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Molecular Design of a PET-based Chemosensor for Uronic Acids and Sialic Acids Utilizing a Cooperative Action of Boronic Acid and Metal Chelate

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Abstract: To exploit new artificial receptors for detection of uronic acids and sialic acids in an aqueous system a novel fluorescent chemosensor molecule bearing both *o*-aminomethylphenylboronic acid group for diol binding and 1,10-phenanthroline-Zn(II) chelate moiety for carboxylate binding has been synthesized. The saccharide-binding process can be conveniently monitored by a fluorescence change which occurs by the photoinduced electron-transfer (PET) mechanism through a unit integrated in this molecule. Fluorescence measurements indicate that association for commonly occurring monosaccharides is little affected by Zn(II) addition whereas that for uronic acids or sialic acids is enhanced in the presence of Zn(II) owing to the two-points interaction. pH Dependence, stoichiometry of the complexes, and complexation modes have been examined. This artificial receptor shows substantial affinity toward uronic acids and sialic acids in aqueous methanol solution.

INTRODUCTION

Saccharides play many crucial roles in the metabolic cycles such as energy storage, cell recognition, enzyme activity control, *etc*. Among them, uronic acids are known to appear in the oxidation process of monosaccharides or in the biosynthetic process of L-ascorbic acid.[1] Furthermore, sialic acids are frequently used as a recognition unit in influenza viruses.[2] Thus, the selective detection of a uronic acid and a sialic acid family is an important research subject in a biological chemistry. The quantitative analysis of uronic acids and sialic acids has so far been accomplished by classical colorimetic methods such as naphthoresorcine-HCl[3] and carbazole-H_2SO_4.[4] Obviously, these methods are quite troublesome and time-consuming. Hence, it seems very important to develop a new, convenient analytical method which is useful in an aqueous system and in addition, shows high selectivity and sensitivity toward selected uronic acids and sialic acids even under the coexistence of neutral saccharides. We have currently been demonstrating the usefulness of the boronic acid

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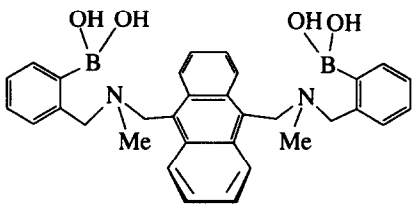
function as a saccharide receptor in an aqueous recognition system.[5] Judging from the structure of uronic acids and sialic acids, a molecule including both boronic acid moiety for the binding of diol groups and metal chelate moiety for the binding of carboxylate groups should lead to an excellent uronic acid and sialic acid receptor. To realize high sensitivity in addition to high selectivity it is desirable to combine this system with the concept of a photoinduced electron-transfer (PET) chemosensor.[6,7] We previously designed compound **1** which selectively binds 1,2- and 4,6-diols of glucose with two boronic acids and reports the binding event by a fluorescence change in the PET mechanism.[8,9] Here, we have rationally designed a molecular chemosensor **2** for the selective binding of uronic acids and sialic acids in which one boronic acid group in **1** is replaced by a metal chelation site. Since a phenanthroline moiety (probably including its 2-phenyl group) serves as a fluorophore, the anthracene moiety in **1** can be replaced by the phenyl group in **2**. We have found that the Zn(II) complex of this new molecular sensor selectively responds to uronic acids and sialic acids in neutral aqueous solution.[10]



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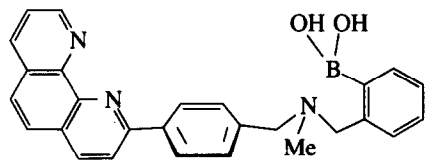
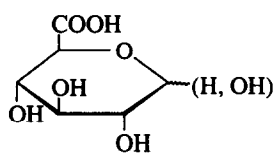


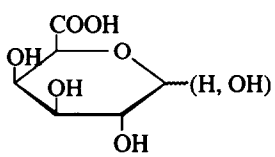
2

RESULTS AND DISCUSSION

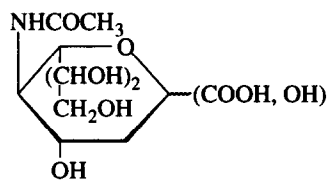
Molecular Design of the Receptor for Uronic Acids and Sialic Acids. Being different from conventional monosaccharides, a family of uronic acids and sialic acids has both a sugar structure and a carboxylic acid group within a molecule. D-Glucuronic acid, D-galacturonic acid, and *N*-acetylneuraminic acid are typical among such saccharide derivatives. In particular, sialic acid frequently occurs as a terminal unit in glycoproteins and glycolipids. Therein, it influences their conformations or physiological functions and sometimes, serves as their identification tags.[11]



D-Glucuronic acid

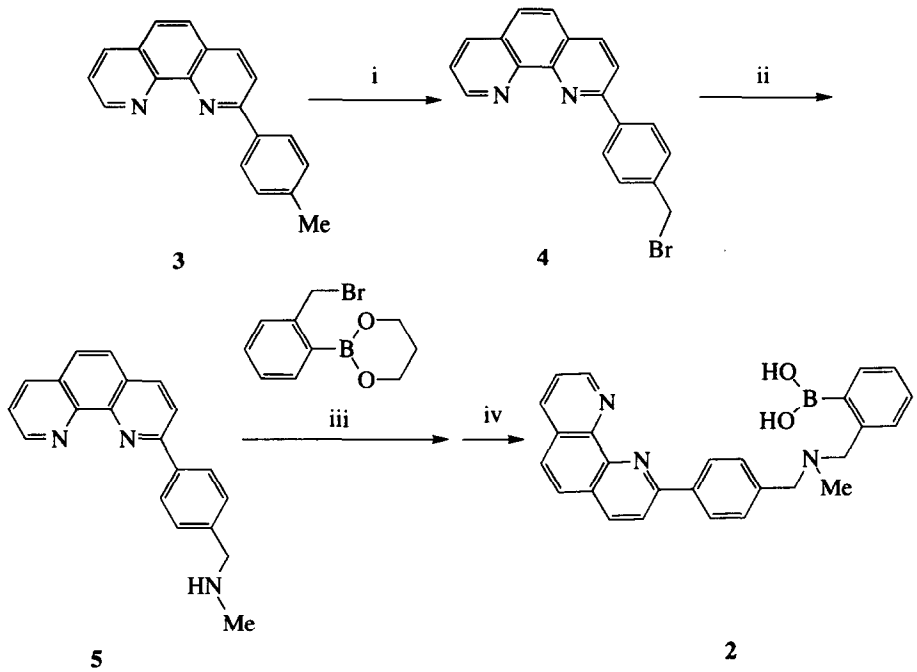


D-Galacturonic acid



Sialic acid
(*N*-Acetylneuraminic acid)

Thus, the synthesis of **2** was achieved from 2-(*p*-tolyl)-1, 10-phenanthroline[12] by the route outlined in Scheme 1. Compound **2** (mp 166-169 °C) was identified by IR and ^{1}H NMR spectroscopic evidence and elemental analysis (see Experimental).



Scheme 1. *Reagents and conditions* (yields): i, NBS, AIBN, CCl_4, reflux ; ii, $MeNH_2$, CCl_4 (30 %, calculated from **3**) ; iii, K_2CO_3, MeCN, reflux ; iv, H_2O (40 %)

Absorption Spectra of 2. Since compound **2** is not so soluble in water, we used a mixed medium of water : MeOH = 1 : 2 (v/v) to avoid the self-aggregation. In this medium, the absorption spectra of **2** satisfied Lambert-Beer's law with a linear A_{290} versus [**2**] plot ([**2**] = 0 ~ 0.50 mmol dm^{-3}). One can thus regard that **2** is discretely dissolved into this medium. As shown in Fig. 1, the absorption maximum of **2** shifted from 301 nm to 290 nm by a pH change from 2.0 to 7.5 with a tight isosbestic point (at 294 nm). This change corresponds to deprotonation of the chromophoric 1,10-phenanthroline moiety ($2H_2 \rightleftharpoons 2H + H^+$: see Scheme 2). The absorption spectra did not change any more at higher pH region. A plot of A_{290} versus pH is shown in Fig. 2. From analysis of this plot the pK_{a1} was estimated to be 4.4. On the other hand, it was difficult to determine the pK_{a2} and pK_{a3} from the absorption spectroscopic measurements. The similar experiment was repeated in the presence of D-fructose (100 mmol dm^{-3}) which is known to form the stablest complex with monoboronic acids.[8a] We obtained the similar spectral change as Fig. 1. The plot is shown in Fig. 2. The result indicates that the boronic acid-D-fructose complexation does not affect the proton dissociation of the 1,10-phenanthroline moiety.

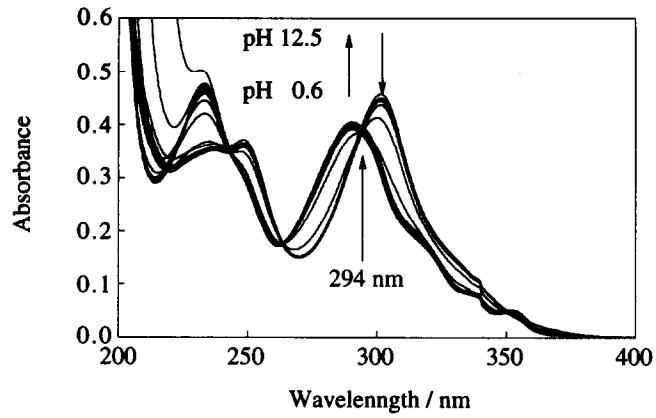


Fig. 1. pH-dependent absorption spectral change in **2** (1.00×10^{-5} mol dm^{-3}): 25 °C, water : MeOH=1 : 2 (v/v). The pH was adjusted with HCl and NaOH. The pH correction was difficult for several reasons.[13] Hence, the "pH" values determined by a glass-electrode pH meter were used herein.

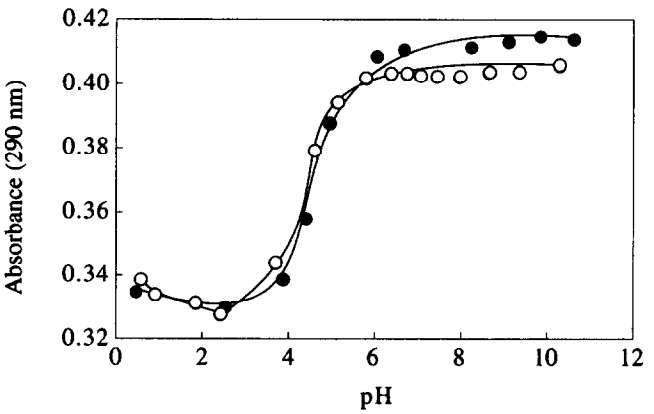
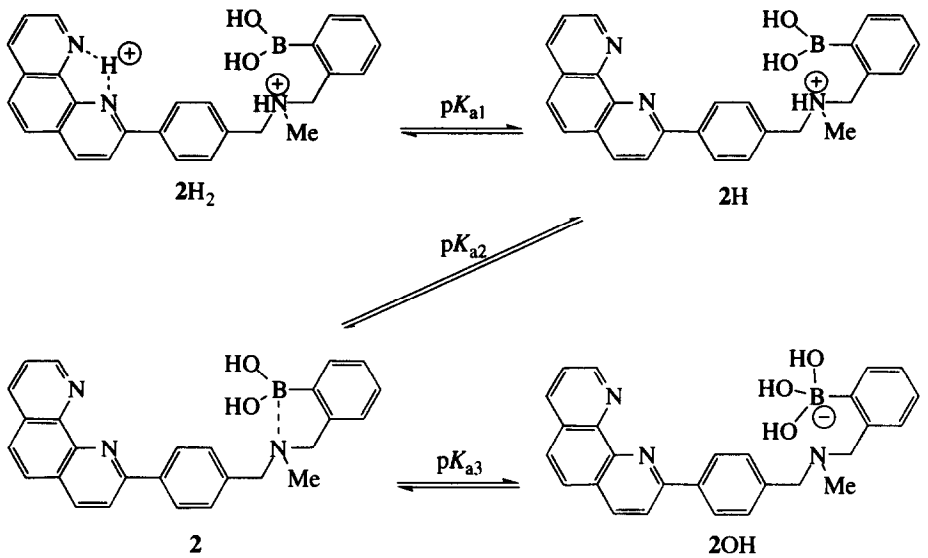
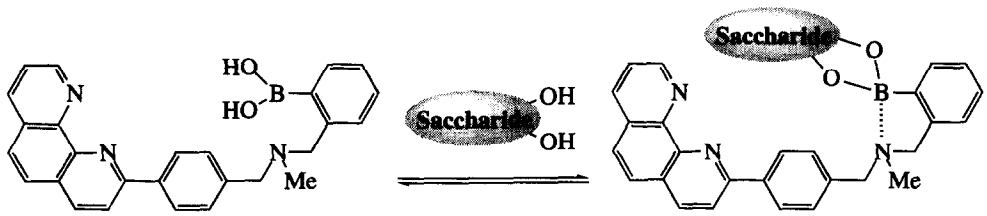


Fig. 2. Plots of absorbance (290 nm) versus pH: O in the absence of saccharide, ● in the presence of D-fructose (100 mmol dm^{-3}).[13]



Scheme 2

Fluorescence Spectra of 2. The foregoing results indicate that absorption spectroscopy is not useful to estimate the association constant (K_{ass}) between the boronic acid group and saccharides. We thus applied fluorescence spectroscopy to the present system. Although the distance between the 1,10-phenanthroline moiety and the *o*-aminomethylphenylboronic acid moiety is relatively long, the nitrogen base may be effective enough to quench the singlet excited state intramolecularly. Particularly, if the 2-phenyl group is included in a conjugation system of the 1,10-phenanthroline moiety, the intramolecular fluorescence quenching should occur efficiently as already observed for other related PET chemosensor systems.[5~8] Since the isosbestic point in the absorption spectra appeared at 294 nm, this wavelength was used for excitation. The typical fluorescence spectra were shown in Fig. 3. The fluorescence spectra in the presence of D-fructose (100 mmol dm^{-3}) were similar to those in Fig. 3. Thus, the fluorescence maximum at 375 nm was plotted as a function of medium pH in Fig. 4. It is seen from Fig. 4 that in the absence of saccharide, the fluorescence intensity increases at pH 2 ~ 6, which corresponds to deprotonation of the 1,10-phenanthroline moiety. This change gives again pK_{a1} 4.4. The fluorescence intensity decrease starting from pH 5.5 and pH 10 corresponds to deprotonation of the tertiary amine (pK_{a2}) and OH^- adduct formation with the boronic acid group (pK_{a3}), respectively. Because of the overlap of the dissociation groups the pK_{a2} could be only approximately estimated to be *ca.* 7 or a little lower than this, whereas the pK_{a3} was too high to be determined accurately ($pK_{a3} > 12$). In the presence of D-fructose, the fluorescence intensity at pH 6 ~ 11 is much stronger than that in the absence of the saccharide. This is due to the B-N interaction intensified by complexation of saccharide with the boronic acid group: that is, saccharide complexation lowers the pK_{a3} and the fluorescence quenching ability of the nitrogen base is weakened because of the intensified B-N interaction.[8]



2
(weakly fluorescent)

2·Saccharide
(strongly fluorescent)

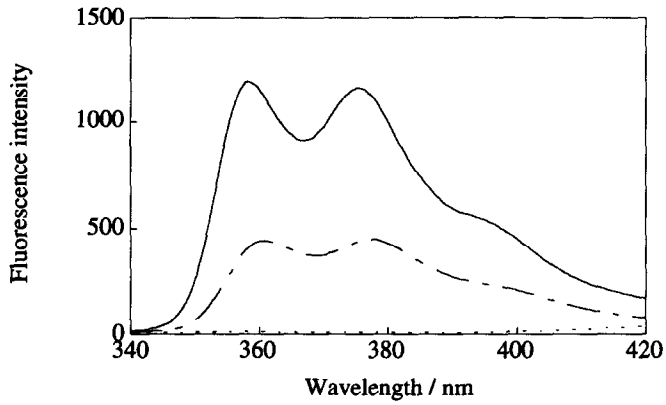


Fig. 3. Fluorescence spectra of **2** (1.00×10^{-5} mol dm^{-3}): 25 °C, water : MeOH=1 : 2 (v/v), excitation 294 nm,------- pH 0.6, ——— pH 5.8, -·-·-· pH 12.5.[13]

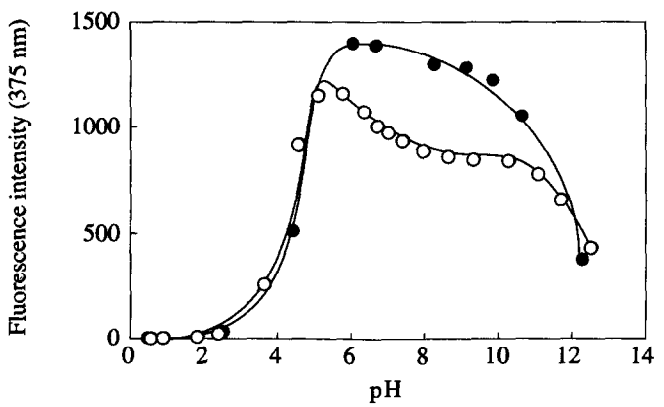


Fig. 4. Plots of the fluorescence intensity (375 nm) versus pH: O in the absence of saccharide, ● in the presence of D-fructose (100 mmol dm^{-3}).[13]

Taking an advantage that the fluorescence intensity increases at pH 6~11, one can estimate the K_{ass} values for the complexation of saccharides with **2**. Here, we chose pH 8.0 for the K_{ass} determination for several reasons: that is, (i) to efficiently utilize the PET mechanism the *o*-aminomethyl group should not be protonated while the boronic acid group should not be a sp^3-hybridized OH^--adduct, (ii) the fluorescent 1,10-phenanthroline moiety should not be protonated, and (iii) in the subsequent experiments the K_{ass} values have to be compared with those in the presence of Zn(II), so that the pH should not be so alkaline but not so acidic as to protonate the 1,10-phenanthroline moiety. The pH 8.0 satisfies these requirements and in fact, the fluorescence intensity gap at pH 8.0 is the largest (see Fig. 4: I_F and I_0 are the fluorescence intensities in the presence and the absence of saccharides, respectively). In Fig. 5, the fluorescence intensity (at 375 nm) was plotted against the saccharide concentration. Unfortunately, the fluorescence intensity change for D-glucuronic acid, sialic acid, and D-glucose was too small to estimate the K_{ass}. For other saccharides, the K_{ass} values could be determined using a Benesi-Hildebrand equation for the formation of a 1:1 complex (correlative coefficient > 0.99).[14] The results are summarized in Table 1.

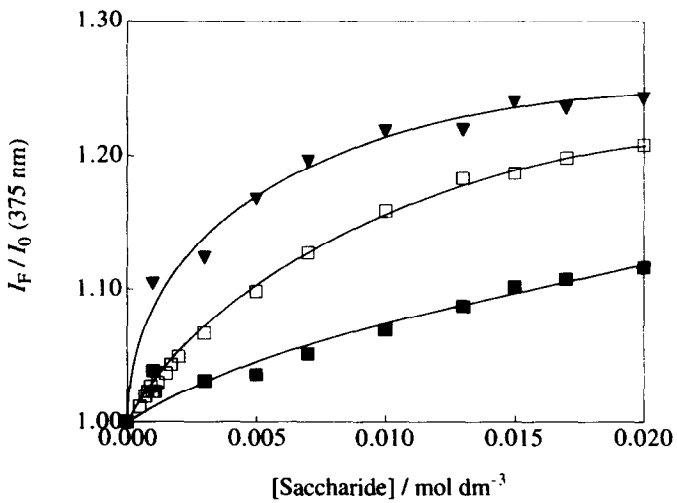


Fig. 5. Plots of the relative fluorescence intensity (I_F / I_0 at 375 nm) versus [saccharide]: [**2**]=1.00 × 10^{-4} mol dm^{-3}, □ D-galacturonic acid, ■ D-galactose, ▼ D-fructose.

Table 1. Association constnts (log K_{ass}) for saccharides with **2** and **2**·Zn(II)[a]

	log K_{ass} (I_{max}/I_0)[c]	
Saccharide	**2**	**2**·Zn(II)
D-Glucuronic acid	_[b]	3.4 (2.11)
D-Galacturonic acid	1.9 (1.15)	3.1 (3.69)
Sialic acid (*N*-Acetylneuraminic acid)	_[b]	2.3 (1.83)
D-Glucose	_[b]	_[b]
D-Galactose	1.7 (1.12)	1.3 (1.50)
D-Fructose	2.5 (1.33)	2.4 (1.90)

[a] pH 8.0 (5.0 mmol dm^{-3} MOPS buffer), water : MeOH = 1 : 2 (v/v).

[b] Too small to determine (log K_{ass}<1).

[c] I_{max} is the fluoresence intensity at [saccharide] = 0.020 mol dm^{-3} where I_F is nearly saturated (except D-glucuronic acid: 0.0050 mol dm^{-3}, D-galactose and D-fructose: 0.10 mol dm^{-3}).

Examination of Table 1 reveals that the affinity of saccharides with the *o*-aminomethylphenylboronic acid is generally low at pH 8.0: only D-fructose (which possesses the high affinity with monoboronic acids),[8a] D-galactose (which show the relatively high affinity),[8a] and D-galacturonic acid provide the fluorescence intensity change large enough to determine the K_{ass}.

Complexation with Zn(II). To serve as a receptor site for the carboxylate anion the relatively "hard" metal ion is suitable. We thus tested Ca(II) and Zn(II). As described below, the complexation behaviors of Zn(II) which have the higher affinity with the 1,10-phenanthroline moiety are simpler than those of Ca(II). Hence, Zn(II) was used in the following experiments.

As preliminary steps to use Zn(II) in the present system, one has to answer several questions: for example, (i) to simply analyze the saccharide-binding process the 1,10-phenanthroline moiety should be entirely converted to the Zn(II) complex, (ii) the stoichiometry of Zn(II) versus **2** under the working conditions must be determined, and (iii) it is undoubted that the 1,10-phenanthroline moiety coordinates to Zn(II) but the coordination of the *o*-aminomethyl group should be also assessed. The addition of Zn(II) (added as $Zn(NO_3)_2 \cdot 6H_2O$) decreased the UV region of the absorption spectrum both in the absence and the presence of D-galacturonic acid (Fig. 6A). Plots of the absorption maximum (290 nm) against [Zn(II)] indicate that the absorbance decrease has been saturated at [Zn(II)]/[**2**] = 4 (Fig. 6B). In fluorescence spectroscopy, the addition of Zn(II) decreased the fluorescence intensity in the absence of D-galacturonic acid but increased it in

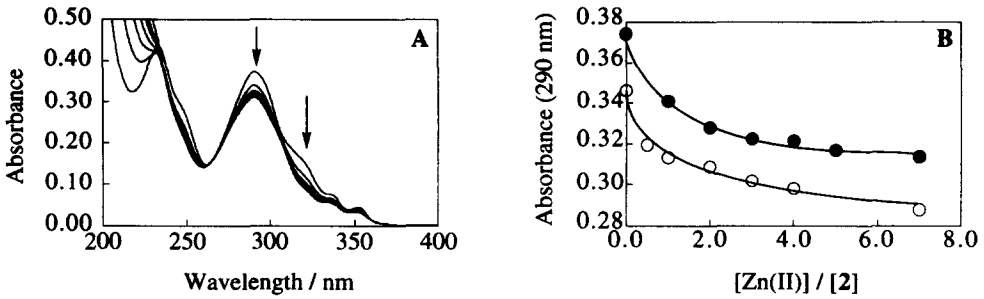


Fig. 6. (A) Influence of added Zn(II) on the absorption spectra of **2** (1.00×10^{-4} mol dm^{-3}) in the presence of D-galacturonic acid (1.00×10^{-2} mol dm^{-3}) and (B) plots of the absorbance (290 nm) versus [Zn(II)] / [**2**], O in the absence of saccharide and ● in the presence of D-galacturonic acid (1.00×10^{-2} mol dm^{-3}): 25 °C, water : MeOH = 1 : 2 (v/v), [Zn(II)] = 0 ~ 7.00×10^{-4} mol dm^{-3}, pH 8.0 with 5.0 mmol dm^{-3} MOPS buffer. The absorption spectral change in the absence of D-galacturonic acid is not shown here, but it is similar to (A). To achieve the saturation of the plot in (B) at the low [Zn(II)] / [**2**] ratio, the concentration of **2** was enhanced up to 1.00×10^{-4} mol dm^{-3}. Instead, a quartz cuvette with 0.1 cm thickness was used here.

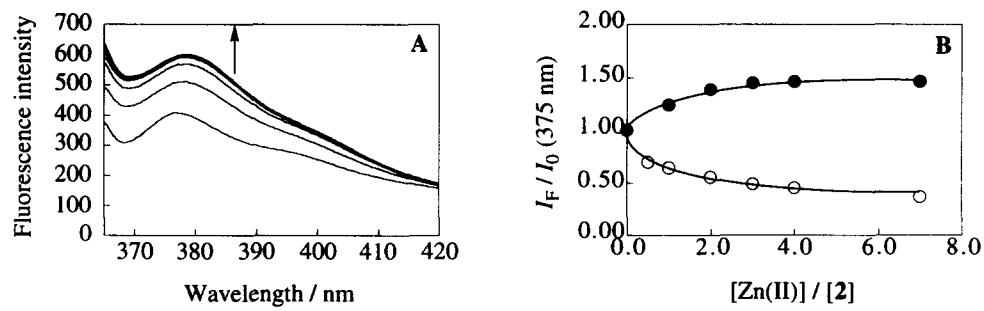


Fig. 7. (A) Influence of added Zn(II) on the fluorescence spectra of **2** (1.00×10^{-4} mol dm^{-3}) in the presence of D-galacturonic acid (1.00×10^{-2} mol dm^{-3}) and (B) plots of the relative fluorescence intensity (375 nm) versus [Zn(II)] / [**2**], O in the absence of saccharide and ● in the presence of D-galacturonic acid (1.00×10^{-2} mol dm^{-3}): excitation 359 nm, the absorbance of which is not affected by the Zn(II) addition. Other measurements conditions are recorded in a caption to Fig. 6. The fluorescence spectral change in the absence of D-galacturonic acid is not shown here: the fluorescence intensity decreased with increasing Zn(II) concentration (as shown in (B)). It was confirmed that MOPS (5.0 mmol dm^{-3}) used to adjust the medium pH does not affect either the interaction between **2** and Zn(II) or the fluorescence intensity.

the presence of D-galacturonic acid (Figs. 7A and 7B). Again, plots of the fluorescence intensity (375 nm) against [Zn(II)] have been saturated at [Zn(II)]/[**2**] = 4. The results mean that addition of 4 equivalents of Zn(II) to **2** suffices to entirely convert **2** to its Zn(II) complex. We thus fixed the ratio to 4 in the following experiments. These findings will be useful as the answer for problem (i).

The final absorption spectra in the presence of excess Zn(II) were very similar to those of $\mathbf{2H_2}$. In fluorescence spectroscopy, Fig. 7B shows that in the absence of saccharide the Zn(II) addition decreases the fluorescence intensity of the 1,10-phenanthroline moiety, like the fluorescence decrease observed for protonation of **2** to $\mathbf{2H_2}$ (see Fig. 4). In contrast, when Zn(II) is useful for the cooperative binding of D-galacturonic acid, the fluorescence intensity increases with increasing Zn(II) concentration: this implies that the PET function at the *o*-aminomethylphenylboronic acid group works so efficiently that the fluorescence decrease induced by Zn(II) complexation is sufficiently offset by the fluorescence increase induced by the PET mechanism. These findings consistently support the view that Zn(II) interacts only with the 1,10-phenanthroline moiety. This is the answer for problem (iii).

The last problem which is not yet solved is (ii). Fig. 8 shows a continuous variation plot on the basis of absorption spectroscopy. As pointed out in Fig. 6, the absorbance at 290 nm decreases with increasing Zn(II) concentration: in Fig. 8, therefore, the absorbance at [**2**] / ([**2**] + [Zn(II)]) > 0.5 (*i. e.*, at the low Zn(II) concentration) is larger than that at [**2**] / ([**2**] + [Zn(II)]) < 0.5. Important is the fact that the break-point appears at 0.5. Based on this result, one can propose that the complex formed from **2** and Zn(I) has the 1:1 stoichiometry under the measurement conditions. Since three problems which are necessitated to be solved before the K_{ass} determination have been clarified, one can now determine the K_{ass}.

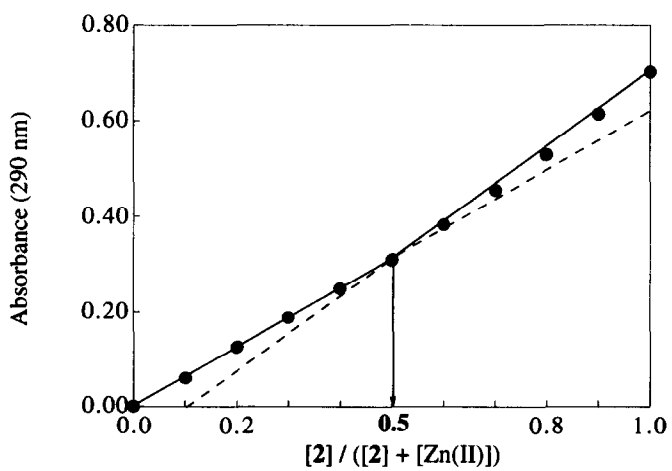


Fig. 8. A continuous variation plot for the absorbance (290 nm) of the **2**·Zn(II) complex. The [**2**] + [Zn(II)] value was maintained constant (2.00×10^{-4} mol dm^{-3}): pH 8.0 with 5.0 mmol dm^{-3} MOPS buffer, 0.1 cm cell, 25 °C, water : MeOH = 1 : 2 (v/v).

Determination of K_{ass} for the Saccharide Complexation. As mentioned later, the ternary mixture of **2**, Zn(II), and D-galacturonic acid yields an CD-active species. As shown in Fig. 9, a continuous variation plot of [**2**·Zn(II)] / ([**2**·Zn(II)] + [D-galacturonic acid]) against the CD intensity (290 nm) afforded a maximum at 0.5. This finding indicates that the **2**·Zn(II) complex forms a 1 : 1 complex with D-galacturonic acid and allows us to assume an equilibrium **2**·Zn(II) + D-galacturonic acid ⟷ **2**·Zn(II)·D-galacturonic acid.

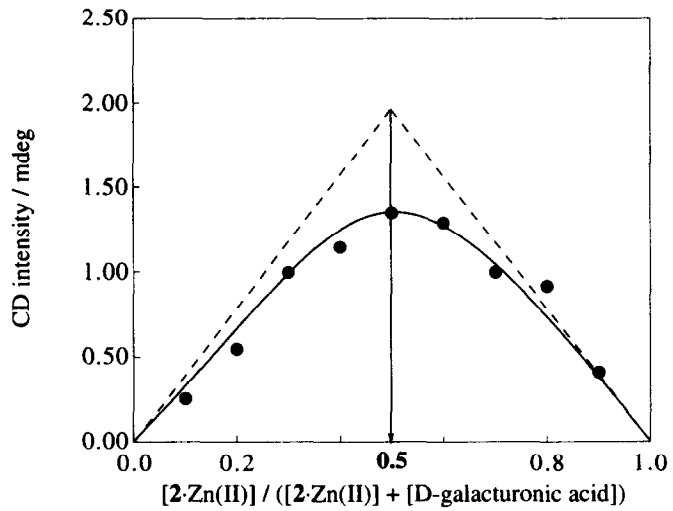


Fig. 9. A continuous variation plot for the CD intensity (290 nm) of the **2**·Zn(II) complex. The concentration ratio of [Zn(II)] / [**2**] was maintained constant (4.0) in order to entirely convert **2** to **2**·Zn(II). The sum of [**2**·Zn(II)] + [D-galacturonic acid] was also maintained constant (5.00×10^{-4} mol dm^{-3}). Other measurement conditions are summarized in a caption to Fig. 8.

To estimate the K_{ass} for the saccharide complexation with **2**·Zn(II) the fluorescence intensities were measured as a function of the saccharide concentrations (Fig. 10). Here, the ratio of [Zn(II)] / [**2**] was fixed to 4.0 where **2** could be entirely converted to **2**·Zn(II) (see Figs. 6B and 7B). We confirmed that even in the presence of excess Zn(II) (*e.g.*, [Zn(II)] / [**2**] = 7.0) the plots in Fig. 10 are scarcely changed. One may thus regard that uncomplexed Zn(II) does not interact so strongly with saccharides as to influence the formation of the **2**·Zn(II)·saccharide ternary complexes or as to influence the fluorescence intensity. The fluorescence intensities increased with increasing saccharide concentrations and were saturated at the high concentration

(Fig. 10). From the analysis of the plots the K_{ass} values for the 2·Zn(II) complexes were determined and are summarized in Table 1.

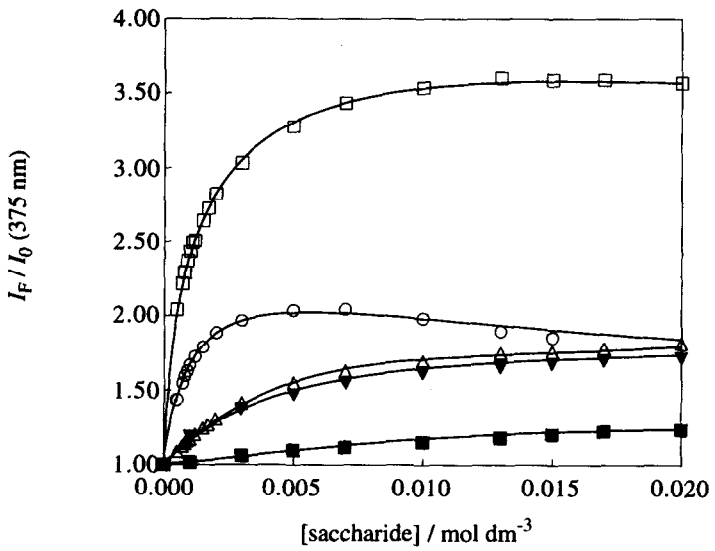


Fig. 10 Plots of the relative fluorescence intensity (375 nm) versus [saccharide]: [**2**] = 1.00×10^{-4} mol dm^{-3}, [Zn(II)] = 4.00×10^{-4} mol dm^{-3}, excitation 355 nm. Other measurement conditions are recorded in a caption to Fig. 8: O D-glucuronic acid, □ D-galacturonic acid, Δ sialic acid, ▼ D-fructose, ■ D-galactose. The K_{ass} for D-glucose could not be determined because of the small fluorescence change.

Examination of Table 1 reveals that as expected,[8] D-fructose gives the largest K_{ass} with **2** in the absence of Zn(II). On the other hand, uronic acids are scarcely bound to **2** (except D- galacturonic acid with K_{ass} = 80 dm^3 mol^{-1}). In contrast, the K_{ass} values for two uronic acids and sialic acid are remarkably improved in the presence of Zn(II) whereas those for three neutral monosaccharides are scarcely affected. The difference clearly supports the view that uronic acids are efficiently bound to 2·Zn(II) by a cooperative action of boronic acid-diol complexation and Zn(II)-carboxylate coordination. The possible complexation mode is illustrated in Fig. 11: that is, the 2·Zn(II) complex can accept uronic acids with the two-points interaction, one at the diol-binding boronic acid site and the other at the carboxylate-binding metal chelate site. According to the best of our knowledge, this is the first and the sole example for recognition of uronic acids with an artificial receptor.

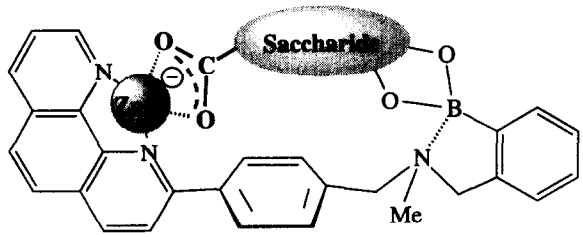


Fig. 11 Complexation mode proposed for the two-points binding of uronic acids to the **2**·Zn(II) complex.

It is known that when saccharides interact with the receptor at two points to from a cyclic structure, the resultant complexes become CD(circular dichroism)-active.[8,15] In fact, D-glucuronic acid and D-galacturonic acid with the large K_{ass} values gave CD-active complexes with **2**·Zn(II): λ_{max} and [θ], 305 nm and 4.0×10^3 deg·cm^{-2}·dmol^{-1} for D-glucuronic acid and 282 nm and 28.0×10^3 deg·cm^{-2}·dmol^{-1} for D-galacturonic acid. The CD-activity can be an additional supporting evidence for the complexation mode as illustrated in Fig. 11.

CONCLUSION

In conclusion, the present study demonstrates a new successful example for molecular design of a saccharide receptor for uronic acids and sialic acids, which features two-points interaction of boronic acid-diol complexation and Zn(II)-carboxylate coordination. The receptor can well discriminate between anionic uronic and sialic acids and neutral monosaccharides and the binding event can be sensitively read out by a fluorescence change. We believe that this is a new concept for molecular design utilizing a cooperative action of boronic acid and metal chelate. Further applications utilizing the concept of multi-point interaction are continued in this laboratory.

EXPERIMENTAL

Materials. 2-(*p*-Tolyl)-1,10-phenthroline (**3**) was prepared according to the method of Goodman *et al.*[12] and identified by IR and ^{1}H NMR spectral evidence and elemental analysis.

2-(*p*-Methylaminomethylphenyl)-1,10-phenanthroline (5). Compound **3** (0.50 g, 1.8 mmol) was treated with *N*-bromosuccinimide (NBS) (0.64 g, 3.6 mmol) and AIBN (0.07 g, 10 wt% of NBS) in CCl_4 at the reflux temperature for 50 min. The progress of the reaction was followed by ^{1}H NMR

spectroscopy (250 MHz, CCl_4 : $CDCl_3$ = 1 : 1 (v/v) with the disappearance of δ_{CH_3} 2.45 ppm and the appearance of δ_{CH_2Br} 4.57 ppm). After cooling, the insoluble materials were removed by filtration, the filtrate being dried over Na_2SO_4. This solution containing **4** was used for the synthesis of **5** without further purification.

Into the CCl_4 solution cooled with an ice bath was introduced methylamine gas for 8 h. The progress of the reaction was followed by ^1H NMR spectroscopy (250 MHz, CCl_4 : $CDCl_3$ = 1 : 1 (v/v)). After 8 h when the peak of δ_{CH_2Br} 4.57 ppm disappeared, the reaction was finished by the addition of aqueous 5 % $NaHCO_3$ solution. The mixture was stirred for one day and then the insoluble materials were removed by filtration. The CCl_4 layer was washed three times with aqueous 5 % $NaHCO_3$ solution and then dried over Na_2SO_4. The solution was concentrated to dryness, the solid residue being crystallized from *n*-hexane. Finally, the product (**5**) was purified by reprecipitation from dichloromethane to diethyl ether: slightly yellow powder, yield 30 % (calculated from **3**), mp 149 ~ 152 °C; ^1H NMR (250 MHz, $CDCl_3$, 27 °C) δ 2.77 (3H, s, CH_3), 4.16 (2H, s, $-CH_2-$), 7.63 (3H, m, H_8 and H_m), 7.81 (2H, s, H_5 and H_6), 8.04 (2H, m, H_3 and H_7), 8.29 (3H, m, H_4 and H_o), 9.18 (1H, d, H_9). Since the ^1H NMR spectral evidence supported the structure of **5**, the product was directly used for the next reaction to synthesize **2**.

1-[*N*-Methyl-*N*-(2-boronophenylmethyl)aminomethyl]-4-(1,10-phenanthrolin-2-yl)benzene (2). Compound **5** (30 mg, 0.10 mmol) and 2-(2-bromomethylphenyl)-1,3-dioxaborinane (33 mg, 0.22 mmol) were treated in refluxing acetonitrile (60 ml) in the presence of K_2CO_3 (30 mg, 0.22 mmol). The progress of the reaction was followed by a TLC method (alumina, MeOH). After 17 h when the spot for **5** (R_f = 0.70) disappeared, the reaction was finished. After cooling, the insoluble materials were filtered off, the filtrate being evaporated to dryness. The solid residue was taken with a mixture of dichloromethane and aqueous 5 % $NaHCO_3$ solution and the phase-separated mixture was stirred for 30 min. The organic layer was separated, washed with water, and dried over Na_2SO_4. The solution was concentrated to dryness, the solid residue being purified by reprecipitation from dichlomethane to diethyl ether: *n*-hexane = 1:1 (v/v): slightly yellow powder, yield 40 %, mp 166 ~ 169 °C; IR (KBr) ν_{B-O} 1340 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$, 27 °C) δ 2.21 (3H, s, CH_3), 3.68 (2H, s, $-CH_2-$), 3.77 (2H, s, $-CH_2-$), 7.24 (1H, boronophenyl H_6), 7.37 (2H, t (7.4 Hz), boronophenyl H_4 and H_5), 7.46 (2H, d (7.9 Hz), H_2), 7.65 (1H, dd (7.9, 4.3 Hz), phen H_8), 7.79 (1H, d (8.8 Hz), phen H_6), 7.83 (1H, d (8.6 Hz), phen H_5), 7.96 (1H, d (6.8 Hz), boronophenyl H_3), 8.09 (1H, d (8.4 Hz), phen H_3), 8.26 (1H, d (7.8 Hz), phen H_7), 8.31 (2H, d (8.0 Hz), H_3), 9.23 (1H, d (3.8 Hz), phen H_9); Anal. Calcd for $C_{27}H_{22}N_3BO+0.5H_2O$: C, 76.43; H, 5.46; N, 9.90 %. Found: C, 76.68; H, 5.55; N, 9.42 %.

Miscellaneous

^1H NMR, Absorption spectra, fluorescence spectra and CD spectra were measured with Bruker AC-250P or Bruker DMX 600, JASCO V-570, Hitachi F-4500, and JASCO J-720 WI, respectively.

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