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Boronic Acid Receptors for α-Hydroxycarboxylates: High Affinity of Shinkai's Glucose Receptor for Tartrate

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Abstract: The glucose receptor **1** developed by Shinkai was synthesized by known methods and with modifications involving the final synthetic step, installation of the phenylboronic acid moieties. Binding of the bis(α-hydroxycarbolxylate), tartrate, was assessed and compared to the corresponding bis(diol), erythritol, as well as the corresponding mono(α-hydoxycarboxylate), malate. These results suggest that bisboronate/bis(α-hydoxycarboxylate) interactions are stronger than the corresponding bisboronate/bis(diol) interactions. Furthermore, we report that the receptor is an order of magnitude more selective for tartrate than malate.

The affinity of boronic acids for 1,2- and 1,3-diols has served as the linchpin for numerous synthetic carbohydrate receptors.[1] Boronates have long been utilized on solid support in affinity columns for glycoproteins and more recently in glucose-sensing polymers.[2] Less exploited is the fact that boronic acids have a much higher affinity for α-hydroxycarboxylic acids than 1,2-alkanediols, and boronates should be applicable in chemosensors for compounds containing these species.[3] Pizer compared the binding of boronic acids to vicinal diols and α-hydroxycarboxylic acids in great detail and determined that the pK_a of the bidentate ligand is an accurate predictor of binding affinity with boric and boronic acids.[4] While boronates interact strongly with individual α-hydroxycarboxylates, we set out to determine if this binding could be amplified through multiple interactions, as has been accomplished with bisboronate receptors for sugars. In 1995, Shinkai demonstrated that the chiral, fluorescent

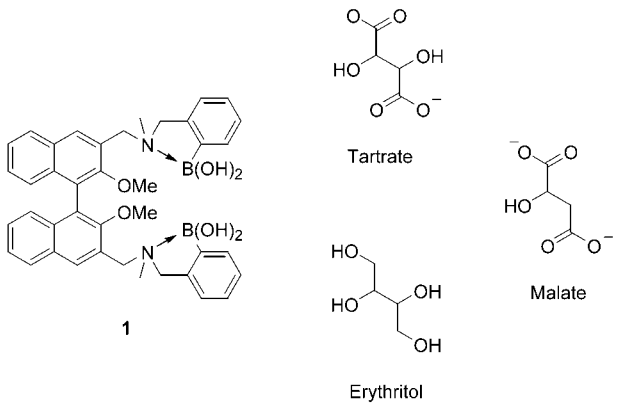

FIGURE 1. Shinkai's glucose receptor (**1**) and substrates investegated herein.

compound **1** (Figure 1) can bind D-glucose selectively over its enantiomer and has a binding constant 2 orders of magnitude higher than that of a monoboronate/glucose complex.[5] This compound offered an opportunity for direct comparison of bisboronate/bis(diol) versus bisboronate/bis(α-hydroxycarboxylate) interactions to determine if similar amplification in affinity occurred.

For the purpose of these studies, we selected tartrate as a model bis(α-hydroxycarboxylate) because it is available in both racemic and enantiomerically pure forms. Molecular modeling of tartrate with Shinkai's receptor suggested that the α-hydroxycarboxylates in tartrate are spaced the correct distance apart to interact with both boronic acids of the receptor without a substantial change in the energy-minimized conformation of the receptor.[6] Additionally, the interaction of tartrate with a diamidinium/monoboronate receptor has been previously investigated. In 1998, Anslyn[7] reported the synthesis of a boronate receptor for tartrate that included amidinium groups for electrostatic interaction and displayed high affinity (K_a = ca. 5 × 10^4) for both tartaric[8] and malic acids. It is important to note that in these studies there was little discrimination between tartrate and malate by the amidininium/boronate receptor. The cooperative interaction of boronate esterification and charge attraction has also been used successfully by both Shinkai's group and Smith and Taylor in receptors for sialic acid (which itself contains an α-hydroxycarboxylate).[9] It appears that boronate/α-hydroxycarboxylate binding is evident in some (e.g., malate and possibly tartrate) but not all of these cases, although alternative binding mechanisms have

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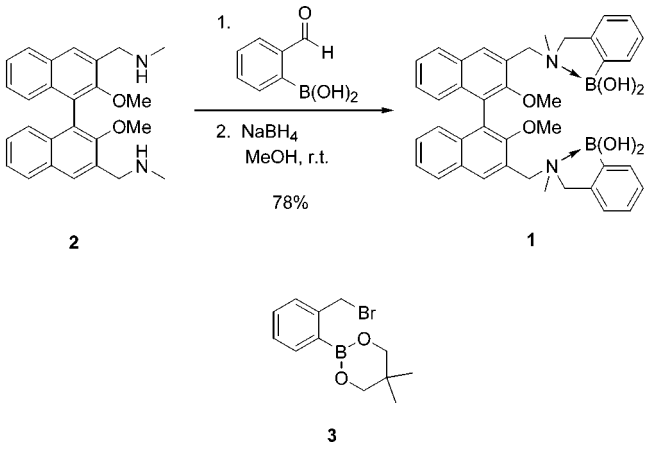
SCHEME 1. Alternative Synthesis of Shinkai's Glucose Receptor



been proposed. Another possibility is that electrostatic binding of the carboxylate portion of the hydroxyacid diminishes its affinity for boronates, as appears to be the case with both sialic acid receptors. Shinkai's receptor **1** exists predominately in charge neutral form around pH 7.8, and the boronate binding can be studied more precisely.

Because efficient synthesis of **1** proved to be quite challenging, we sought ways to improve the procedure in both yield and number of synthetic steps. To this end, **1** was synthesized from racemic **2** by reductive amination with *o*-formylphenylboronic acid after **2** was synthesized by reported procedures (Scheme 1). Previously, the last step of the receptor synthesis included alkylation of **2** with **3**, a procedure that first required protection of the boronic acid with neopentylglycol and then bromination of the corresponding tolylboronate. Reductive amination substantially increases the yield of the final step from 29% to 78%. More importantly, the bisboronate receptor is directly accessible in one synthetic step from the corresponding bisamine through this reductive amination procedure, a feat that required three steps by previously published procedures.

The binaphthol-derived receptor functions through the enhanced fluorescence that occurs when both boronic acids are esterified upon binding of a bisdiol or bis(α-hydroxycarboxylate). Because boronate esters are more Lewis acidic than their parent boronates, this strengthens the coordination of the nitrogen lone pairs and prevents photoinduced electron transfer (PET) that quenches fluorescence in the free boronate.[10] An enhanced fluorescent signal is achieved when both boronates on a single receptor bind to diols or α-hydroxycarboxylates. It is assumed that once one boronate binds to a diol or α-hydroxycarboxylate on a molecule containing more than one of these moieties, the corresponding intramolecular reaction to bind a second moiety on the same molecule (1:1 ligand/receptor complex) will be sufficiently fast to avoid a 2:1 ligand/receptor complex. At higher concentrations, a 2:1 complex is quite possible as a result of the reversible nature of boronate ester formation in aqueous solution and one would expect a

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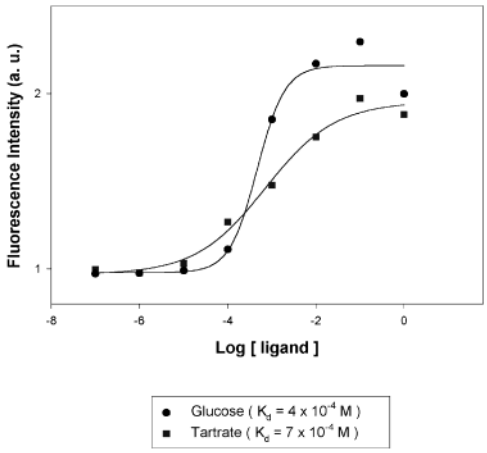

FIGURE 2. Fluorescence measurements and dissociation constants for glucose vs tartrate affinity for receptor **1**.

broad binding curve for this type of bimodal binding. As can be seen from the fluorescence data (Figure 2), D,L-Tartrate binds quite strongly to racemic **1** (apparent $K_d = 7 \times 10^{-4}$ M) with an affinity approaching that of D-glucose ($K_d = 5 \times 10^{-4}$ M). The results for glucose are consistent with the results previously published by Shinkai et al. Also noteworthy is the broad binding curve of tartrate, which would seem to suggest a 1:1 ligand/receptor interaction at lower concentrations of ligand and a 2:1 interaction at much higher concentrations.[11] At concentrations approaching 1 M, fluorescence quenching was observed, and this effect was consistent in repetitive trials of the same experiment.

Unlike glucose, the four tartrate oxygens that bind to the two borons of the receptor are located on contiguous carbons. Glucose does not offer an adequate comparison of simple bisdiol affinity relative to tartrate. Shinkai has shown that the high affinity of the receptor for glucose is the result of one boronic acid of the receptor interacting with the 1,2-diol at the anomeric center and the second with the 1,3-diol present at carbons 4 and 6 of glucose. The rotational freedom associated with the 6-hydroxyl of glucose allows glucose to adopt a conformation that fits the receptor, while the vicinal diol at carbons 3 and 4 are in a trans relationship and have a much lower affinity for the boronate. For a direct comparison of bisdiol binding versus tartrate binding, increase of fluorescence signal with erythritol was measured (Figure 3). While erythritol elicits a much stronger fluorescence increase than tartrate or glucose, its binding affinity ($K_d = 3 \times 10^{-3}$ M) is much less. Thus, in this direct comparison, affinity of these bidentate species does again parallel the pK_a of the ligand.

The absence of a single hydroxyl group in malate compared to tartrate means that the receptor should not be able to bind to both carboxylates and thus should not have high affinity for **1**. The data in Figure 3 show this to be the case, although malate still elicits a fluorescent response ($K_d = 8 \times 10^{-3}$). It is likely that a 2:1 complex

(11) Pure L-tartrate gives a more distinctly bimodal binding curve (data not shown) indicative of selective chiral recognition by **1** as seen with this receptor for glucose.[5] Hamilton has previously demonstrated the utility of binaphthol systems in chiral recognition of tartaric acid derivatives.[8]

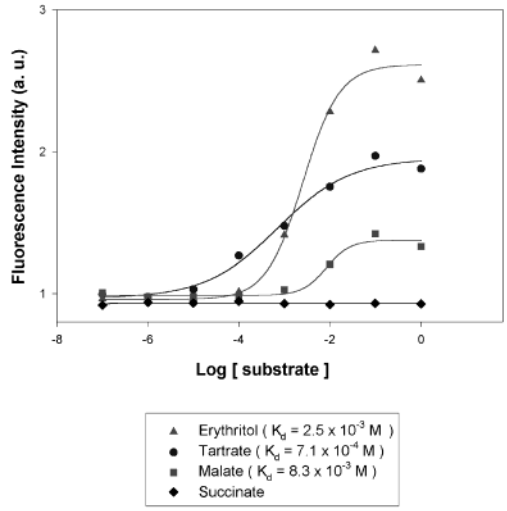

FIGURE 3. Fluorescence measurements and dissociation constants for tartrate, erythritol, malate, and succinate with receptor **1**.

may be forming at higher concentrations of the monovalent ligand. This would also suggest that at low concentrations, a 1:1 complex is present for tartrate binding. Nonetheless, there is an order of magnitude increase in the binding affinity of tartrate over malate. Succinate was chosen as a control because it does not contain diols or α-hydroxycarboxylates and thus should not elicit a fluorescent response. As expected, succinate did not interact with the boronates of the receptor.

In conclusion, the synthesis of Shinkai's glucose receptor becomes much more efficient when installation of the boronic acid functionalities is accomplished through reductive amination with *o*-formylphenylboronic acid. The strong binding of the receptor to tartrate and experiments performed with erythritol and malate have several implications. First, the binding constant of the receptor to tartrate is very similar to both literature values for the binding of the receptor to glucose and also experiments performed herein. To our knowledge, this is the first bisboronate receptor shown to bind contiguous α-hydroxycarboxylates. Second, a comparison of binding between tartrate and erythritol confirms that α-hydroxycarboxylates exhibit stronger affinity for boronic acids than do 1,2-diols. Finally, this work offers design elements for selective sensing of tartrate in the presence of malate, each an important constituent of wine.[7] Furthermore, a fluorescence assay for tartrate based on this system may allow for direct determination of the amount of tartrate present in calcium tartrate rich precipitates of the wine industry, a value that has most recently been measured using time-consuming HPLC, Fourier transform (FT) IR, and capillary electrophoresis methods.[12] Currently, these byproducts are recycled as a useful source of (*R*,*R*)-(+)-tartaric acid, which is important in both the pharmaceutical and food industries.[13] Further investigation into the design of boronate receptors for α-hydroxycarboxylates is underway.

Experimental Section

To a stirred solution of **2** (0.250 g, 0.624 mmol) in 25 mL of methanol at room temperature was added 2.1 equiv of *o*-formylphenylboronic acid (0.197 g, 1.31 mmol). The mixture was allowed to react for 2 h, at which time 3 equiv of sodium borohydride (0.071 g, 1.87 mmol) was added. The mixture was stirred for an additional 1 h. The solvent was removed in vacuo, and the resulting solid was redissolved in 15 mL of methylene chloride. Remaining particulate sodium salts were vacuum filtered, and the filtrate was collected. Hexanes were added to the filtrate dropwise until a pale white solid precipitated. The solid was triturated in the solvent mixture and collected by vacuum filtrate. Remaining residual solvent was removed in vacuo to give the desired producat as a pale white solid (0.326 g, 78%): ^{1}H NMR (CD_3OD) δ 2.50 (s, 6H), 3.19 (s, 6H), 4.09 (d, J = 12.6 Hz, 2H), 4.25 (d, J = 14.3 Hz, 4H), 4.43 (d, J = 12.6 Hz, 2H), 7.05 (m, 2H), 7.19 (m, 4H), 7.30 (t, J = 6.6 Hz, 2H), 7.41 (m, 2H), 7.45 (t, J = 7.1 Hz, 2H), 7.57 (d, J = 7.2 Hz, 2H), 8.01 (d, J = 7.7 Hz, 2H), 8.23 (s, 2H); ^{13}C NMR (CD_3OD) δ 39.67, 53.69, 60.33, 62.13, 69.08, 119.72, 124.68, 124.88, 125.32, 125.44, 126.78, 127.02, 127.32, 128.37, 128.95. 130.55, 132.74, 133.76, 134.64, 155.60; FAB-HRMS ($C_{40}H_{42}B_2N_2O_6$ + H^+, diglycerol boronate adduct) calcd 785.3831, found 785.3836.

Fluorescence measurements were performed using an excitation wavelength of 289 nm and measuring emission at 361 nm. Binding was assessed in 50 mM HEPES (33% MeOH/H_2O) buffered to pH 7.77. In all measurements, the concentration of the receptor (**1**) was fixed at 10^{-5} M. Data points for 0.1 to 10^{-8} M substrate concentrations were obtained through a serial dilution of the 0.1 M solution with a stock solution of buffer, which was 10^{-5} M in receptor (**1**). A separate solution, 1 M in substrate, was prepared because of solubility difficulties at high concentration of the substrate, which was remedied by heating. Plots were generated using SigmaPlot, and K_d values were obtained by substituting the fluorescence value at half the maximum into the regression formula.

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