Pergamon

Tetrahedron Vol. 51, No. 7, pp. 1893-1902, 1995

0040-4020/95 $9.50+0.00

0040-4020(94)01055-2

Spectroscopic Sugar Sensing by a Stilbene Derivative with Push (Me_2N-)-Pull ($(HO)_2B$-)-Type Substituents

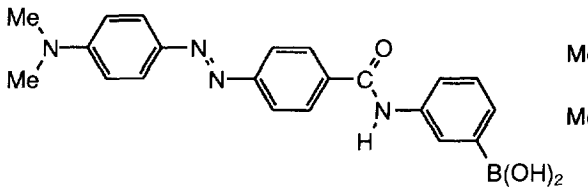
Hideyuki Shinmori, Masayuki Takeuchi, and Seiji Shinkai*

Department of Chemical Science & Technology, Faculty of Engineering,
Kyushu University, Fukuoka 812, Japan

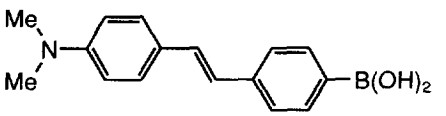
Abstract: A stilbene derivative (**2**) with a push (Me_2N-) and a pull ($(HO)_2B$-) substituent at 4,4'-positions was synthesized. **2** aggregated in aqueous solution (water:DMSO = 30:1 v/v) but was dispersed nearly homogeneously in water:DMSO = 1:1 v/v. Spectroscopic studies established that **2** is useful to detect saccharides by both absorption and fluorescence spectroscopic methods. Particularly, in aqueous solution at pH 8.0 a large increase in the fluorescence intensity was observed upon complexation with D-fructose. This is a novel sugar sensing system using boronic acid as an interface.

Introduction

Boronic acids, which have been known since a long time ago to form covalently-bonded complexes with diols, now attract a great deal of attention as a new interactive tool for sugar recognition.[1-10] We previously synthesized an azobenzene dye bearing a phenylboronic acid moiety (**1**).[11] In aqueous solution this dye changes its color in response to added saccharides.[11] The spectroscopic studies revealed that the color change stems from a change in the saccharide-induced aggregation-deaggregation equilibrium.[11] The results suggest that the color of synthetic dyes may be controllable by added saccharides, like flower colors in nature.[12] Based on these lines of novel information, we newly designed compound **2**. The following ideas are stored in **2**: (i) since **2** has a pair of push (Me_2N-) and pull ($(HO)_2B$-) substituents, a large dipole moment which facilitates molecular aggregation exists, (ii) the saccharide binding can change a sp^2-hybridized neutral boron to a sp^3-hybridized anionic boron, the dipole moment changes upon complexation with saccharides, (iii) since the stilbene moiety is fluorescent, the binding process can be monitored by a change in the fluorescence intensity, and (iv) since the $(HO)_2B$- group conjugates with the Me_2N- group, one can expect a large change in the fluorescence intensity for the sp^2-B $\rightarrow$ sp^3-B^- process. As expected, the spectroscopic studies have established that **2** is a very useful molecule to detect saccharides in an aqueous system by absorption and fluorescence spectroscopic methods.



1



2

Results and Discussion

Solvent Effect and Concentration Dependence. The absorption spectra of **2** were measured in several solvents. The aim of this experiment is to clarify whether or not **2** aggregates in aqueous solution. In an aqueous system (*i.e.*, water:DMSO = 1:1 v/v and 30:1 v/v) neutral **2** with $RB(OH)_2$ was generated by using an aqueous solution buffered to pH 8.0 with 0.10 M phosphate whereas anionic **2** with $RB^-(OH)_3$ was generated by using an aqueous solution buffered to pH 10.5 with 0.10 M carbonate. As evidenced later, these pH's suffice to generate each species. The results are summarized in Table 1.

As expected for a chromophore with a push-pull substituent couple, the λ_{max} for neutral **2** shifts to longer wavelength with increasing E_T value. This is due to the stabilization of the charge-separated excited state in polar solvents.

Table 1. Absorption maxima of **2** in various solvents (25 °C)

Solvent	λ_{max} (nm)[a]		E_T [b]
	Neutral **2** ($RB(OH)_2$)	Anionic **2** ($RB^-(OH)_3$)	
Carbon tetrachloride	359	–	32.5
Benzene	362	–	34.5
THF	360	–	37.4
Chloroform	361	–	39.1
DMSO	367	–	45.0
Water:DMSO (1:1 v/v)	366	344	–
Water:DMSO (30:1 v/v)	318	331	63.1
Water:DMSO (30:1 v/v) with 0.10 M D-fructose	–	331	–

[a] [**2**] = 1.00×10^{-5} M.

[b] Cited from Dimroth, K.; Reichardt, C.; Siepmann, T.; Bohlmann, F. *Justus Liebigs Ann. Chem.* **1963**, *661*, 1. The E_T for water:DMSO = 30:1 v/v is that for water.

In water:DMSO = 1:1 v/v the λ_{max} does not move further to longer wavelength from that in DMSO in spite of the increase in the solvent polarity. In water:DMSO = 30:1 v/v, on the other hand, the λ_{max} shifts to *shorter wavelength* by 49 nm from that in DMSO. The results imply that **2** aggregates totally (in water:DMSO = 30:1 v/v) or partially (in water:DMSO = 1:1 v/v). It is known that boronic acids as

Lewis acids interact with intramolecular tertiary amines as bases.[10] We thus presume that the aggregation is induced by the B···N interaction as well as by the hydrophobic interaction between the stilbene moieties.

For anionic **2** such a B···N interaction is no longer expected and the aggregation must be hampered by electrostatic repulsion between anionic charges. As shown in Table 1, the λ_{max} in water:DMSO = 30:1 v/v shifts to shorter wavelength only by 13 nm from that in water:DMSO = 1:1 v/v. Furthermore, in water:DMSO = 1:1 v/v the λ_{max} for anionic **2** (344 nm) appears at shorter wavelength than that for neutral **2** (366 nm) whereas in water:DMSO = 30:1 v/v the λ_{max} for anionic **2** (331 nm) appears at longer wavelength than that for neutral **2** (318 nm). These results suggest that anionic **2** is not or only slightly aggregated in water:DMSO = 30:1 v/v.

The concentration dependence of neutral **2** and anionic **2** was examined in water:DMSO = 30:1 v/v at [**2**] = $1.00 \times 10^{-6} \sim 5.00 \times 10^{-5}$ M. Although neutral **2** is aggregated and anionic **2** is nearly monomeric, both systems apparently obey the Lambert-Beer's law as evidenced by a good linear relationship in Fig. 1. This implies that at this concentration range the aggregation mode of neutral **2** is not so changed as to affect the absorption spectrum.

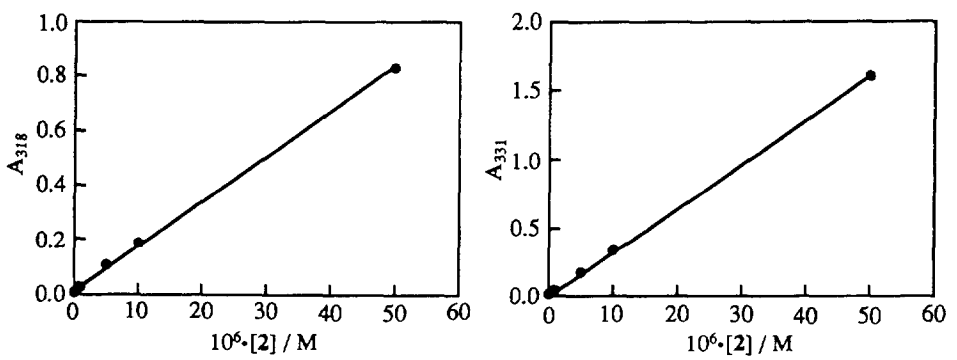


Fig. 1. Plots of absorbance versus concentration for neutral **2** (left) and anionic **2** (right): water:DMSO = 30:1 v/v, 25 °C.

Effects of Added Saccharides on the Absorption Spectra. Firstly, the effect of medium pH on the absorption spectra was examined in water:DMSO = 1:1 v/v. As shown in Fig. 2 (the pH is not corrected: it shows the value of a pH meter), the λ_{max} (366 nm at pH 8.5) shifts to shorter wavelength with the increase in pH. This indicates that the excitation energy of **2** with an electron-withdrawing $RB(OH)_2$ group is smaller than that with a less or non electron-withdrawing $RB^-(OH)_3$ group. One can regard that this is a common characteristic of dyes with a push-pull substituent couple. The apparent pK_a was estimated to be 12.1.

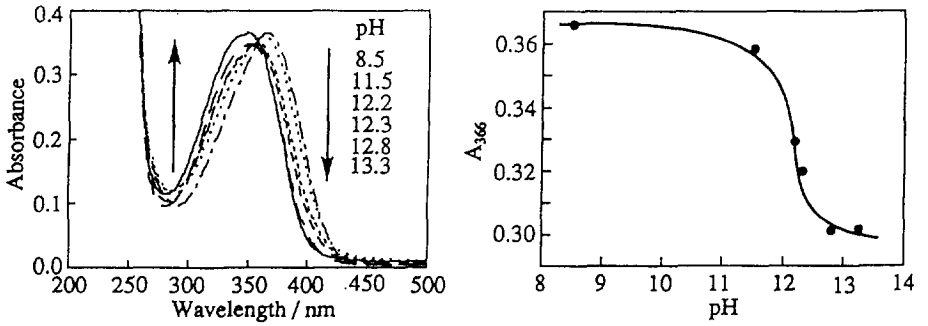


Fig. 2. Absorption spectra (left) and plots of A_{366} versus pH (right) of **2** (1.00×10^{-5} M) in water:DMSO = 1:1 v/v, 25 °C.

In water:DMSO = 30:1 v/v the λ_{max} shifts from 318 nm (at pH 8.0) to 331 nm (at pH 10.5) and moreover, the absorbance increases conspicuously with the increase in pH (Fig. 3). The spectral change reflects the deaggregation process of **2**. The similar spectral change was also observed for **2** in the presence of D-fructose but it occurred at much lower pH region. From plots of absorbance versus pH (Fig. 4) we obtained pK_a 9.6 for **2** and 7.1 for **2** plus D-fructose. Apparently, D-fructose lowers the pK_a by 2.5 pK unit.

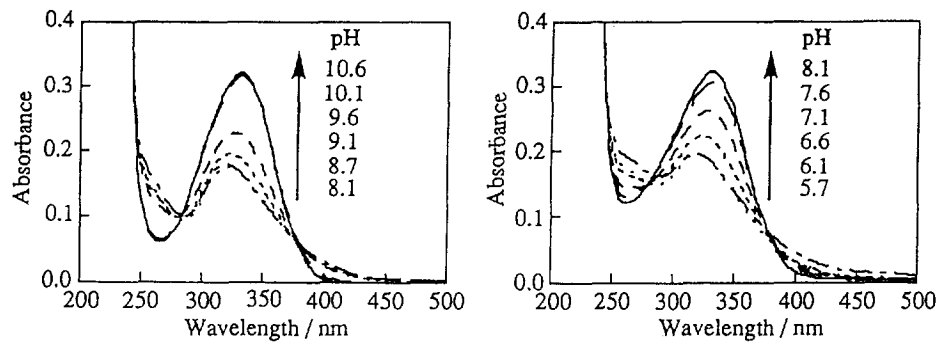


Fig. 3. pH Dependence of the absorption spectra of **2** (left) and **2** plus D-fructose (right) in water:DMSO = 30:1 v/v at 25 °C: [**2**] = 1.00×10^{-5} M, [D-fructose] = 0.10 M.

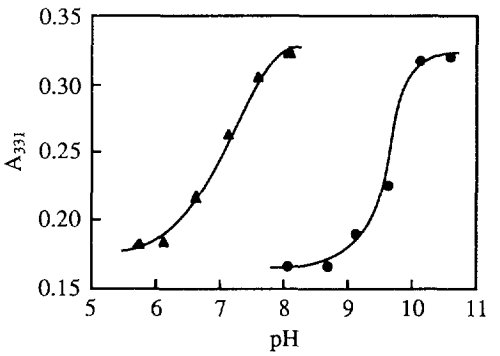


Fig. 4. Plots of A_{331} versus pH: ● **2**, ▲ **2** plus D-fructose.

It is seen from Fig. 4 that at pH 8.0 neutral $RB(OH)_2$ can be converted to anionic $RB^-(OH)(OR')_2$ only by addition of D-fructose. Hence, one can expect a large spectral change when saccharides are added to the **2** solution at pH 8.0. Fixing the solution pH to 8.0, we added monosaccharides to the water:DMSO = 30:1 v/v solution of **2** (Fig. 5). As expected, the absorbance at 331 nm increased with increasing saccharide concentration. One can consider that this spectral change reflects conversion of neutral dimeric **2** to anionic monomeric **2**•saccharide complexes. As shown in Fig. 5, the largest spectral change is observed for D-fructose and the next for D-arabinose. The order of the change magnitude (D-fructose > D-arabinose > D-mannose > D-glucose) is well consistent with the order of the association constant for phenylboronic acid.[14] It is intriguing to estimate how these changes in the absorption spectra are reflected by the fluorescence spectra.

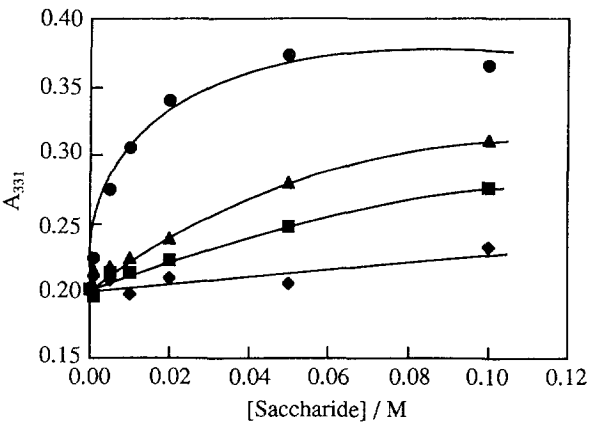
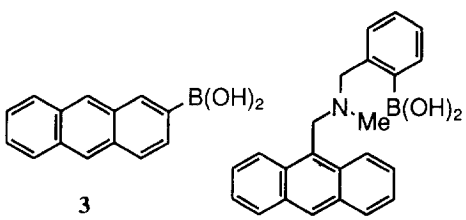
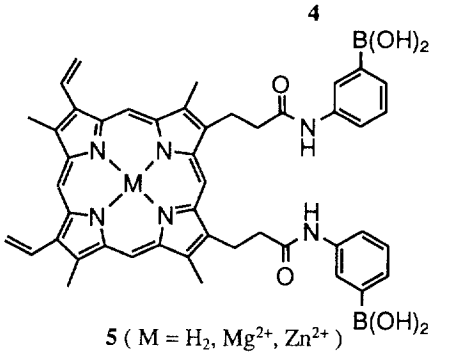


Fig. 5. Plots of A_{331} versus [saccharide] in water:DMSO = 30:1 v/v at pH 8.0 and 25 °C: [**2**] = 1.00 x 10^{-5} M; ● D-fructose, ▲ D-arabinose, ■ D-mannose, ♦ D-glucose.

Effects of Added Saccharides on the Fluorescence Spectra. Yoon and Czarnik[1] showed that the fluorescence intensity (I) of 2-anthrylboronic acid (**3**) decreases upon complexation with saccharides. However, the magnitude of the intensity decrease (I / I_0 = ca. 0.7) is not so large, reflecting the relatively low quenching efficiency of the boronate group. James *et al.*[13] showed that a very large change in the fluorescence intensity can be induced in **4** which has a contrivance of the photo-induced electron transfer mechanism within a molecule. On the other hand, Murakami *et al.*[7] reported a new saccharide sensing system using a boronic-acid-appended porphyrin (**5**): complexation of saccharides changes the aggregation state of the porphyrin, which can be read out as a change in the fluorescence intensity. Compound **2** is unique in that the fluorescent stilbene is sandwiched by a push(Me_2N-)-pull($(HO)_2B$-) substituent couple and that the electron-withdrawing ability of the pull substituent changes in response to the saccharide-binding.





5 (M = H_2, Mg^{2+}, Zn^{2+})

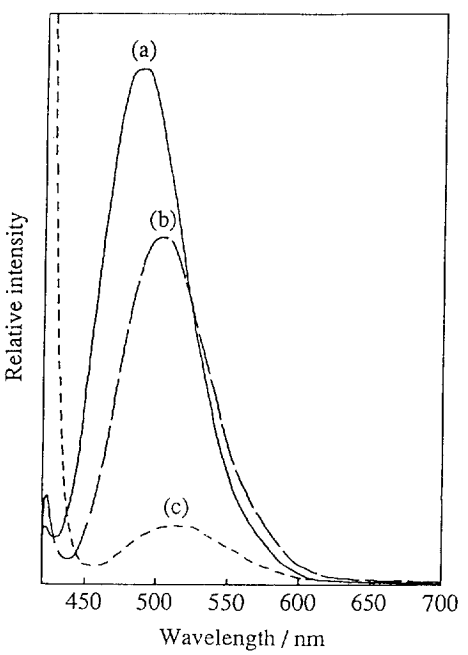


Fig. 6. Fluorescence spectra of **2** in water (pH 8.0 with 0.10 M phosphate):DMSO mixtures: [**2**] = 1.00 x 10^{-5} M, 25 °C, excitation wavelength 419 nm; water:DMSO (v/v) = 0:1 (a), 1:1 (b), 30:1 (c).

Firstly, we examined the solvent effect on the fluorescence spectra in water:DMSO mixtures. The excitation wavelength is 419 nm which is an isosbestic point in the absorption spectra. As shown in Fig. 6, the fluorescence intensity decreases in the order of DMSO > water:DMSO = 1:1 v/v > water:DMSO = 30:1 v/v. Although the fluorescence intensity is affected by the solvent polarity, this decrease is mainly due to the concentration quenching in the aggregate formed weakly in water:DMSO = 1:1 v/v and strongly in water:DMSO = 30:1 v/v.

The fluorescence spectra were measured as a function of pH in water:DMSO = 1:1 v/v and 30:1 v/v. In Fig. 7 for water:DMSO = 1:1 v/v, a tight isosbestic point appears at 494 nm. If both

deaggregation and $RB(OH)_2 \rightarrow RB^-(OH)(OR')_2$ conversion occur simultaneously, such an isosbestic point should not be observed (as in Fig. 8). Hence, the spectral change in Fig. 7 mainly reflects the effect of $RB(OH)_2 \rightarrow RB^-(OH)(OR')_2$ conversion on the fluorescence spectra. The λ_{max} shifts from 504 nm to 453 nm with increasing pH. This change is the same as that in the absorption spectra (Fig. 2). One can thus consider that anionic **2** has a transition energy level higher than neutral **2**. This is rationalized in terms of the stabilization of neutral **2** by conjugation between push and pull substituents. From a plot of I_{453} versus pH the apparent pK_a is estimated to be 11.9 (the pH is not corrected). This value accords to that determined by absorption spectroscopy (12.1). It is also worthwhile to mention that in **3** the fluorescence decreases at high pH region whereas in **2** it inversely increases. The system in which the fluorescence intensity is increased upon the guest-binding is more convenient for the sensing.

Figure 8 shows the pH dependence of the fluorescence spectra in water:DMSO = 30:1 v/v. The absence of an isosbestic points suggests that both deaggregation and $RB(OH)_2 \rightarrow RB^-(OH)(OR')_2$ conversion are induced at high pH region. The similar spectral change was also observed for **2** plus D-fructose. Plots of I_{463} versus pH are shown in Fig. 8. From the plots the pK_a values were estimated to be 9.7 for **2** and 7.1 for **2** plus D-fructose. The values accord to those determined by absorption spectroscopy (9.6 and 7.1, respectively).

Judging from Fig. 8, the large effect of added saccharides on the fluorescence intensity will appear at pH 8.0. Plots of I_{469} versus [saccharide] are illustrated in Fig. 9. Although the order of the fluorescence change appears in the order of D-fructose > D-arabinose > D-mannose > D-glucose, the particularly large change is observable only for D-fructose. Conceivably, the ordinate in Fig. 9 reflects not only complexation with **2** but also deaggregation induced by complexation. D-Fructose which shows the highest affinity with boronic acids forms a complex with **2** and efficiently deaggregates the **2** aggregate owing to the developed anionic charge and the enhanced hydrophilicity. Figure 9 suggests that this system is useful for selective detection of D-fructose in the presence of other saccharides.

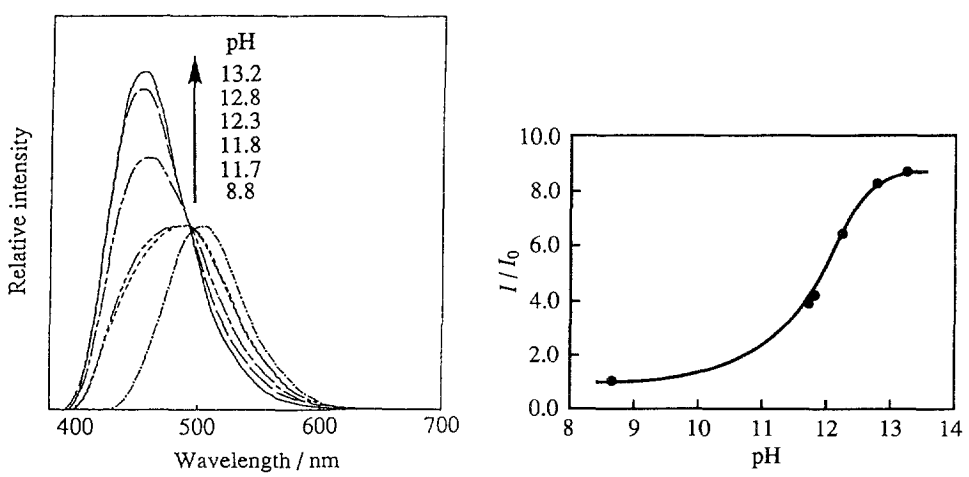


Fig. 7. Fluorescence spectra (left) and I / I_0 (at 453 nm) *vs.* pH plot (right) in water:DMSO = 1:1 v/v: [**2**] = 1.00 x 10^{-5} M, 25 °C, excitation wavelength 356 nm (an isosbestic point in Fig. 2).

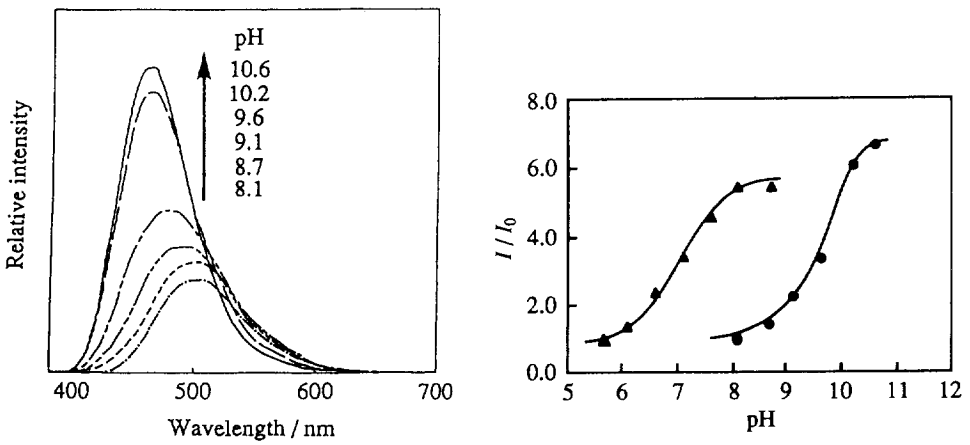


Fig. 8. Fluorescence spectra for **2** (left) and I / I_0 (at 463 nm) *vs.* pH plot for **2** and **2** plus D-fructose (right) in water:DMSO = 30:1 v/v: [**2**] = 1.00 x 10^{-5} M, [D-fructose] = 0.10 M, 25 °C, excitation wavelength 284 nm (an isosbestic point in Fig. 3): ● **2**, ▲ **2** plus D-fructose.

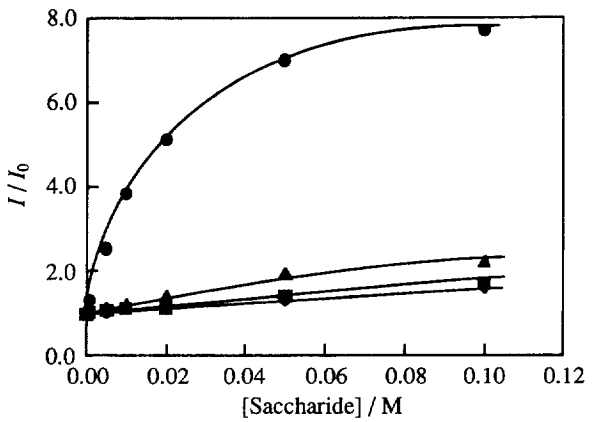


Fig. 9. Plots of I / I_0 (at 469 nm) versus [saccharide] in water:DMSO = 30:1 v/v at pH 8.0 and 25 °C, excitation wavelength 383 nm (an isosbestic point for the spectral change in the presence of saccharides): [**2**] = 1.00 x 10^{-5} M; ● D-fructose, ▲ D-arabinose, ■ D-mannose, ♦ D-glucose.

Conclusions. Compound **2** is a fluorescent molecule with a push-pull substituent couple and the pull substituent is the boronic acid which can bind saccharides. The foregoing spectroscopic studies demonstrate that **2** acts as a unique probe molecule for sugar sensing: *i.e.*, (i) saccharides can be detected by both absorption and fluorescence spectroscopic methods, (ii) in aqueous solution **2** aggregates because of hydrophobic and B···N interactions whereas it deaggregates when it complexes

saccharides; this change can be sensitively detected by a fluorometric methods, (iii) the magnitude of the fluorescence change in **2** is much larger than that in **3**, and (iv) upon the saccharide-binding the fluorescence intensity *decreases* in **3** whereas it *increases* in **2**; this situation is more favorable for sugar sensing. Further elaboration of molecular design and further application to other saccharides are currently continued in this laboratory.

Experimental

Materials

2-(4-Bromomethylphenyl)-1,3-dioxa-2-borinane (6). 2-(4-Methylphenyl)-1,3-dioxa-2-borinane (2.5 g, 14.2 mmol), *N*-bromosuccinimide (2.78 g, 15.6 mmol), and benzoyl peroxide (50 mg, 0.206 mmol) were dissolved in carbon tetrachloride (100 ml) and the solution was refluxed for 2 h under a nitrogen stream. After cooling the solution was filtered to remove the precipitate. The precipitate was washed with carbon tetrachloride, the filtrate being combined with the mother filtrate. The solution was washed with water and dried over $MgSO_4$. The concentration of the solution *in vacuo* gave white powder, which was recrystallized from ether-petroleum ether: mp 233-234 °C, yield 71%; IR(KBr)$\nu_{B\text{-}O}$ 1340 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$, 25 °C) δ 2.05 (CCH_2C, m, 2H), 4.15 (OCH_2, t, 4H), 4.49 ($BrCH_2$, s, 2H), 7.35 and 7.73 (ArH, d each, 2H each). Anal. Calcd for $C_{10}H_{12}BBrO_2 \cdot 1/15C_6H_{14}$: C, 47.91; H, 5.01%. Found: C, 48.22; H, 4.48%

2-(4-Diethylphosphorylmethyl)-1,3-dioxa-3-borinane (7). Compound **6** (2.0 g, 7.85 mmol) was dissolved in triethyl phosphate (40 ml) and the solution was refluxed for 7 h under a nitrogen stream. Unreacted triethyl phosphate was removed by distillation *in vacuo* and the residue was subjected to column chromatography (silica gel, ethyl acetate: *n*-hexane = 2:1 v/v). The product was oil: yield 70%; ^1H NMR (250 MHz, $CDCl_3$, 25 °C) δ 1.23 (CH_3, t, 6H), 2.01-2.10 (CCH_2C, m, 2H), 3.16 (PCH_2, d(J = 21.8 Hz), 2H), 3.92-4.04 ($POCH_2$, m, 4H), 4.16 ($BOCH_2$, t, 4H), 7.28 and 7.71 (ArH, d each, 2H each). Since the ^1H NMR spectrum is satisfactorily assigned to **7**, which was used for the next reaction without further purification.

[β-(4-Dimethylaminophenyl)]styryl-4-boronic Acid (2). To a DMF solution (100 ml) containing **7** (1.5 g, 4.81 mmol) was added *tert*-BuOK (0.54 g, 4.81 mmol). After cooling in an ice-water bath, a DMF solution (20 ml) containing 4-dimethylaminobenzaldehyde (0.72 g, 4.81 mmol) was added dropwise. The mixture was stirred at room temperature for 15 h. It was diluted with water (500 ml) and the precipitate was recovered by suction. The solid product was recrystallized from benzene: yellow powder, mp (dec) 278 °C, yield 17%; IR (KBr) ν_{OH} 3110-3600 cm^{-1}, $\nu_{B\text{-}O}$ 1340 cm^{-1}, $\nu_{C=C}$ 1590 cm^{-1}; ^1H NMR (250 MHz, DMSO-d_6, 25 °C) δ 2.95 (NCH_3, s, 6H), 6.80 and 7.47 (N-ArH, d each, 2H each), 6.99 and 7.20 (CH=CH, d each, 1H each), 7.50 and 7.77 (B-Ar$(OH)_2$, d, 4H). Anal. Calcd for $C_{16}H_{18}BNO_2 \cdot 1/6C_6H_6$: C, 72.87; H, 6.85; N, 5.00%. Found: C, 73.33; H, 6.55; N, 5.30%
λ_{max} (nm) (ε 10^4 dm^3 $mole^{-1}$ cm^{-1}) DMSO: 367 (3.41)

References

(1) Yoon, J.; Czarnik, A. W. *J. Am. Chem. Soc.* **1992**, *114*, 5874.

(2) Paugan, M. -F.; Smith, B. D. *Tetrahedron Lett.* **1993**, *34*, 3723.

(3) Nagai, Y.; Kobayashi, K.; Toi, H.; Aoyama, Y. *Bull. Chem. Soc. Jpn.* **1993**, *66*, 2965.

(4) (a) Tsukagoshi, K.; Shinkai, S. *J. Org. Chem.* **1991**, *56*, 4089. (b) Shiomi, Y.; Saisho, M.; Tsukagoshi, K.; Shinkai, S. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2111.

(5) (a) Kondo, K.; Shiomi, Y.; Saisho, M.; Harada, T.; Shinkai, S. *Tetrahedron* **1992**, *48*, 8239. (b) Shiomi, Y.; Kondo, K.; Saisho, M.; Harada, T.; Tsukagoshi, K.; Shinkai, S. *Supramol. Chem.* **1993**, *2*, 11.

(6) James, T. D.; Harada, T.; Shinkai, S. *J. Chem. Soc., Chem. Commun.* **1993**, 857 and 1176. (corrigendum)

(7) (a) Murakami, H.; Nagasaki, T.; Hamachi, I.; Shinkai, S. *Tetrahedron Lett.* **1993**, *34*, 6273. (b) Murakami, H.; Nagasaki, T.; Hamachi, I.; Shinkai, S. *J. Chem. Soc., Perkin Trans. 2* **1994**, 975. (c) Imada, T.; Murakami, H.; Shinkai, S. *J. Chem. Soc., Chem. Commun.* **1994**, 1557.

(8) (a) Sandanayake, K. R. A. S.; Shinkai, S. *J. Chem. Soc., Chem. Commun.* **1994**, 1083. (b) Sandanayake, K. R. A. S.; Nakashima, K.; Shinkai, S. *Ibid.* **1994**, 1621. (c) James, T. D.; Sandanayake, K. R. A. S.; Shinkai, S. *Ibid.* **1994**, 477.

(9) Deng, G.; James, T. D.; Shinkai, S. *J. Am. Chem. Soc.* **1994**, *116*, 4567.

(10) Wulff, G. *Pure & Appl. Chem.* **1982**, *54*, 2093.

(11) Nagasaki, T.; Shinmori, H.; Shinkai, S. *Tetrahedron Lett.* **1994**, *35*, 2201.

(12) Goto, T.; Kondo, T. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 17.

(13) James, T. D.; Sandanayake, K. T. A. S.; Shinkai, S. *J. Chem. Soc., Chem. Commun.* **1994**, 477.

(14) Lorand, J. P.; Edwards, J. O. *J. Org. Chem.* **1959**, *24*, 769.

(*Received in Japan* 30 *September* 1994; *accepted* 28 *November* 1994)