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A Colorimetric Boronic Acid Based Sensing Ensemble for Carboxy and Phospho Sugars

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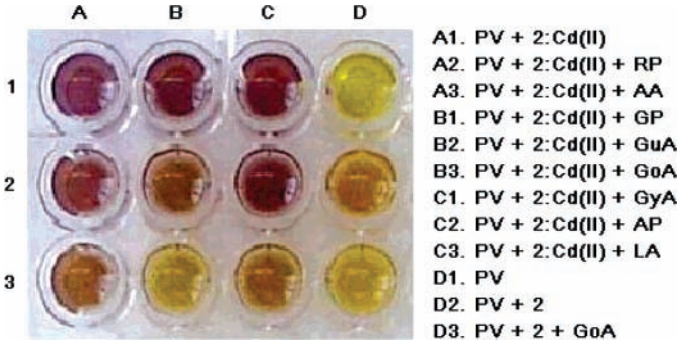
ABSTRACT



A cadmium-centered tris-boronic acid receptor was synthesized, and its binding properties toward various anionic sugars were determined. This receptor shows high affinity for different anionic sugars, especially gluconic acid, which has an association constant near $\sim 10^7$ M^{-1} at neutral pH. Further, using an indicator displacement assay, a color change of pyrocatechol violet was observed upon addition of anionic sugars. This colorimetric test was used as a facile screening technique to qualitatively analyze guest affinities.

The design of artificial receptors that have high selectivity/affinity and can compete with those in natural systems is an attractive area of research.[1] Sugar recognition, especially in aqueous solution at neutral pH, has been a particular focus because of its potential applications in the development of therapeutic agents, chemosensors, and glycomics tools.[2]

The boronic acid functional group is known to form reversible covalent linkages with diols, α-hydroxy acids, and some α-amino acids, thereby making it a common recognition moiety used in sugar-sensing systems.[3] The vast majority of current detection methods rely on fluorescence modulations that occur upon addition of carbohydrates. Yet, the receptors often show relatively low binding affinities in aqueous solution at neutral pH.

Transition-metal ions provide coordination modes for anionic molecules. This offers the possibility of preorganizing a receptor by metal ligation and also enhancing the binding

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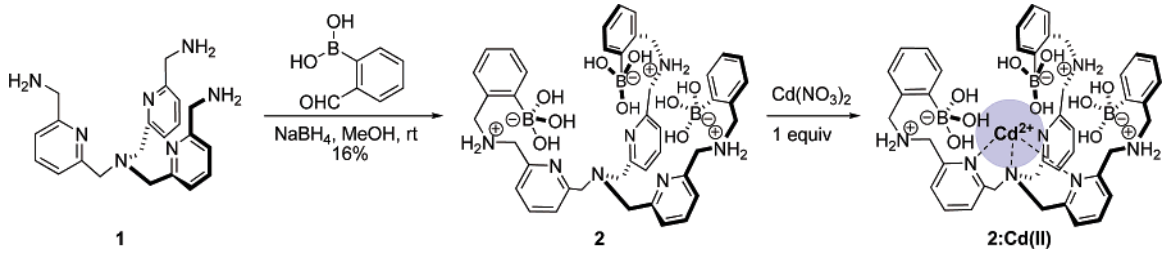
Scheme 1. Synthesis of Boronic Acid Receptor



properties for anionic guest molecules.[4] Many receptors have been designed to exploit these dual properties,[5] but metalloboronic acid receptors are rare.[6] In this paper we report a C_{3v}-symmetric tris-boronic acid receptor, **2**:Cd(II), that binds various carboxy and phospho sugars with high affinity in protic media at neutral pH.

Compound **2** was synthesized from tris-amino compound **1**[7] by using 2-formylbenzene boronic acid and $NaBH_4$ in dry MeOH with added molecular sieves[8] (Scheme 1).

Several metal ions were analyzed for their association with compound **2**. Neither zinc nor lanthanide ions were found to have significant affinity for **2** via analysis of absorbance changes. However, Cu(II) promoted UV−vis shifts upon titration with **2**, but the stoichiometry of binding was found to be 2:1 metal:ligand. This is in contrast to a previously reported receptor for phosphate that had guanidinium groups in the place of the boronic acids in **2**.[9] With this previous receptor, Zn(II) and Cu(II) were found to bind with a 1:1 stoichiometry. As an explanation for this difference, we refer to recent studies from our group. We have found that α-aminomethyl boronic acids exists predominately in zwitterionic forms,[10] as indicated in the manner that we illustrate **2**. With this form, free-based N-atoms of the aminomethyl groups are available to coordinate with metals, and hence with Cu(II) we propose that these nitrogens are involved in binding along with the pyridines, creating higher order stoichiometries.

In contrast, Cd(II) bound receptor **2** with a 1:1 stoichiometry. We tested this metal because of literature reports of its binding properties toward and crystal structure with the tris(2-pyridylmethyl)amine (TPA) core ligand.[11] Due to the larger size of Cd(II), the cavity created upon ligation with TPA is more open than with Cu(II), and this may effect the stoichiometry found. However, it is also well-known that softer metals such as Cd(II) have weaker coordination properties to nitrogen ligands than do harder metals such as Cu(II). This lower affinity may also be part of the reason for the different stoichiometries found, because the Cd(II) would not gain as much binding energy upon increased nitrogen ligation as would Cu(II), hence allowing the zwitterionic form of the α-aminomethyl boronic acid ligand to persist. Hence, cadmium was chosen as the metal center.

Various carboxy and phospho sugars in aqueous solution were examined as guests (Figure 1). A protocol for screening

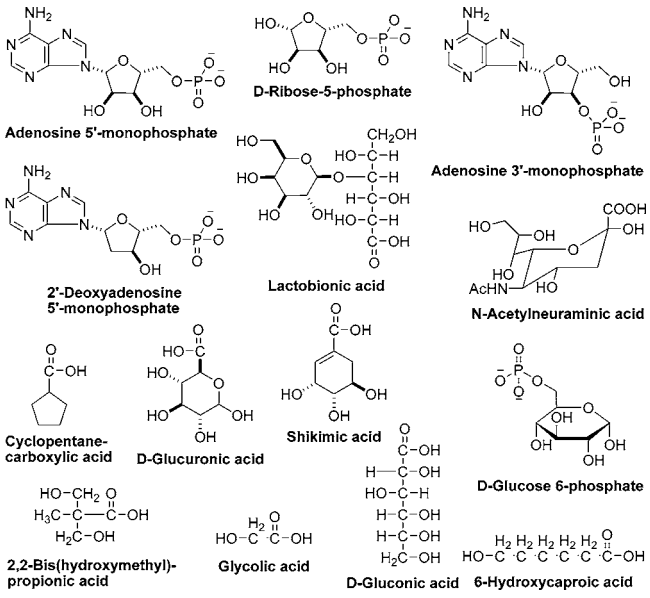


Figure 1. Anionic sugars.

the guests prior to full thermodynamic analyses was developed. Indicator displacement assays were applied to screen the binding affinities of **2**:Cd(II) to various sugar moieties at neutral pH (~7.4). Pyrocatechol violet (PV) was chosen as the indicator, because it created a color change in the UV−vis spectra. The experiments were carried out in 3:1 MeOH/water mixture due to the low water solubility of compound **2**:Cd(II).

As shown in the abstract figure of this paper (columns A−D), in each well, **2**:Cd(II), PV, and one of the guests

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were placed at 0.1, 0.1, and 0.5 mM, respectively, in ~10 mM HEPES buffer. The pure PV gives yellow solution (D1), whereas **2**:Cd(II) + PV is purple (A1). With addition of the different guest molecule, PV would be partially displaced from the receptor molecules, and hence the color of the solutions changes to different extents from purple to yellow dependent upon the strength of the associations. By examining the abstract graphic, it is instantly clear that glucuronic acid and lactobionic acid give intermediate colors, whereas gluconic acid exhibits a color that close to a free PV, meaning it is the best guest.

A typical UV−vis titration for quantitative analysis is shown in Figure 2. A clear isosbestic point exists in the

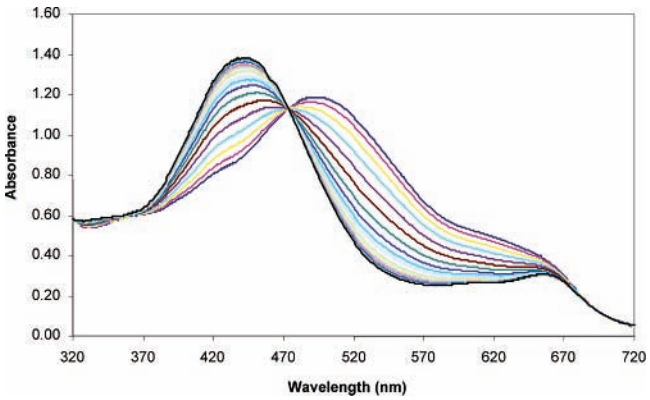

Figure 2. Typical UV−vis binding curves obtained upon titration of anionic sugars into **2**:Cd(II)-indicator solution. Titration conditions: 3:1 methanol/water; HEPES buffer 50 mM, pH = 7.4; pyrocatechol violet indicator; [**2**:Cd(II)] = [PV] = 0.1 mM; 25 °C.

overlaid spectra. The titration results were readily fit by 1:1 isotherms[12] (Figure 3). The association constants of sugars were calculated based on previous determined association constants: $K_{\mathbf{2}:\mathrm{Cd(II)}:\mathrm{PV}} = 3.18 \times 10^5\ \mathrm{M}^{-1}$ and $K_{\mathbf{2}:\mathrm{PV}} = 2.54 \times 10^3\ \mathrm{M}^{-1}$ (Supporting Information) (Table 1).

On the basis of the analysis of Figure 2 and the 96-well screening studies, it is clear that **2**:Cd(II) displays selectivity in its binding among the anionic sugars, likely due to arrangement of the boronic acid groups. Among the phospho sugars, only ribose 5-phosphate and adenosine 5′-monophosphate were found to have relatively strong binding. The lack of a 2′ hydroxyl group on the primary phosphate (2′-deoxyadenosine 5′-phosphate) or a secondary phosphate (adenosine 3′-phosphate) or even a one carbon extension in the ring (glucose 6-phosphate) dramatically decreased binding. Second, among carboxy sugars, a carboxylate group with an α- and/or β-hydroxyl group was found to have large affinities. Other guests such as shikimic acid, cyclopentanecarboxylic acid, 6-hydroxycaproic acid, and 2,2-bis(hydroxymethyl)-propionic acid, did not give observable UV−vis changes, even with large excesses of guest. Third, linear

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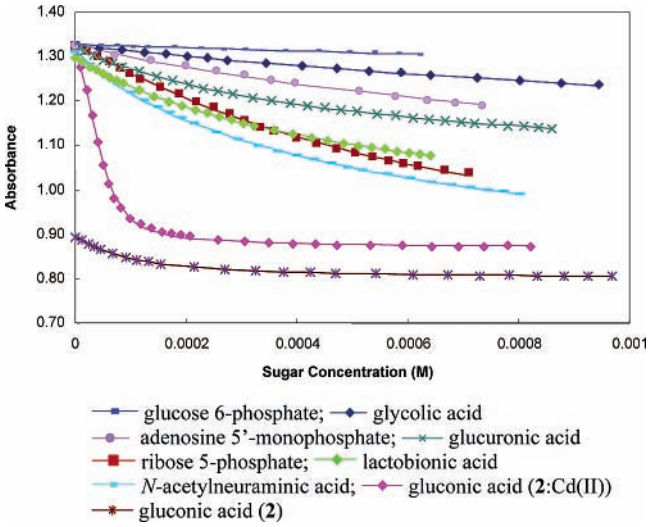

Figure 3. Relative absorbance of **2**:Cd(II)-indicator system upon addition of anionic sugars, modulated at 500 nm.

carboxy sugars were found to have stronger binding by 2 orders of magnitude relative to that of cyclic sugars. One interesting comparison was that of **2** with or without metal coordination. In the gluconic acid titration experiment, both association constants (log K_a) were determined: 6.75 with and 4.32 without the metal center, respectively. Obviously, the reversible covalent bond formation between boronic acid groups and sugars played a significant role in the recognition process. However, the cadmium metal center not only preorganized the C_{3v} ligand core molecule but likely also coordinated to the guest, which further enhances the receptor affinity for the carboxy and phospho sugars.

Table 1. Association Constants Determined for the Binding of Anionic Sugars in MeOH/H_2O (3:1 v/v)

sugars	log K_a
glucose 6-phosphate (GP)	
glycolic acid (GyA)	3.76[a]
adenosine 5′-monophosphate (AP)	3.95[a]
ribose 5-phosphate (RP)	4.76[a]
N-acetylneuraminic acid (AA)	4.77[a]
glucuronic acid (GuA)	5.34[a]
lactobionic acid (LA)	5.44[a]
gluconic acid (GoA)	6.75[a]; 4.32[b]

[a] Association constants to **2**:Cd(II). [b] Association constants to **2**.

In conclusion, we have synthesized a rigid cadmium-centered tris-boronic acid receptor and screened its binding properties toward various carboxy and phospho sugars with a quick 96-well plate analysis. We then analyzed the thermodynamics of a variety of the analytes from the screening technique. This preorganized and functionalized receptor displays high affinities toward specific carboxy and

phospho sugars in protic media at neutral pH. Particularly large affinity was found for gluconic acid, which has an association constant on the order of 10^7 M^{-1}.

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Supporting Information Available: Experimental procedures, ^1H and ^{13}C NMR spectra of **1** and **2**, **2**:Cd(II) association, and predetermination of $K_{\mathbf{2}:Cd(II):PV}$ and $K_{\mathbf{2}:PV}$. This material is available free of charge via the Internet at http://pubs.acs.org.

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