Selective Detection of D-Lactulose by a Porphyrin-based Diboronic Acid

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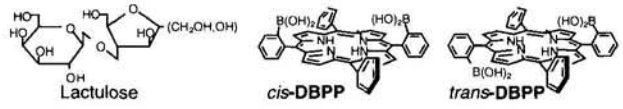
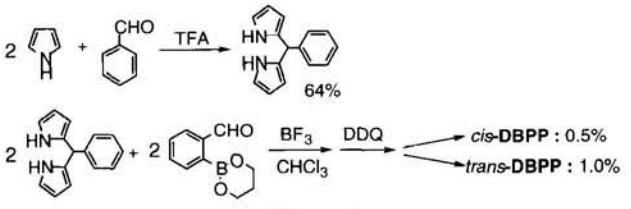
Cis-5,15-bis[2-(dihydroxyboronyl)phenyl]-10,20-diphenylporphine (*cis*-DBPP) bearing two boronic acid groups in a 9 Å distance has been synthesized. The absorption, fluorescence, and CD spectral changes were induced only by D-lactulose among 10 disaccharides tested herein. One can readily sense D-lactulose by the spectroscopic methods.

We have recently demonstrated the usefulness of the boronic acid function as a saccharide receptor in an aqueous system.[1] It is known that among monosaccharides, D-fructose shows the highest affinity with monoboronic acids[1-5] while D-galactose is frequently bound to diboronic acids forming macrocyclic 1:1 complexes.[6] Careful examination of the D-lactulose structure teaches us that this disaccharide consists of D-galactose and D-fructose. Hence, D-lactulose should be favorably bound to a diboronic acid receptor if the distance between the two boronic acids is appropriately designed. The possible boronic-acid-binding sites in D-lactulose are the 1,2- or 2,3-diol in the D-fructose moiety and the 4',6'-diol in the D-galactose moiety, the distance being *ca.* 9 Å. It is not so easy, however, to immobilize two boronic acid groups with a 9 Å distance on the rigid matrix. We noticed that this requirement may be satisfied with *cis*-5,15-bis[2-(dihydroxyboronyl)phenyl]-10,20-diphenylporphine (*cis*-DBPP): the porphyrin moiety can serve as a rigid matrix[7] and two boronic acid groups exactly construct a cleft with a 9 Å distance. We have found that the spectroscopic properties of *cis*-DBPP respond only D-lactulose among 10 disaccharides tested herein.



Cis-DBPP and *trans*-DBPP were synthesized according to Scheme 1. Two isomers were separated by a preparative TLC method (silica gel, $CHCl_3$:EtOAc = 5:1: R_f = 0.23 for *cis*-isomer and 0.73 for *trans*-isomer). The products (mp > 300 °C for *cis*-isomer and > 300 °C for *trans*-isomer) were assigned by IR, ^1H NMR, and Mass[8] spectral evidence and elemental analyses. The structural difference between two isomers could not be discriminated by 400 MHz ^1H NMR but a significant difference did appear in 600 MHz ^1H NMR.[9] It was confirmed that as long as the solution is kept below 15 °C, *cis-trans* isomerization can be suppressed.



Scheme 1.

Firstly, we tested many commercially-available monosaccharides but none of them could change the spectroscopic properties of *cis*-DBPP.

Here, we tested commercially-available 10 disaccharides.[10] Among them, only D-lactulose induced significant spectral changes. In CD spectroscopy, only D-lactulose gave a CD-active species with λ_{max} 416.5 nm (Figure 1). As already shown in the related systems,[1,6] the result is indicative that D-lactulose bridges the two boronic acid groups, forming an intramolecular 1:1 complex. As expected, the Job plot ([*cis*-DBPP] + [D-lactulose] = 5.00×10^{-5} mol dm^{-3} (constant)) afforded a maximum CD intensity at 0.5, supporting the formation of the 1:1 complex. On the basis of previous work, it is expected that *cis*-DBPP and D-lactulose form an intramolecular complex using 2,3- and 4',6'-diols as the binding sites.[11,12] Such a CD-active species did not appear from a *trans*-DBPP + D-lactulose system.

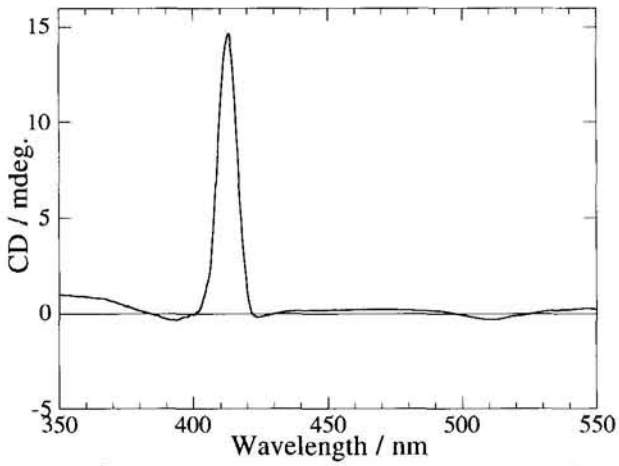


Figure 1. CD spectrum of *cis*-DBPP (4.0×10^{-5} mol dm^{-3}) in the presence of D-lactulose (2.7×10^{-2} mol dm^{-3}): methanol, 15 °C, cell length 1.0 cm.

The absorption spectra of *cis*-DBPP is shown in Figure 2. Again, a significant increase in the absorption bands was observed only for D-lactulose. Although the spectral change is relatively small, one can estimate the association constant (K_{ass}) from Figure 2. By analyzing of *A* and [D-lactulose] using a Nagakura's method,[13] the K_{ass} for the 1:1 complex was estimated to be 560 ± 30 dm^3 mol^{-1}.

It is known that when a fluorescent diboronic acid is intramolecularly bridged by a saccharide, the fluorescence intensity increases owing to the rigidification effect.[14,15] Such a phenomenon should be also observable for the 1:1 complex of *cis*-DBPP and D-lactulose. The 417 nm in the Soret band was used for the excitation where an isosbestic point appeared in the D-lactulose-dependent absorption spectral change. The fluorescence spectral change is shown in Figure 3. As expected, the fluorescence intensity increased only when D-lactulose was

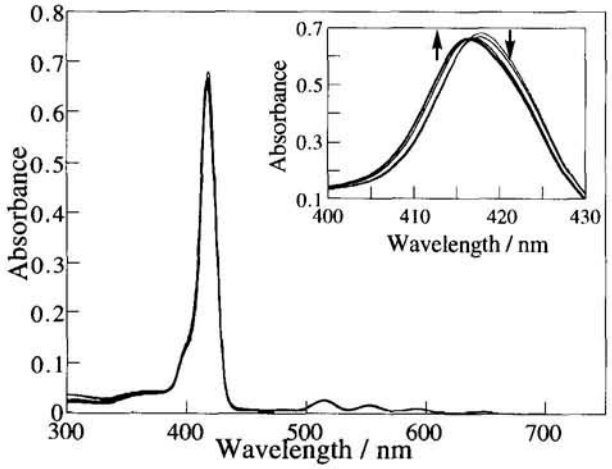


Figure 2. Absorption spectrum of *cis*-DBPP (2.0×10^{-6} mol dm^{-3}) and the spectral change in the presence of D-lactulose ($0\sim6.0 \times 10^{-3}$ mol dm^{-3}): methanol, 15 °C.

added. From the analysis of a plot of the fluorescence intensity *vs.* [D-lactulose][16] we obtained the K_{ass} 560 ± 50 dm^3 mol^{-1}. Although the *cis*-DBPP concentration used here is lower by 25-fold than that used for the measurements of the absorption spectra, the K_{ass} value shows a good agreement with that determined by the absorption spectral change.

In conclusion, *cis*-DBPP has turned out to act as a potential diboronic-acid-based receptor for D-lactulose. This means that as described in introduction, D-lactulose consisting of D-fructose and D-galactose is spacially recognizable among disaccharides with the similar molecular size. The binding process forming the macrocyclic 1:1 complex can be readily estimated by the spectroscopic methods using the porphyrin moiety as a chromophore. D-Lactulose (4-*O*-*β*-D-galactopyranosyl-D-fructose) is one of the most important and well-known oligosaccharides which affects the proliferation of *Lactobacillus bifidus* and lactic acid bacteria and controls the pH by the production and absorption of ammonia in the lowels.[17] It is also useful as an effective medicine for suppression of the urea production and treatment of hepatic encephalophathy.[18] In spite of its importance in the medical chemistry field, the effective artificial receptor which can selectively bind D-lactulose has not yet developed. Obviously, it seems very important to design a new analytical system which is useful under physiological conditions. We are now planning to prepare the metal complex derivatives and apply them to a catalytic decomposition of D-lactulose.

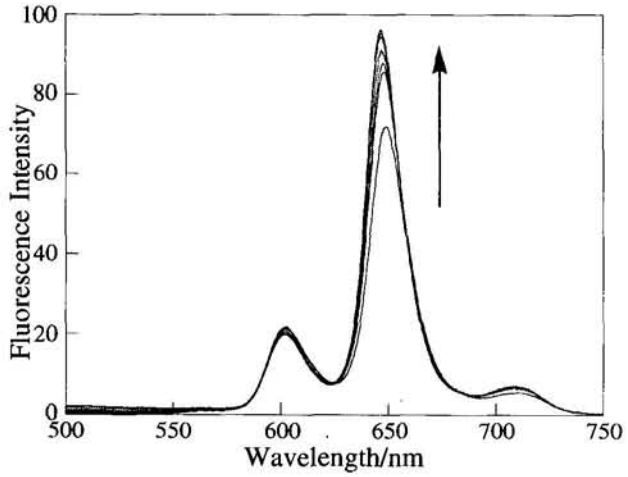


Figure 3. Fluorescence spectral change in *cis*-DBPP (2.0×10^{-6} mol dm^{-3}) in the presence of D-lactulose ($0\sim2.9 \times 10^{-2}$ mol dm^{-3}): methanol, 15 °C, excitation 417 nm.

References and Notes

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8 Positive SIMS: m/z 815, $[MH + 2glycerol\text{-}4H_2O]^+$.
9 600 MHz ^1H NMR spectrum of *cis*-DBPP ($CDCl_3$, TMS): δ = -2.70 (s, 2H, NH of pyrrole), 7.70-7.80 (m, 8H, ArH), 7.84 (t, 2H, ArH), 8.04 (d, 2H, ArH), 8.12 (d, 2H, ArH), 8.23-8.25 (m, 2H, ArH), 8.33 (d, 2H, ArH), 8.72 (d, 4H, CH of pyrrole), 8.85 (d, 4H, CH of pyrrole).
10 Disaccharides tested herein are D-maltose, D-cellobiose, D-lactose, D-saccharose, D-isomaltose, D-melibiose, D-trehalose, D-gentiobiose, D-turanose, and D-xylosyl-D-fructoside.
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