solution were necessarily carried out. Ten carbohydrates adding to the sensor were studied and the interferences of different carbohydrates on the process of sensor to recognize D-sorbitol were included. In the presence of 6×10^{-5} M of D-arabinose, D-galactose, D-mannose, D-ribose, D-maltose, D-xylose, D-glucose and D-glucosamine, respectively, the fluorescence intensity of sensor **15c** was almost unchanged. The same amount of D-sorbitol caused a 5-fold fluorescence enhancement of sensor **15c**, while D-fructose had a 2-fold enhancement. In addition, all sensor **15c** solutions (1×10^{-5} M) containing different carbohydrates (6×10^{-5} M) respectively had remarkable fluorescence enhancement after addition 6×10^{-5} M of D-sorbitol. This may be ascribed to D-sorbitol competitively binding sensor **15c**, resulting in fluorescence enhancement. Owing to the sensor **15c** has a certain fluorescence response to D-fructose, the mixture of D-sorbitol and D-fructose causes the most significant increase in the fluorescence of sensor compared to other carbohydrates and D-sorbitol mixtures. Nonetheless, the fluorescence change of sensor **15c** is most pronounced in the presence and absence of D-sorbitol. Furthermore, some D-sorbitol prescription drugs do not contain D-fructose, therefore, it isn't necessary to consider the influence of D-fructose on the detection of D-sorbitol. The results shown in Fig. 6 indicated that sensor **15c** works well as a D-sorbitol-specific fluorescence sensor in both the absence and presence of carbohydrates interferent.[28, 34]

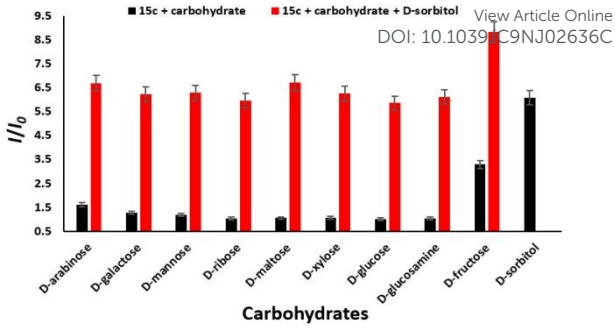

Fig. 6. Relative fluorescence intensity of **15c** to carbohydrates (black bar) or D-sorbitol in the presence of other carbohydrate interference (red bar) in phosphate buffer at pH 9.

Application of sensor 15c in D-sorbitol analysis in real samples

Due to remarkable selectivity and competition of sensor **15c** toward D-sorbitol over other carbohydrates, we wonder if we can detect the concentration of D-sorbitol (for oral administration). Due to the sensor has a good fluorescence response at pH 9, a real sample solution to be tested is prepared by 0.1 M phosphate buffer (pH 9). The fluorescence intensity of the real sample was measured and used the linear regression equation obtained in Fig 2B. A series of real samples' concentrations were converted by the linear regression equations processing the fluorescence intensity. As shown in Fig. 2B, the linear regression equation was determined to be I=17508000c+394.88005. The recoveries and the error bars were calculated by using the concentrations of calculated D-sorbitol and spiked D-sorbitol. It was found that the concentrations of spiked D-sorbitol in D-sorbitol (for oral administration) can be determined with good recovery, as shown in Table 2. The results indicated that sensor **15c** could be applied to the analysis in real samples.[2, 17]

Table 2 Determination of D-sorbitol concentration in D-sorbitol (for oral administration)

Sample	D-sorbitol added (μM)	D-sorbitol found (μM)	Recovery%	RSD[a]%
D-sorbitol (for oral administration)	0	1.46	-	3.66
	1.5	3.20	116	1.87
	4.5	6.62	115	3.39
	7.5	9.10	102	2.24

[a] Relative standard derivation was calculated based on three times of measurement.

Comparison with other boronic acid-based sensors

Owing to different boronic acid-based sensors were prepared under different experimental conditions, we summarized and compared some key information of boronic acid-based sensors reported in the literature. It was found that our reported sensor **15c** that has a high binding constant and a low LOD. Although most sensors reported were carried out in pH 7.4, low binding constant need to be further improved. The results indicated that sensor **15c** is significantly greater than that of sensors summarized in Table 3. Much boronic acid sensors reported earlier to selectively recognize D-sorbitol [18-20, 35, 36] did not have a good linear relationship between fluorescence intensity and carbohydrates concentration due to the use of a high D-sorbitol concentration resulting fluorescence intensity varied

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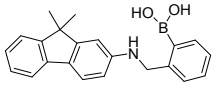
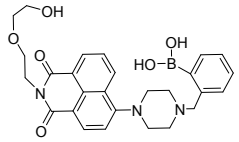
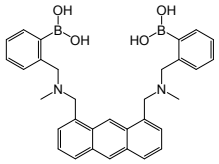
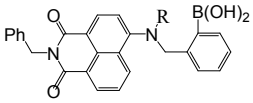
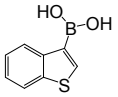
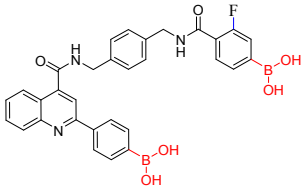
greatly. Therefore, the LOD was not further studied. Furthermore, other key conditions are also ignored, including a suitable pH and response time. Designing and screening a highly selective and sensitive D-sorbitol sensor, a low LOD is a very important aspect. Because it involves practical applications, such as whether it can detect commercially available D-sorbitol-containing foods, D-sorbitol-related drugs used in hospitals, and even apply biological fluid detection and cellular fluorescence imaging. Compared to previous methods for the detection of D-sorbitol,[2, 14-16] we reported that boronic acid sensors may offer some very significant advantages. The selectivity to D-sorbitol is increased by increasing the boronic acid groups. Due to the rapid forming a five-membered ring of boronic acid groups and *cis*-diols, the reaction can be completed quickly in the time required for one measurement, thereby reducing the response time. The sensors we reported are water-soluble and suitable for food and drug testing. However, there are some limitations to the sensors. For example, the pH value is required a litter higher. The designed sensor has a high binding constant and low LOD for D-sorbitol under the condition of pH 9 and the pH value of body fluid is less than 9. Therefore, when the sensor is applied to detect D-sorbitol in biological fluid for early diagnosis of diabetic complications, it needs to increase the work of regulating biological fluid pH value. However, our sensors could satisfy the determination of D-sorbitol in drug and food products, which have no limits on the pH value.

Table 3 key information of reported sensor for D-sorbitol

Authors	Sensors	Buffer; pH	Binding constant (K_a / M^{-1})	λ_{ex}; λ_{em}	LOD(M)
Hosseinzadeh *et al.* reported[2]		MeOH/H_2O (1:49, v/v); pH 8.31	1107±48	293 nm; 362 nm↑[a]	7.04×10^{-6}
Liu *et al.* reported[17]		PBS buffer; pH 7.4	140.8	400 nm; 530 nm↑	2.8×10^{-5}
Swamy *et al.* reported[20]		MeOH/ PBS buffer (1:1, v/v); pH 7.4	1060	-; 365 nm↑	-
Wang *et al.* reported[18]		MeOH/ PBS buffer (1:1, v/v); pH 7.4	340	446 nm; 543 nm↑	-
Akay *et al.* reported[19]		PBS buffer; pH 7.4	4561±90	274 nm; 303 nm↑, 334 nm↑	-
Akay *et al.* reported[19]		PBS buffer; pH 7.4	1883±32	274 nm; 317 nm↓[b]	-
Present work		DMSO/ PBS buffer (1:99, v/v); pH 9	10922±776	337 nm; 418 nm↑	6.79×10^{-7}

[a] ↑: turned on the fluorescence; [b] ↓: turned off the fluorescence

A series of fluorescent sensors with suppressing photoinduced electron transfer (PET) mechanism were reported by T. D. James, J. Yoon, *et al.*[20, 37], which are based on fluorophores, amines and boronic acid groups. While the boronic acid group forms a five-membered ring with the *ortho*-dihydroxy group of the carbohydrate, the lone pair electrons of amine group (N) is speculated to run into the empty orbital of B to form an N-B bond, thereby suppressing the photoinduced electron transfer process and releasing fluorescence. The sensors we designed and reported are based on these structures, and the fluorescent changes of sensors caused by D-sorbitol are extremely similar to those sensors reported by Yoon *et al.* Therefore, the mechanism of those sensors bind to D-sorbitol

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or D-fructose is speculated to suppress photoinduced electron transfer.

Conclusions

In summary, we synthesized and reported several water-soluble diboronic acid sensors *via* an improved synthetic route of diboronic acid compounds, which selectively recognize D-sorbitol in aqueous solution. Among them, sensor **15c** not only has remarkably fast response time toward D-sorbitol under mild conditions (at room temperature), but also has a high binding constant (10922±776 M^{-1}) and a low LOD (6.79×10^{-7} M) in phosphate buffer (0.1M, pH 9) among investigated sensors toward D-sorbitol. In addition, competition experiments showed that sensor **15c** works well as a D-sorbitol-specific fluorescence sensor in both the absence and presence of carbohydrates interferent. Furthermore, sensor **15c** was applied to the analysis in real samples (D-sorbitol for oral administration). These studies indicated that sensor **15c** may be served as a potential tool to detect the content of D-sorbitol in drug and food products, as well as the early diagnosis of diabetic complications *via* D-sorbitol detection.

Experimental

Material and method

All of the materials and solvents for synthesis were purchased from commercial suppliers without further purification. All aqueous solutions were prepared using pure water. The NMR spectra were recorded on a Bruker AM-600 spectrometer (Billerica, MA), and resonances (δ) are given in parts per million relative to tetramethylsilane (TMS). High-resolution mass spectrometry (HRMS) spectra were recorded on an Agilent 1290LC-6540 Accurate-Mass Q-TOF by using electrospray ionization (ESI). Fluorescence data were collected on RF5301PC Fluorescence Spectrophotometer (Shimadzu, Japan).

Synthesis

Compound **2** is a known compound which was prepared according to the established literature procedure.[22]

Synthesis of compound **2**:

Compound **1** (0.15 g, 5.1×10^{-4} mol) was dissolved using methanol (20 mL) in a round bottom flask and 30% H_2O_2 (3 mL) was added, then stirred for 4 h at room temperature, and gradually a yellow solid precipitated. Compound **1** was shown to be completely reacted by TLC. The solvent was evaporated under vacuum and obtained brown solid. The crude product was washed with cold ethyl ester and ethanol and dried to get a yellow powder compound **2** (100 mg, 74%). The color was the same as described in the literature.[22] ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) (Fig. S11): δ 9.95 (s, 1H), 8.65 – 8.59 (m, 1H), 8.38 (s, 1H), 8.19 – 8.14 (m, 2H), 8.10 (d, J = 8.0 Hz, 1H), 7.81 (ddd, J = 8.3, 6.9, 1.3 Hz, 1H), 7.64 (ddd, J = 8.3, 6.9, 1.2 Hz, 1H), 6.97 – 6.92 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ (ppm) (Fig. S12): δ 168.20 (s), 159.94 (s), 156.23 (s), 148.89 (s), 137.74 (s), 130.49 (s), 129.94 (s), 129.26 (s), 127.55 (s), 125.82 (s), 123.45 (s), 119.06 (s), 116.26 (s). HRMS m/z (Fig. S13): Calculated 266.0812, found 266.0797.

Synthesis of compound **3**:

Compound **1** (0.20 g, 6.8×10^{-4} mol) was dissolved using methanol (20 mL) in a round bottom flask. Subsequently, 1-(N-Boc-aminomethyl)-4-(aminomethyl)benzene (0.14 g, 7.5×10^{-4} mol), 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium (0.200 g, 7.5×10^{-4} mol) and a drop of N-methylmorpholine were added. The bottom flask was protected with nitrogen atmosphere, stirred at room temperature for 16 h (25 °C). After the reaction was completed, the reaction mixture was added to ice water and stirred. A yellow powder solid was obtained by filtration, which was washed three times with water and recrystallized from methanol-water. After vacuum drying, a light yellow powder product was obtained. The product obtained in the previous step was dissolved using ethyl acetate (30 mL) in a round bottom flask. Subsequently, 1mL of hydrochloric acid was added slowly and stirred at room temperature for 4 h at room temperature. The reaction mixture changed from a yellow clarified state to a yellow turbid state. After vacuum drying, a light yellow powder compound **3** was obtained (0.202 g, 66%). ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) (Fig. S14): δ 9.64 (t, J = 5.7 Hz, 1H), 8.54 (s, 3H), 8.32 – 8.21 (m, 5H), 8.02 (d, J = 7.9 Hz, 2H), 7.89 (t, J = 7.5 Hz, 1H), 7.70 (t, J = 7.6 Hz, 1H), 7.50 (dd, J = 22.3, 8.0 Hz, 4H), 4.62 (d, J = 5.8 Hz, 2H), 4.01 (d, J = 5.6 Hz, 2H). ^{13}C NMR (151 MHz, DMSO) δ (ppm) (Fig. S15): δ 166.84, 156.17, 147.11, 144.16, 139.78, 138.84, 136.88, 135.15, 133.22, 131.34, 129.60, 128.89, 128.14, 127.99, 127.14, 125.89, 124.08, 117.94, 42.92, 42.37. HRMS m/z (Fig. S16): Calculated for $C_{24}H_{23}BN_3O_3^+$ $[M+H]^+$: 412.1827, found 412.1807.

Synthesis of compound **15a**:

Compound **3** (0.11 g, 2.5×10^{-4} mol) was dissolved using methanol (15 mL) in a round bottom flask. Subsequently, triethylamine (0.042 mL), 3-carboxybenzeneboronic acid (0.046 g, 2.75×10^{-4} mol), 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium (0.076 g, 2.75×10^{-4} mol) and a drop of N-methylmorpholine were added. The bottom flask was protected with nitrogen atmosphere, stirred at room temperature for 16 h at room temperature. After the reaction was completed, the reaction mixture was added to ice water and stirred. A yellow powder solid was obtained by filtration, which was washed three times with water and recrystallized from methanol-water. After vacuum drying, a light yellow powder compound **15a** was obtained (0.054 g, 39%). ^{1}H NMR (600 MHz, MeOD) δ (ppm) (Fig. S17): δ 8.26 – 8.07 (m, 5H), 8.02 (d, J = 19.1 Hz, 1H), 7.94 (d, J = 6.4 Hz, 1H), 7.89 – 7.70 (m, 4H), 7.56 (t, J = 7.6 Hz, 1H), 7.51 (s, 1H), 7.42 – 7.30 (m, 4H), 4.70 (s, 2H), 4.63 (s, 2H). ^{13}C NMR (151 MHz, MeOD) δ (ppm) (Fig. S18): δ 168.48, 156.96, 148.28, 143.15, 139.49, 138.67, 136.69, 136.31, 134.10, 133.74, 133.39, 132.34, 132.01, 130.17, 130.09, 128.99, 128.52, 127.13, 126.44, 126.38, 126.32, 126.30, 126.27, 124.85, 123.51, 116.91, 43.20, 43.04. HRMS(ESI) (Fig. S19): Calculated for $C_{31}H_{28}B_2N_3O_6^+$ $[M+H]^+$: 560.2159 found 560.2105.

Synthesis of compound **15b**:

The synthesis operation is the same as that of **15a**. 4-Carboxybenzeneboronic acid (0.046 g, 2.75×10^{-4} mol) was instead of 3-carboxybenzeneboronic acid and a white powder compound **15b** was obtained (0.082 g, 59%). ^{1}H NMR (600 MHz, MeOD) δ (ppm) (Fig. S20): δ 8.14 (d, J = 8.4 Hz, 4H), 8.02 (s, 1H), 7.94 (s, 1H), 7.83 – 7.72 (m, 5H), 7.61 (s, 1H), 7.55 (dd, J = 11.3, 4.0 Hz, 1H), 7.49 (s, 1H), 7.37 (d, J = 6.4 Hz, 2H), 7.31 (d, J = 6.3 Hz, 1H), 4.69 (s, 2H), 4.61 (s, 2H). ^{13}C NMR (151 MHz, MeOD) δ (ppm) (Fig. S21): δ

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168.45, 148.29, 143.11, 139.43, 138.68, 133.75, 133.17, 130.08, 129.01, 128.52, 127.13, 126.43, 126.27, 125.94, 124.85, 123.50, 116.85, 43.18, 43.03. HRMS(ESI) (Fig. S22): Calculated for $C_{31}H_{28}B_2N_3O_6^+$ $[M+H]^+$: 560.2159 found 560.2115.

Synthesis of compound **15c**:

The synthesis operation is the same as that of **15a**. 3-Carboxy-4-fluorobenzeneboronic acid (0.050 g, 2.75 × 10^{-4} mol) was instead of 3-carboxybenzeneboronic acid and yellow powder compound **15c** was obtained (0.060 g, 42%). ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) (Fig. S23): δ 9.41 (t, J = 5.9 Hz, 1H), 8.88 (dd, J = 5.9, 4.6 Hz, 1H), 8.36 (s, 2H), 8.30 (d, J = 8.2 Hz, 2H), 8.24 – 8.13 (m, 5H), 8.00 (d, J = 8.2 Hz, 2H), 7.88 – 7.80 (m, 1H), 7.63 (ddd, J = 18.3, 13.3, 5.4 Hz, 4H), 7.40 (dd, J = 34.1, 8.1 Hz, 4H), 4.61 (d, J = 5.8 Hz, 2H), 4.48 (d, J = 5.9 Hz, 2H). ^{13}C NMR (151 MHz, MeOD) δ (ppm) (Fig. S24): δ 168.46, 165.58, 160.29, 158.64, 148.29, 143.11, 139.10, 138.69, 130.08, 129.10, 129.01, 128.54, 127.12, 126.42, 126.32, 126.23, 126.21, 124.89, 123.52, 116.88, 43.18, 43.05. HRMS(ESI) (Fig. S25): Calculated for $C_{31}H_{27}B_2FN_3O_6^+$ $[M+H]^+$: 578.2065 found 578.2015.

Synthesis of compound **15d**:

The synthesis operation is the same as that of **15a**. 4-Carboxy-3-fluorobenzeneboronic acid (0.050 g, 2.75 × 10^{-4} mol) was instead of 3-carboxybenzeneboronic acid and yellow powder compound **15d** was obtained (0.62 g, 43%). ^{1}H NMR (600 MHz, MeOD) δ (ppm) (Fig. S26): δ 8.08 (d, J = 66.6 Hz, 6H), 7.92 (s, 1H), 7.77 (s, 3H), 7.53 (d, J = 27.9 Hz, 2H), 7.35 (d, J = 21.7 Hz, 3H), 7.06 (s, 1H), 4.69 (s, 2H), 4.62 (s, 2H). ^{13}C NMR (151 MHz, MeOD) δ (ppm) (Fig. S27): δ 168.53, 148.31, 143.17, 139.15, 138.70, 130.09, 128.98, 128.55, 127.11, 126.38, 126.32, 126.25, 124.89, 123.52, 116.91, 114.93, 114.79, 43.20, 43.05. HRMS(ESI) (Fig. S28): Calculated for $C_{31}H_{27}B_2FN_3O_6^+$ $[M+H]^+$: 578.2065 found 578.2018.

Synthesis of compound **15e**:

The synthesis operation is the same as that of **15a**. 3-Carboxy-5-fluorobenzeneboronic acid (0.050 g, 2.75 × 10^{-4} mol) was instead of 3-carboxybenzeneboronic acid and yellow powder compound **15e** was obtained (0.070 g, 49%). ^{1}H NMR (600 MHz, MeOD) δ (ppm) (Fig. S29): 8.20 – 8.10 (m, 4H), 8.04 (s, 1H), 7.82 (dd, J = 52.3, 44.8 Hz, 4H), 7.60 – 7.44 (m, 4H), 7.40 – 7.30 (m, 3H), 4.70 (s, 2H), 4.62 (s, 2H). ^{13}C NMR (151 MHz, MeOD) δ (ppm) (Fig. S30): 168.50, 148.30, 143.14, 139.32, 138.71, 133.74, 130.08, 129.01, 128.54, 127.11, 126.41, 126.35, 126.32, 126.30, 124.84, 123.51, 123.49, 116.83, 43.18. HRMS(ESI) (Fig. S31): Calculated for $C_{31}H_{27}B_2FN_3O_6^+$ $[M+H]^+$: 578.2065, found 578.2060.

Conflicts of interest

The authors conform that this article content has no conflict of interest.

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