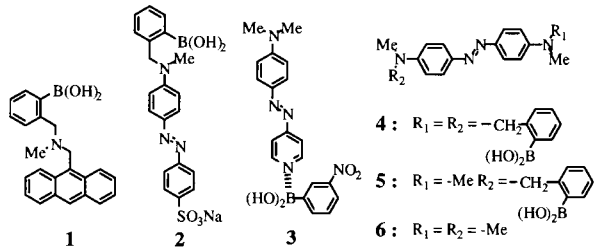
Colorimetric Sugar Sensing Method Useful in "Neutral"Aqueous Media

Kazuya Koumoto and Seiji Shinkai*
Department of Chemistry and Biochemistry, Graduate School of Engineering, Kyushu University, Fukuoka 812-8581

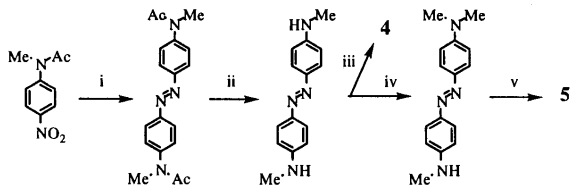
(Received April 21, 2000; CL-000380)

Azobenzene derivatives bearing one or two aminomethylphenylboronic acid groups were synthesized: they were confirmed to be useful for practical colorimetric saccharide sensing in "neutral" aqueous media.

The specific interaction between phenylboronic acids and saccharides or related compounds has been attracting increasing attention as a novel force for sugar recognition in an aqueous system.[1–8] Among them, the most successful example is based on fluorometric sensing coupled with photoinduced electron-transfer (PET).[1] Compound **1** is the typical example: the boronic acid group has an sp^3-hybridized orbital suitable to the saccharide-binding due to the intramolecular B–N interaction and the fluorescence quenching efficiency of the nitrogen base is sensitively affected by the saccharide-binding.[1,9] In fact, **1** acts as a practical saccharide sensor useful in 'neutral' aqueous media. Judging from convenience and simplicity of the reading-out system, however, colorimetric sensing seems to have many advantages over fluorometric sensing. To the best of our knowledge, there exist only a few colorimetric sensing methods using the boronic acid function. Compound **2**, in which the B–N interaction affects the intramolecular charge-transfer band in response to the saccharide-binding, changes it color in 'neutral' pH region, but the color change is not so large.[10] This is due to the low basicity of the amino group acting as an electron-donor group. To avoid this drawback compound **3** was developed, in which the pyridine nitrogen acting as an electron-acceptor group can interact with the boronic acid group more strongly.[11] As expected, a large color change was in fact induced in this intermolecular system[11] but the reformation of this system into a more convenient intramolecular system is not yet achieved. More recently, Strongin et al.[12] reported an interesting finding that a boronic acid-containing resorcin[4]arene results in various colors when it is heated with saccharides. However, this reaction is irreversible and cannot be applied as a saccharide sensor.



Taking these backgrounds into consideration, we newly designed compounds **4** and **5**: as the pK_a of the amino groups should be higher than that in **2**, one may expect the sufficient B–N interaction which eventually induces a large color change. Compound **6** was used as a reference compound.



Scheme 1. Reagents and conditions: i, $NH_2NH_2 \cdot H_2O$, Fe_2O_3, MeOH, 60 °C, 4 h then manganese(IV) oxide, chemicals treated (CMD) Molecular Sieb 4A, benzene, reflux, overnight (51%); ii, KOH in aqueous EtOH, reflux, 5 h (65%); iii, 2-(2-bromobenzyl)-1,3-dioxa-2-borinane, K_2CO_3, CH_3CN, overnight (1.7%); iv, MeI, K_2CO_3, $CHCl_3/CH_3CN$, 1 day (17%); v, 2-(2-bromobenzyl)-1,3-dioxa-2-borinane, K_2CO_3, CH_3CN, 2 h (8%).

Compounds **4** and **5** were synthesized from 4-nitro-*N*-methylacetanilide according to Scheme 1. The products were identified by IR, ^1H NMR and Mass (ESI TOF) spectral evidence and elemental analyses[13]. When the absorption spectra of these compounds were measured in MeOH/water mixtures at 25 °C, the distinct spectral change was observed in water-rich region. This spectral change is attributed to the aggregation equilibria. We thus employed a MeOH/water (pH 7.50 with 50 mmol dm^{-3} phosphate buffer) = 1:1 (v/v) mixture where the spectral changes satisfied the Lambert–Beer's law ([**4** or **5**] = $(0–5.00) \times 10^{-5}$ mol dm^{-3}).

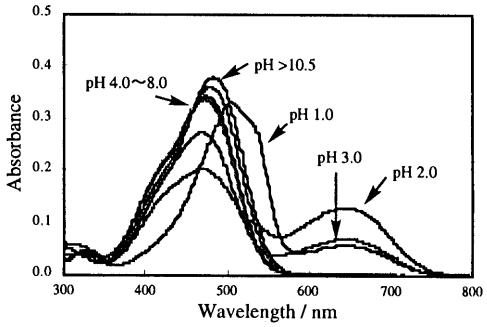


Figure 1. Typical examples of the pH-dependent absorption spectra of **5** (1.00×10^{-5} mol dm^{-3}).

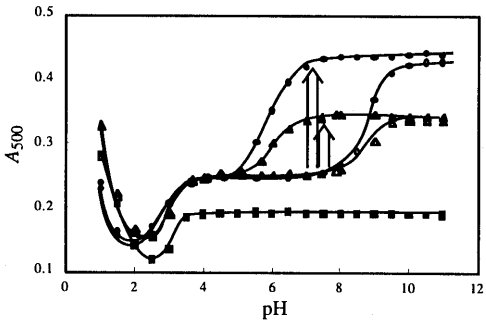


Figure 2. Phototitration of **4**, **5** and **6** (1.00×10^{-5} mol dm^{-3}): ○ and ● for **4**, △ and ▲ for **5** and □ and ■ for **6**. The filled points are those in the presence of D-fructose (0.100 mol dm^{-3}).

The typical example of the pH-dependent spectral change in **5** is shown in Figure 1. A plot of A_{500} vs pH (Figure 2) was obtained from this spectral change. Thus, the pK_{a1} (deprotonation of Me_2NH^+) and pK_{a2} [conversion of $B(OH)_2$ to $B^-(OH)_3$] were estimated to be 2.8 and 8.9, respectively. Phototitration of **4** gave rise to a similar pH-A_{500} profile with pK_{a1} = 3.1 and pK_{a2} = 8.7. In phototitration of **5** a few tight isosbestic points (e.g., at 457 nm) appeared in pK_{a2} region whereas in phototitration of **4** they were significantly divergent. The difference indicates that in **4** two boronic acid groups form the OH^--adducts in a stepwise manner. However, the difference in their pK_{a2} values is so small that the titration curve is apparently analyzable as a single acid dissociation process.

In the presence of 0.100 mol dm^{-3} D-fructose (which shows the highest affinity with monoboronic acids[1–4,7–11]), the pK_{a2} values largely shifted to lower pH region: pK_{a2} = 5.8 for **4** and 5.9 for **5** (Figure 2). Also in the presence of D-fructose, phototitration of **5** resulted in a few tight isosbestic points whereas that of **4** resulted in significantly divergent isosbestic points. The difference implies that the saccharide-binding to the two boronic acid groups in **4** also occurs in a stepwise manner. It is clearly seen from Figure 2 that large spectral changes are induced by the saccharide-binding at pH 6–9. The findings support the view that these boronic acid-containing azobenzene derivatives are useful for practical colorimetric saccharide sensing in "neutral" pH region. We have found that a distinct color change (from yellow to pinkish red) is observable upon addition of saccharides. In particular, both of the saccharide-induced pK_{a2} shift and the absorbance change are much larger in diboronic acid-containing **4** than in monoboronic acid-containing **5**. For **6** which has no boronic acid group, the perceptible spectral change was not induced at pH 4–11 by the saccharide addition (Figure 2).

To estimate the association constants with monosaccharides the absorption spectra were measured as a function of saccharide concentrations. The spectral change in **5** again held tight isosbestic points. The plots of A_{480} vs [saccharide] are shown in Figure 3. Assuming the formation of 1:1 complexes, the association constants (K_1 / dm^3mol^{-1}) were estimated to be 433 ± 8 for D-fructose, 13 ± 0.2 for D-glucose, and 127 ± 3 for D-ribose. The K_1 values appear in the order of D-fructose > D-ribose > D-glucose, which is in line with the general affinity order of monoboronic acids for monosaccharides.[1,2,4,14] The absorption spectral change in **4** again featured the divergent isosbestic points, suggesting that the saccharide-binding occurs in a stepwise manner. It is known that when a diboronic acid forms a macrocyclic 1:1 structure with a saccharide, the resultant complex

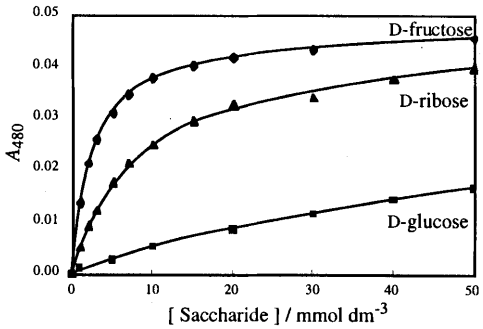


Figure 3. Plots of A_{480} *vs.* [saccharide]: 25 ° C, MeOH/water (pH 7.50 with 50 mmol dm^{-3}) = 1:1 (v/v), [**5**] = 1.00×10^{-5} mol dm^{-3}, [saccharide] = $(1.00–50)\times10^{-3}$ mol dm^{-3}.

becomes CD-active.[1,7–10] Under the present spectral measurement conditions, no CD-active species was recognized. The finding implies that both 1:1 and 1:2 complexes with **4** are noncyclic species. Thus, the K_1 for the formation of 1:1 complexes (= [**4**·saccharide]/[**4**] [saccharide]) and K_2 for the formation of 1:2 complexes from 1:1 complexes (= [**4**·$(saccharide)_2$]/[**4**·saccharide] [saccharide]) were estimated by a nonlinear least-squares computation method, assuming that the ε values of the 1:1 complexes are approximated by those of the **5**·saccharide complexes and that the 1:2 complexes are formed at the plateau region in phototitration of **4**: K_1 = 797 ± 74 and K_2 = 320 ± 29 for D-fructose, K_1 = 16 ± 4 and K_2 (too small to determine) for D-glucose, and K_1=355 ± 10 and K_2 = 69 ± 2 for D-ribose. Again, both the K_1 and K_2 values appear in the order of D-fructose > D-ribose > D-glucose. Although observed saccharide selectivity is common between **4** and **5**, one can raise a merit of **4** over **5**: as seen from Figure 2, Δ*A* at pH 7.5 induced by the saccharide addition in **4** (e.g., 0.13 at maximum wavelength for D-fructose) is greater than that in **5** (0.05). This leads to the high sensitivity in colorimetric saccharide sensing.

In conclusion, this paper demonstrates the first example for the practical colorimetric sensing system useful even in "neutral" aqueous media. The breakthrough has been brought forth owing to the preparation of an electron-rich dye-conjugated nitrogen for the B–N interaction. We believe that this basic concept can be further elaborated to "selective" saccharide sensing by a colorimetric method.

References and Notes

1 For recent comprehensive reviews see a) T. D. James, K. R. A. S. Sandanayake, and S. Shinkai, *Supramol. Chem.*, **6**, 141 (1995). b) T. D. James, P. Linnane, and S. Shinkai, *Chem. Commun.*, **1996**, 281 c) T. D. James, K. R. A. S. Sandanayake, and S. Shinkai, *Angew. Chem., Int. Ed. Engl.*, **35**, 1910 (1996).
2 a) J. Yoon and A. W. Czarnik. *J. Am. Chem. Soc.*, **114**, 5784 (1992). b) L. K. Mohler and A. W. Czarnik, *J. Am. Chem. Soc.*, **115**, 2998 (1993).
3 a) M. F. Paugam and B. D. Smith, *Tetrahedron Lett.*, **34**, 3723 (1993). b) G. T. Morin, M. P. Hughes, M. -F. Paugam, and B. D. Smith, *J. Am. Chem. Soc.*, **116**, 8895 (1994). c) P. R. Westmark and B. D. Smith, *J. Am. Chem. Soc.*, **116**, 9343 (1994).
4 Y. Nagai, K. Kobayashi, H. Toi, and Y. Aoyama, *Bull. Chem. Soc. Jpn.*, **66**, 2965 (1993).
5 a) G. Wulff, B. Heide, and G. Helfmeier, *J. Am. Chem. Soc.*, **108**, 1089 (1986). b) G. Wulff and H. -G. Poll, *Makromol. Chem.*, **188**, 741 (1987).
6 C. R. Cooper and T. D. James, *Chem. Commun.*, **1997**, 1419.
7 M. Takeuchi, M. Yamamoto, and S. Shinkai, *Chem. Commun.*, **1997**, 1731.
8 T. Mizuno, M. Takeuchi, I. Hamachi, K. Nakashima, and S. Shinkai, *Chem. Commun.*, **1997**, 1793.
9 T. D. James, K. R. A. S. Sandanayake, and S. Shinkai, *J. Chem, Soc., Chem. Commun.*, **1994**, 477.
10 K. R. A. S. Sandanayake and S. Shinkai, *J. Chem. Soc., Chem. Commun.*, **1994**, 1083.
11 K. Koumoto, M. Takeuchi, and S. Shinkai, *Supramol. Chem.*, **9**, 203 (1998).
12 C. J. Davis, P. T. Lewis, M. E. McCarroll, M. W. Read, R. Cueto, and R. M. Strongin, *Org. Lett.*, **1**, 331 (1999).
13 **4**: mp = 221.1–223.3 °C. ^{1}H NMR ($CD_3OD/CDCl_3$) δ 3.04 (s, 6H, NCH_3), 6.89, 7.25–7.33, 7.68 (m, 16H, ArH); benzyl protons are overlapped with a solvent peak. ESI-MASS (positive mode, $CHCl_3$/MeOH) Calcd *m/z* 509.25, 523.26, 537.28, 551.29, 565.31, Obsd *m/z* 509.54, 523.52, 537.57, 551.60, 565.58. Found: C, 65.86; H, 5.98; N, 10.72%. Calcd for $C_{16}H_{20}N_4\cdot0.1H_2O$: C, 65.93; H, 5.98; N, 10.98%.
5: mp= 134.5–139.0 °C. ^{1}H NMR ($CDCl_3$) δ 2.75, 3.08 (s, 9H, NCH_3), 4.36 (s, 2H, NCH_2Ar), 6.76, 7.23, 7.23, 7.40, 7.82, 7.84, 7.90 (m, 12H, ArH). ESI-MASS (positive mode, MeOH) Calcd *m/z* 388.21, 402.22, 416.24, Obsd *m/z* 388.05, 402.06, 416.07. Found: C, 67.72; H, 6.49; N, 13.44%. Calcd for $C_{16}H_{20}N_4\cdot0.25H_2O$: C, 67.75; H, 6.88; N, 13.17%.
14 J. P. Lorand and J. O. Edwards, *J. Org. Chem.*, **24**, 769 (1959).