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Full Paper

Ratiometric Fluorescent Receptors for Monosaccharides Based on the Intramolecular Charge Transfer in *p*-Dialkylaminobenzanilides

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Two dual fluorescent receptors (**1** and **2**) for monosaccharides based on 4-dialkylaminobenzanilides (alkyl= methyl and *n*-butyl) containing boronic acid group at the amido aniline were synthesized and their spectral properties were investigated. These receptors exhibited dual fluorescence with the long-wavelength band displaying strong solvent-polarity dependence, indicating the occurrence of the excited-state intramolecular charge transfer (ICT). With increasing pH value in aqueous solutions, the hybridization of the boron atom changed from sp^2 to sp^3, inducing a decrease in the total fluorescence quantum yield. The experimental results indicated that the anionic form of the boronate group acted as an electron donor and the benzanilide-like charge transfer was promoted upon hybridization change. In the presence of monosaccharides, the boronic acid in **1** and **2** changed from neutral to anionic form. The intensity of the locally excited (LE) state emission decreased in the presence of sugars while a slight increase in the intensity at the charge transfer (CT) emission occurred. Based on the change in the CT to LE intensity ratios of **1** and **2** due to sugar binding, ratiometric fluorescent assays for monosaccharide sensing were established.

Keywords dual fluorescence, ratiometric receptor, intramolecular charge transfer, boronic acid, monosaccharide

Introduction

Given the importance of sugar recognition in biosciences and its wide applications, the design of artificial receptors for sugars is of fundamental interest and may have practical applications.[1,2] Because it has the ability to bind *cis*-diol via reversible boronate formation, boronic acid is suitable for the construction of sugar receptors.[3] Direct connection of boronic acid group with a fluorophore provides a fluorescent sugar-receptor if these two components are in electronic interaction in the ground- and/or excited-state. The sensing is based on the modification of the interaction when sugar binds to the boronic acid. Numerous boronic acid containing fluorescent receptors for sugars have been reported which employ mostly the photoinduced electron transfer (PET) signaling mechanism.[4-6] Sugar receptors based on other signaling mechanisms, such as charge transfer (CT),[7,8] energy transfer,[9] ligand exchange[10] and polymer matrix change[11] were also reported. The CT-based receptors are promising since the formation and emission of the CT state are highly sensitive to subtle structural and/or environmental perturbations[12] that afford the receptors with high sensitivity. It is possible to establish a dual fluorescent ratiometric receptor for sugars where the energies of the locally excited (LE) state and the followed CT state are well separated and both of them are emissive. This is important for overcoming the influence of the excitation source fluctuation encountered in normal single-wavelength receptors, and therefore it has obvious advantage in fabricating sugar receptors of practical use. The CT-based dual fluorescent receptors, however, has actually received limited attention.[7,8,13]

We have recently shown that the CT dual fluorescence of *p*-dimethylaminobenzanilides[14] could be observed in solvents over a large polarity range from nonpolar cyclohexane (CHX) to highly polar acetonitrile (ACN), and importantly, the CT dual emission is sensitive to the electronic effect of the amino anilino substitution. This inspired us to develop new CT dual fluorescent receptors for monosaccharides by incorporating boronic acid as a substituent into the amido anilino phenyl ring. Based on Hammett constant,[15] *meta*-boronic acid group (*m*-$B(OH)_2$, $\sigma_m = -0.01$, **I** in Scheme 1) acts as a substituent similar to —H. Upon interaction with a monosaccharide molecule under alkaline condition, the boronic acid group converts to its anionic form ($-B(OH)_3^-$, **II** in Scheme 1) which displays stronger electron-donating ability ($\sigma_m = -0.48$),

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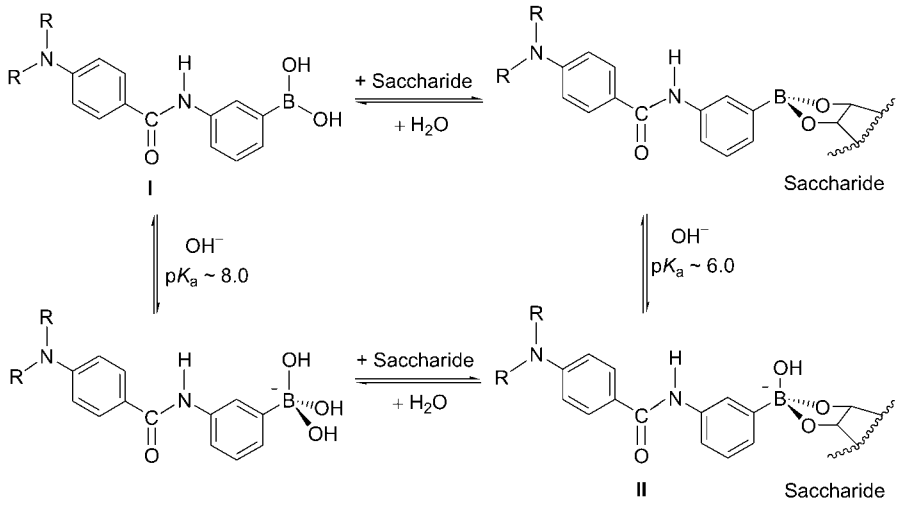
Scheme 1 Equilibria involved in the binding reactions of boronic acid with saccharides

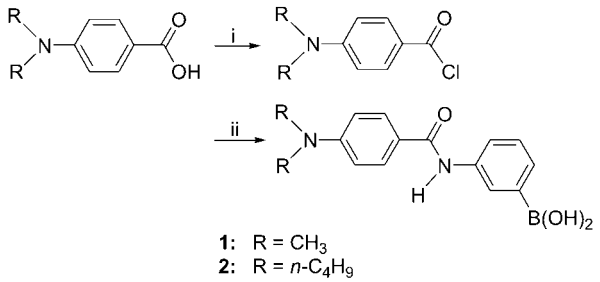


with the boron atom hybridization changing from sp^2 in $-B(OH)_2$ to sp^3 in $-B(OH)_3^-$. The drastically enhanced electron donating ability of the boron acid substituent upon sugar binding would lead to changes in the spectroscopic and photophysical properties of the receptor and hence allow for sugar sensing. Here we report our first attempt in this regard by designing **1** and **2** as new dual fluorescent receptors for monosaccharides.

Materials

1 and **2** were synthesized by reactions of *p*-dialkylaminobenzoyl chlorides (1 mmol) with 3-aminophenylboronic acid hemisulfate (1 mmol) in CH_2Cl_2 in the presence of triethylamine (2.2 mmol) under stirring at room temperature for 6 h (Scheme 2). The crude products were subject to silica column chromatography by using the mixture of ethyl acetate and petroleum ether (4∶3, *V*/*V*) as eluent. The spectral data for receptors **1** and **2** are shown in the following.

Scheme 2 Syntheses of **1** and **2**



Reagents and conditions: (i) $SOCl_2$ (3 mmol), CH_2Cl_2, r.t.; (ii) 3-aminophenylboronic acid hemisulfate, NEt_3, CH_2Cl_2, r.t.

4-Dimethylamino-*N*-(3-boronophenyl)benzamide (**1**): ^{1}H NMR (DMSO-d_6) δ: 2.99 (s, 6H), 6.75 (d, J=8.5 Hz, 2H), 7.28 (t, J=8 Hz, 1H), 7.49 (d, J=7 Hz, 1H), 7.83 (d, J=7 Hz, 1H), 7.88 (d, J=8.5 Hz, 2H), 8.01 (s, 3H), 9.82 (s, 1H). ESI-MS for $C_{15}H_{17}BN_2O_3$ calcd 284.1 (M^+), found 285.1 ($M+H^+$), 335.2 ($M+2CH_3OH-2H_2O+Na^+$).

4-Dibutylamino-*N*-(3-boronophenyl)benzamide (**2**): ^{1}H NMR (DMSO-d_6) δ: 0.93 (t, J=7 Hz, 6H), 1.32—1.36 (m, 4H), 1.49—1.55 (m, 4H), 3.32—3.35 (m, 4H), 6.67 (d, J=8.5 Hz, 2H), 7.27 (t, J=7.5 Hz, 1H), 7.48 (d, J=7 Hz, 1H), 7.82 (t, J=8 Hz, 3H), 7.96—8.04 (m, 3H), 9.75 (s, 1H). ESI-MS for $C_{21}H_{29}BN_2O_3$ calcd 368.3 (M^+), found 369.2 ($M+H^+$), 419.3 ($M+2CH_3OH-2H_2O+Na^+$).

D-Fructose (A.R.), *D*-galactose (A.R.) and *D*-glucose (A.R.) were purchased from Shanghai Chemicals Company (Shanghai, China). Solvents for spectroscopic investigation were purified before use and checked to have no fluorescent impurity at the used excitation wavelength.

Experimental techniques

Corrected fluorescence spectra were recorded on a Hitachi F-4500 spectrophotometer using excitation and emission slits of 5 and 10 nm, respectively. In the fluorescence titration experiments, the excitation wavelength of 300 nm was employed. Fluorescence quantum yields were measured using quinine sulfate as a standard (Φ_F=0.546 in 0.5 mol•L^{-1} H_2SO_4).[16] Absorption spectra were taken on a Varian Cary 300 absorption spectrophotometer using a 1 cm quartz cell. ^{1}H NMR (500 MHz) data were acquired in DMSO-d_6 on a Varian Unity$^+$ 500 MHz NMR spectrometer using TMS as the internal reference. ESI-MS data were obtained on a Bruker Dalton Esquire 3000 plus and Finnigan Mat-LCQ (ESI direct injection).

Results and discussion

Spectroscopic and photophysical properties of receptors 1 and 2

Similar to *p*-dimethylaminobenzanilides, dual fluorescence was observed with **1** and **2** in solvents of varied polarity (Figure 1). The short-wavelength emission

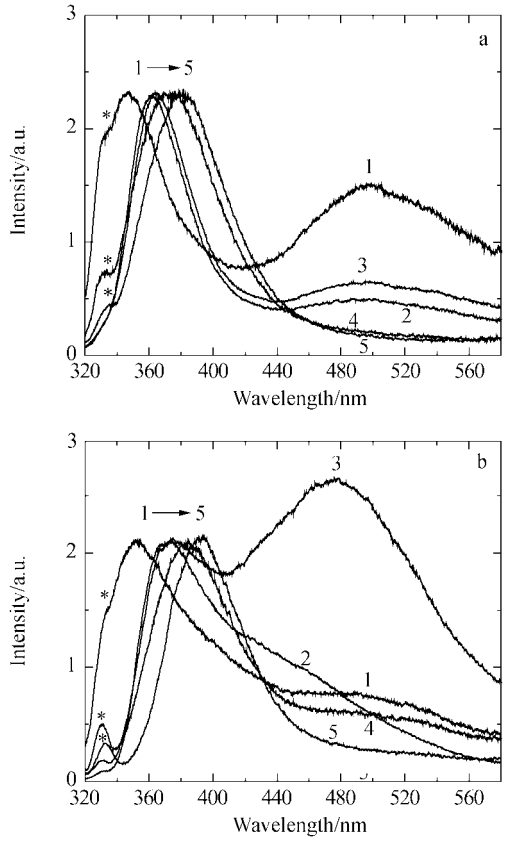


Figure 1 LE emission normalized fluorescence spectra of **1** (a) and **2** (b) in a variety of solvents at room temperature under excitation of 300 nm. Solvents: 1, DEE (diethyl ether); 2, CH_2Cl_2 (dichloromethane); 3, ACN (acetonitrile); 4, MeOH (methanol); 5, H_2O+MeOH (V∶V=1∶1). Asterisks point to solvent Raman scattering.

at around 370 nm and the long-wavelength emission at around 500 nm can be ascribed to the LE and CT states, respectively.[14] No fluorescence spectra of **1** and **2** in cyclohexane (CHX) can be obtained due to their poor solubility. However, a substantial blue shift was observed in the long-wavelength band in cyclohexane-diethyl ether (CHX-DEE) binary solvents with in creasing content of CHX, revealing the CT nature of the long-wavelength emission of **1** and **2**. Photophysical parameters of **1** and **2** in several solvents are summarized in Table 1. With *p*-dimethylaminobenzanilides, it was shown[14] that there existed two competitive CT reaction channels, one from amido aniline to benzoyl as in other benzanilides (the BA-like CT, low fluorescence quantum yield),[17,18] and the other one from dimethylamine to benzamide moiety as that with *p*-dimethylaminobenzamide (the DMABA-like CT, high fluorescence quantum yield).[19] As expected, the two competitive CT reactions also existed within **1** and **2**. Comparing photophysical properties of **1** or **2** in DEE and ACN, it was found that with increasing solvent polarity, the CT emission band position experienced a blue-shift. This 'abnormal' solvatochromism could be assigned to the CT direction reversal in *p*-dimethylaminobenzanilides based on our previous results with this series of molecules.[14] The BA-like CT channel occurred predominantly in DEE, whereas the DMABA-like CT was switched on with increasing solvent polarity and the emission of two competitive CT states became overlapped in the long-wavelength range.

Comparing emission spectra of **1** and **2** in the same solvent, it was found from Figure 1 and Table 1 that, replacing dimethylamino by di(*n*-butyl)amino group led to an obvious increase of the CT to LE intensity ratio and the LE band showed a 10 nm red-shift. The changes in the photophysical properties were explained in terms of that, with the DMABA-like CT channel, the overall efficiency of the LE to CT reaction was increased by lengthening the amino substituent.[20,21] The efficiency of the LE to CT reaction of **2** higher than that of **1** would enhance the fluorescence quantum yield and show more obviously optical changes upon sugar binding.

pH value and sugar effects on the optical spectra of 1 and 2

The titration curves of the fluorescence intensity against pH value for **1** and **2** in the absence and presence of monosaccharides are shown in Figure 2. With

Table 1 Spectroscopic parameters of **1** and **2**

Comp.	Solvent	λ_{LE}/nm	λ_{CT}/nm	$\Delta\nu_{1/2}{}^{a}$/cm^{-1}	Φ_F	λ_{Abs}/nm	ε/(L•mol^{-1}•cm^{-1})
	DEE	347	500	5881	0.0052	307	12800
	CH_2Cl_2	363	496	—[c]	0.0079	318	12600
1	ACN	366	496	—[c]	0.0086	312	17900
	MeOH	373	—[b]	—[c]	0.0017	314	18500
	H_2O+MeOH (1∶1)	382	—[b]	—[c]	0.0041	319	16900
	DEE	352	490	—[c]	0.0221	314	21760
	CH_2Cl_2	373	458	—[c]	0.0418	327	19650
2	ACN	374	477	5848	0.0536	322	20970
	MeOH	386	500	—[c]	0.0091	323	20900
	H_2O+MeOH (1∶1)	392	—[b]	—[c]	0.0105	329	19370

[a] Full width at the half maximum of the CT emission band. [b] Too weak to ascertain. [c] Unable to accurately determine because of its overlap with the LE emission.

increasing pH value, a significant decrease in the fluorescence intensity was observed, while the LE band position remained unchanged. A decrease in the fluorescence intensity at pH below 4.00 was also observed, which was assigned to the protonation of the dialkylamino group. The pK_a[22] of 7.99 obtained for **1** and 7.84 for **2** are characteristic of the substituted phenyl boronic acid. The increase of the acidity of **1** and **2** due to sugar binding was indicated by lowered pK_a, as was observed with other boronic acid-sugar complexes. This decrease in pK_a of the receptor-sugar complex compared to that of the receptor itself allowed for the detection of sugar at a neutral pH of 6.70, where the maximum intensity changes were obtained. At this pH value, **1** and **2** existed predominantly in their neutral form in the absence of sugar (**I** in Scheme 1), but not in their anionic form in the presence of sugar (**II** in Scheme 1). This explains the observed spectral changes.

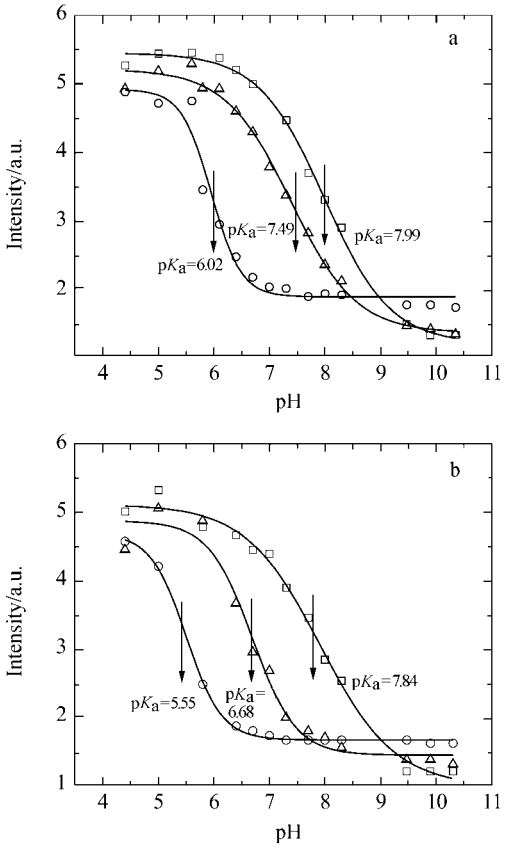


Figure 2 Titration curves of the fluorescence intensity of **1** (a) and **2** (b) vs. solution pH value in the absence and presence of monosaccharides, respectively. Fluorescence intensities were measured at emission wavelength of 382 nm for **1** and 392 nm for **2**. □, Concentration of receptor: 0.02 mmol/L; △, Concentration of receptor plus glucose: 25 mmol/L; ○, Concentration of receptor plus fructose: 25 mmol/L.

Effect of fructose on the emission spectra of **1** and **2** in buffered solution of pH 6.70 is displayed in Figure 3. It was found that the LE fluorescence intensity decreased in the presence of fructose while the intensity at longer wavelength increased slightly. With **1**, an isoemissive point was observed at 430 nm. In the LE emission normalized spectra, such increase in the intensity in the long wavelength region can be obviously observed (insets of Figure 3). These spectral changes can be explained in terms of the sugar-binding promoted BA-like CT. Because of the increased contribution of the BA-like CT, the fluorescence quantum yields substantially decreased. The absorption spectra, however, showed only a very minor change under the same conditions employed for fluorescence measurements (data not shown), indicating that the variations in fluorescence intensity upon sugar binding were mainly due to the changes of quantum yield.

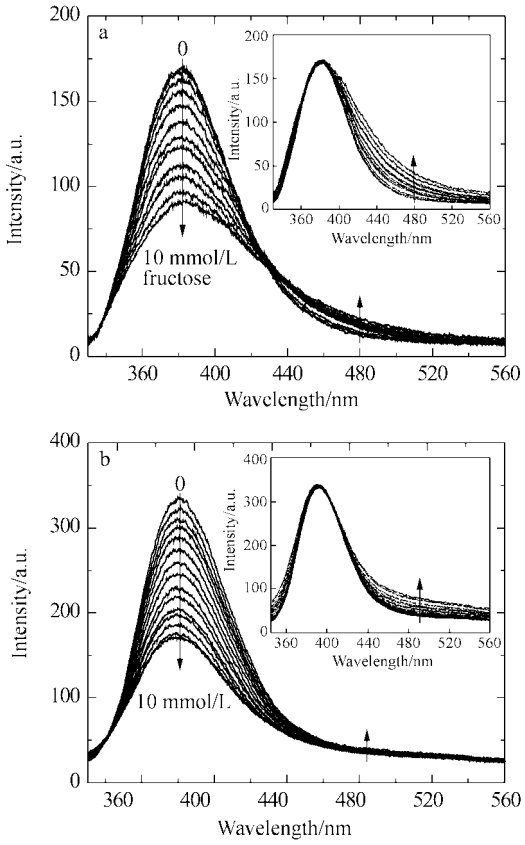


Figure 3 Fluorescence spectra of **1** (a) and **2** (b) ($c=1.0\times10^{-5}$ mol•L^{-1}) in the presence of *D*-fructose in phosphate buffer-methanol (1∶1, *V*/*V*) mixture of pH 6.70 under excitation of 300 nm. Insets show the LE normalized spectra.

Titration curves against sugar concentration were obtained at pH 6.70 (Figure 4). Response to sub-millimole monosaccharides such as *D*-fructose was observed. The sugar binding constants (*K*'s) were determined by fitting the CT to LE fluorescence intensity ratio of **1** (482 nm/382 nm) and **2** (492 nm/392 nm) vs. sugar concentration by a nonlinear regression method.[23,24] Nice fittings supported the 1∶1 binding stoichiometry. The obtained binding constants varied in the order of *D*-fructose＞*D*-glucose＞*D*-galactose (Table 2) which

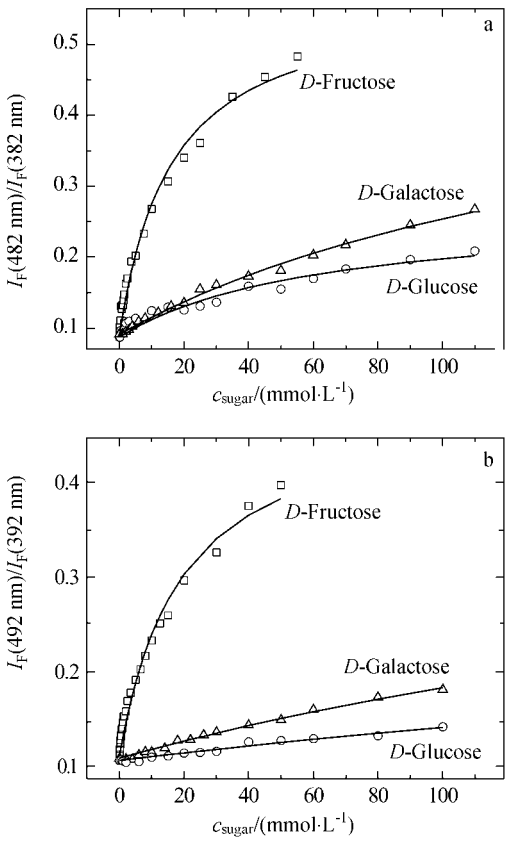


Figure 4 Titration curves of the CT to LE intensity ratio of **1** (a) and **2** (b) ($c=1.0\times10^{-5}$ mol•L^{-1}) against sugar concentration in phosphate buffer-methanol (1∶1, *V*/*V*) mixture at pH=6.70 under excitation of 300 nm.

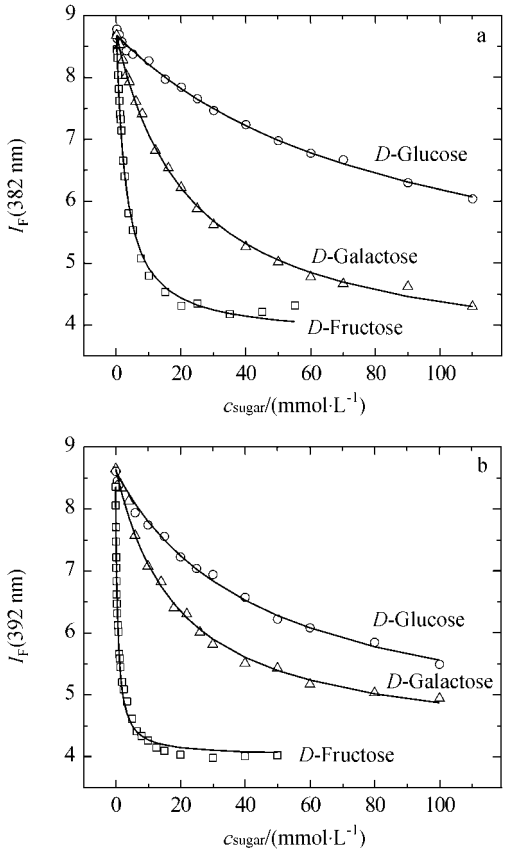


Figure 5 Titration curves of the fluorescence intensity at 382 nm for **1** (a) and at 392 nm for **2** (b) ($c=1.0\times10^{-5}$ mol•L^{-1}) against sugar concentration in phosphate buffer-methanol (1∶1, *V*/*V*) at pH=6.70 under excitation of 300 nm.

was in agreement with those observed with other monoboronic acids.[7,8] These results showed that the two CT dual fluorescent receptors provide a promising way to develop wavelength ratiometric fluorescent probes for monosaccharides sensing.

Table 2 Binding constants K (mol^{-1}•L) obtained by ratiometric method for monosaccharides with **1** and **2** in phosphate buffer-methanol (1∶1, *V*/*V*) mixture at pH=6.70 under excitation of 300 nm

Comp.	K/(mol^{-1}•L)	
	1	**2**
D-Fructose	63.0±6.9	52.0±6.0
D-Galactose	5.6±0.7	2.9±0.9
D-Glucose	16.0±4.7	2.1±1.1

Meanwhile, the plots of the LE fluorescence intensity of **1** (at 382 nm) and **2** (at 392 nm) against monosaccharide concentration were also fitted (Figure 5) and the obtained binding constants (Table 3) were in the order of *D*-fructose>*D*-galactose>*D*-glucose. Similar fluorescence spectral behavior but distinctly different binding constants between **1**+fructose (389.0±15.0 mol^{-1}•L) and **2**+fructose (1549.5±64.8 mol^{-1}•L) indicated that the amino substitution changed from methyl to *n*-butyl could indeed improve the binding ability of the receptors toward monosaccharides, and **2** sensed fructose with a higher selectivity than **1** did.

Table 3 Binding constants K (mol^{-1}•L) obtained using LE fluorescence intensity of **1** (at 382 nm) and **2** (at 392 nm) against monosaccharides concentration in phosphate buffer-methanol (1∶1, *V*/*V*) at pH=6.70 under excitation of 300 nm

Comp.	K/(mol^{-1}•L)	
	1	**2**
D-Fructose	389.0±15.0	1549.5±64.8
D-Galactose	44.0±1.6	50.4±3.4
D-Glucose	11.0±0.9	22.2±2.0

The ratiometric method has advantages of eliminating a number of possible side-effects such as variations in probe concentration and illumination instability. Because of the weak CT emission, the CT to LE intensity ratio of **1** and **2** were only slightly increased from 0.1 to 0.4 upon sugar binding. Therefore, the single-wavelength intensity nonlinear fitting is another complementary method to the ratiometric method for sugar sensing reported in this paper.

Conclusions

Two *p*-dialkylaminobenzanilide-based dual fluorescent receptors were developed for monosaccharide recognition. It could be assumed that the response in the dual fluorescence by increasing the CT to LE intensity ratio was due to the electron donor/acceptor variations in the CT fluorophores. This result is instructive for developing dual fluorescent sugar-receptors with higher CT to LE intensity ratio. Continued work is now underway to enhance the relative quantum yield of the CT emission and the total quantum yield as well, in order to achieve better sensing performance.

References

1 James, T. D.; Sandanayake, K. R. A. S.; Shinkai, S. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1910.

2 Davis, A. P.; Wareham, R. S. *Angew. Chem., Int. Ed.* **1999**, *38*, 2979.

3 Lorand, J. P.; Edwards J. D. *J. Org. Chem.* **1959**, *24*, 769.

4 James, T. D.; Sandanayake, K. R. A. S.; Shinkai, S. *Nature* **1995**, *374*, 345.

5 James, T. D.; Shinmori, H.; Takeuchi, M.; Shinkai, S. *Chem. Commun.* **1996**, 705.

6 Yang, W.-Q.; Yan, J.; Fang, H.; Wang, B.-H. *Chem. Commun.* **2003**, 792.

7 DiCesare, N.; Lakowicz, J. R. *J. Phys. Chem. A* **2001**, *105*, 6834.

8 DiCesare, N.; Lakowicz, J. R. *Chem. Commun.* **2001**, 2022.

9 Arimori, S.; Bell, M. L.; Oh, C. S.; James, T. D. *Org. Lett.* **2002**, *24*, 4249.

10 Arimori, S.; Ward, C. J.; James, T. D. *Tetrahedron Lett.* **2002**, *43*, 303.

11 Asher, S. A.; Alexeev, V. L.; Goponenko, A. V.; Sharma, A. C.; Lednev, I. K.; Wilcox, C. S.; Finegold, D. N. *J. Am. Chem. Soc.* **2003**, *125*, 3322.

12 Retting, W. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 971.

13 Cao, H.; Diaz, D. I.; DiCesare, N.; Lakowicz, J. R.; Heagy, M. D. *Org. Lett.* **2002**, *9*, 1503.

14 Zhang, X.; Wang, C.-J.; Liu, L.-H.; Jiang, Y.-B. *J. Phys. Chem. B* **2002**, *106*, 12432.

15 Hansch, C.; Leo, A.; Taft, R. W. *Chem. Rev.* **1991**, *91*, 165.

16 Demas, J. N.; Crobys, G. A. *J. Phys. Chem.* **1971**, *75*, 991.

17 Heldt, J.; Gormin, D.; Kasha, M. *J. Am. Chem. Soc.* **1988**, *110*, 8255.

18 Zhang, X.; Sun, X.-Y., Wang, C.-J.; Jiang, Y.-B. *J. Phys. Chem. A* **2002**, *106*, 5577.

19 Braun, D.; Rettig, W.; Delmond, S.; Letard, J.-F.; Lapouyade, R. *J. Phys. Chem. A* **1997**, *101*, 6836.

20 Schuddeboom, W.; Jonker, S. A.; Warman, J. M.; Leinhos, U.; Kühnle, W.; Zachariasse, K. A. *J. Phys. Chem.* **1992**, *96*, 10809.

21 Il'ichev, Y. V.; Kűhnle, W.; Zachariasse, K. A. *J. Phys. Chem. A* **1998**, *102*, 5670.

22 Titration curves against pH value were fitted and pK_a values were obtained following the procedure reported in Ref. 7.

23 Connors, K. A. *Binding Constants-The Measurement of Molecular Complex Stability,* John Wiley & Sons, New York, **1987**.

24 Valeur, B.; Pouget, J.; Bouson, J.; Kaschke, M.; Ernsting, N. P. *J. Phys. Chem.* **1992**, *96*, 6545.

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