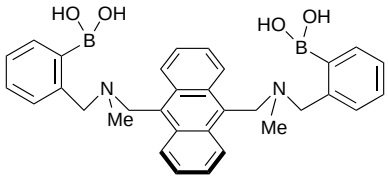
Fluorescent sensing of uronic acids based on a cooperative action of boronic acid and metal chelate

Masayuki Takeuchi, Masashi Yamamoto and Seiji Shinkai*

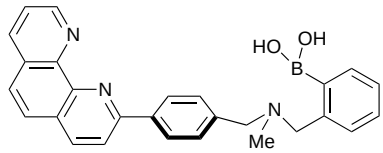
Department of Chemical Science & Technology, Faculty of Engineering, Kyushu University, Fukuoka 812, Japan

A new fluorescent molecular sensor for uronic acids is designed, which features two-point interactions of boronic acid–diol complexation and zinc(II)–carboxylate coordination.

Saccharides are nature's conveyors of energy and play many crucial roles in metabolic cycles. Uronic acids are known to appear in the oxidation process of monosaccharides or in the biosynthetic process of L-ascorbic acid. The quantitative analysis of uronic acids has so far been accomplished by classical colorimetic methods such as naphthoresorcine–HCl[1] and carbazole–H_2SO_4.[2] Obviously, it seems very important to develop a new analytical method which is useful under physiological conditions and in addition, shows high selectivity and sensitivity towards uronic acids in the presence of concomitant neutral saccaharides. We have recently demonstrated the usefulness of the boronic acid function as a saccharide receptor in a saccharide recognition system.[3] Judging from the structure of uronic acids, molecular design including both a boronic acid moiety for diol binding and a metal chelate moiety for carboxylate binding within a molecule should lead to an excellent uronic acid receptor. To achieve high sensitivity it is desirable to combine this system with the concept of a photoinduced electron-transfer (PET) sensor.[4,5] We previously designed compound L′ 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.[6] Here, we have rationally designed a molecular sensor L for uronic acids in which one boronic acid group in L′ is replaced by a metal chelation site. We have found that the zinc(II) complex of this new molecular sensor selectively responds to uronic acids in neutral aqueous solution.



L′

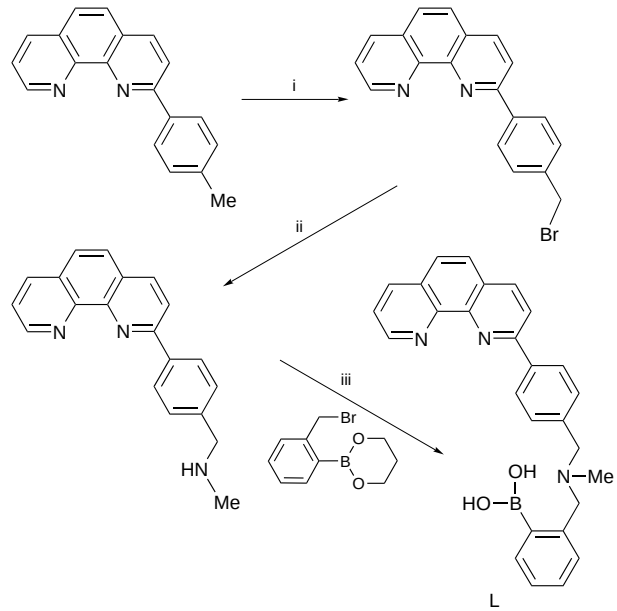


L

The synthesis of L was achieved from 2-(*p*-tolyl)-1,10-phenanthroline[7] by the route outlined in Scheme 1. The product (mp 166–169 °C) was identified by IR and ^{1}H NMR spectroscopic evidence and elemental analysis.

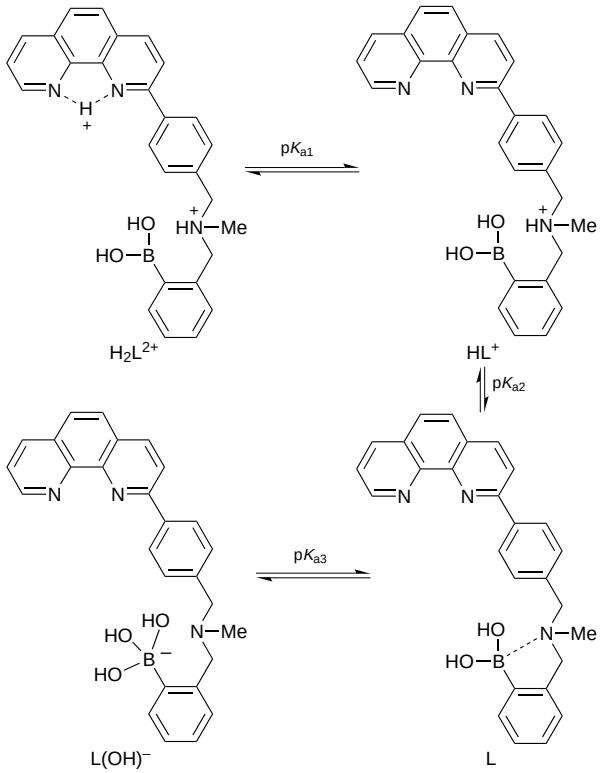
Since the water solubility of L was not good, we used a mixed medium of water–MeOH (1 : 2 v/v) to avoid self-aggregation. The absorption maximum of L shifted from 301 to 290 nm upon a pH change from 2.0 to 7.5 with a tight isosbestic point (at 294 nm). This change corresponds to deprotonation of the chromophoric 1,10-phenanthroline moiety (pK_{a1} 4.4, Scheme 2). The absorption spectra did not change further at higher pH. Using 294 nm as the excitation wavelength we measured the fluorescence spectra. Fig. 1 shows plots of the fluorescence intensity (λ_{max} at 375 nm) *vs.* pH. In the absence of saccharide, the fluorescence intensity increase at pH 2–6 corresponds to deprotonation of the 1,10-phenanthroline moiety, which again gives 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, whereas the pK_{a3} could not be determined accurately. In the presence of saccharide ([D-fructose] = 100 mmol dm^{-3}, Fig. 1), the fluorescence intensity is increased at pH 6–11. This is due to the B–N interaction intensified by complexation of saccharide with the boronic acid group.[6] The association constants (K_{ass}) in the absence of Zn^{II} were estimated from plots of the fluorescence intensity against the saccharide concentration. The results are summarized in Table 1.

Addition of Zn^{II} [added as $Zn(NO_3)_2 \cdot 6H_2O$] decreased the absorbance in the UV region of the absorption spectrum and the visible region of the fluorescence spectrum and the final spectra were very similar to those of H_2L^{2+}. The results suggest that Zn^{II} is primarily associated with the phenanthroline moiety. Since the spectral changes were saturated at [Zn^{II}]/[L] = 4.0, we fixed the ratio to this value and then added saccharides. The fluorescence intensity increased with increasing saccharide concentration and was saturated at high concentration. From the



L

Scheme 1 *Reagents and conditions* [yields]: i, NBS, AIBN, CCl_4, reflux; ii, $MeNH_2$, CCl_4 [30%, calculated from 2-(*p*-tolyl)-1,10-phenanthroline]; iii, K_2CO_3, MeCN, reflux [40%]



Scheme 2

analysis of the plots the K_{ass} values in the presence of Zn^{II} were determined and are summarized in Table 1.

Examination of Table 1 reveals that as expected,[6] D-fructose gives the largest K_{ass} with L in the absence of Zn^{II}. On the other hand, uronic acids are scarcely bound to L (except D-galacturonic acid with K_{ass} = 80 dm^3 mol^{-1}). In contrast, the K_{ass} values for three uronic acids 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 bound to $[Zn^{II}L]^{2+}$ by a cooperative action of boronic acid–diol complexation and zinc(II)–carboxylate coordination.

It is known that when saccharides interact with the receptor at two points to form a cyclic structure, the resultant complexes become CD-active.[6,8] In fact, D-glucuronic acid and D-galacturonic acid with large K_{ass} values gave CD-active

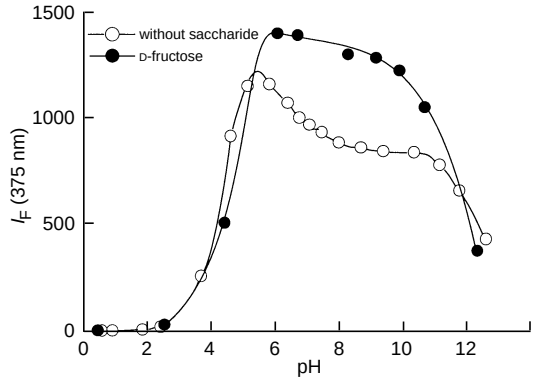


Fig. 1 Fluorescence intensity *vs.* pH profile of L (1.00 × 10^{-5} mol dm^{-3}); 25 °C [D-fructose] = 0 or 100 mmol dm^{-3}, λ_{ex} = 294 nm

Table 1 Association constants (log K_{ass})[a] for saccharides with L and $[Zn^{II}L]^{2+}$

	Log K_{ass} (I_{max}/I_0)	
Saccharide	L	$[Zn^{II}L]^{2+}$
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).

complexes with $[Zn^{II}L]^{2+}$: λ_{max} and $[\theta]$ = 305 nm and 4.0 × 10^3 ° cm^{-2} dmol^{-1} for D-glucuronic acid and 282 nm and 28.0 × 10^3 ° cm^{-2} dmol^{-1} for D-galacturonic acid. Job plots using the CD intensity provided a maximum at $[ZnL^{2+}]/([ZnL^{2+}] + [\text{uronic acid}]) = 0.50$, indicating that the stoichiometry of these complexes is 1 : 1.

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

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Footnote and References

* E-mail: seijitcm@mbox.uc.kyushu-u.ac.jp

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