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Specific sensing between inositol epimers by a bis(boronate)

Charles W. Gray, Jr.,[a,b] Leland L. Johnson, Jr.,[a,b] Brian T. Walker,[a] Mark C. Sleevi,[b,*] A. Stewart Campbell,[b] Robert Plourde[b] and Todd A. Houston[a,c,*]

[a]*Department of Chemistry, Virginia Commonwealth University, Richmond, VA 23284, USA*
[b]*Insmed, Incorporated, Glen Allen, VA 23060, USA*
[c]*School of Science and Eskitis Institute, Griffith University, Nathan, QLD 4111, Australia*

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Abstract—Bis(boronates) that utilize internal photoinduced electron transfer (PET) quenching mechanisms can specifically signal the binding of *chiro*-inositol without responding to its epimer, *myo*-inositol.

One important aspect of drug development, especially for drugs involved in clinical trials, is the monitoring of metabolism in vivo. Concentrations of the drug in serum and measurements of the amount of secreted drug are parameters that can be used to adjust the dosage of administrated drug and drug delivery formulations so that the full potential of the drug may be realized. The compound D-(+)-*chiro*-inositol (DCI, **1**) has been investigated in clinical trials for two therapeutic applications: as an oral sensitizer for type-2 diabetes[1] and as a first line therapy for polycystic ovarian syndrome (PCOS). Currently, serum concentrations and excretion rates for DCI are monitored using time-consuming HPLC assays. A fluorescence assay that is selective for DCI over its biologically more-prevalent epimer *myo*-inositol (**2**) would significantly increase the speed with which biological samples could be screened for DCI, and work to develop such a method is described here. To date, a chemosensor that responds specifically to only one of two cyclitol epimers has not been reported.

DCI is a naturally occurring cyclitol found in substantial quantities in a number of plants including the pine tree and soybean plant. It was first cited in 1993 that there was a strong correlation between insulin resistance and DCI deficiencies in the urine and serum of diabetics.[2] Furthermore, it was found that DCI excretion is an index marker for insulin sensitivity.[3] Similarly, the same deficiencies were observed for women diagnosed with PCOS, which has a known relationship to insulin resistance.[4,5] Recently, a putative insulin mediator (PIM) containing DCI linked through the 4-OH to a β-galactosamine residue was reported by Insmed and collaborators at the University of Virginia.[6] It appears to be the first known β-1,4-linked inositol glycan from animals that displays insulin mimetic properties.

Cyclitols and their phosphates are abundant in nature and fulfill many different biochemical roles and this has inspired chemists to create synthetic receptors for these systems.[7,8] For example, in addition to his seminal work on inositol triphosphate receptors,[7] Anslyn and co-workers have developed receptors with preorganized hydrogen bonding sites that accommodate simple cyclitol guests.[9] The *chiro*-inositols are two of nine possible inositol isomers and the only chiral isomers, thus the prefix indicating this.[10] Contributing to its C_2-symmetry is the presence of contiguous *cis*-1,2-diols, a structural feature that can be exploited by the affinity of boronic acids for vicinal diols. Boronic acids have become widely used as carbohydrate sensors due to their ability to exchange covalent bonds with polyols in aqueous solution.[11] To date, these have not previously been incorporated into the design of receptors for cyclitols.

Aminoboronates are useful photoinduced electron transfer (PET) sensors of polyol binding. Upon esterification, the boron generally becomes more electrophilic and interaction with the nitrogen lone pair is strengthened.[12] This interrupts the PET quenching pathway and allows increased fluorescence that can be measured

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* Corresponding authors. Tel.: +61 7 3735 4115; fax: +61 7 3735 6572; e-mail: t.houston@griffith.edu.au

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quantitatively as a signal for binding. James et al. first described the use of this method with a δ-aminoboronate coupled to an anthracene core[13] and showed that a bis(boronate) analog of this system displayed nearly two orders of magnitude greater affinity for glucose.[14] Using a similar strategy with the binaphthol receptor **3**, they first reported chiral discrimination in the sensing of D-glucose with a single enantiomer of this bis(boronate).[15] Previously, we showed that Shinkai's glucose receptor **3** also has high affinity for tartaric acid[16] and sought to apply this compound to the fluorescent detection of DCI.

Use of a bis(boronate) such as **3** for sensing DCI offers several advantages. First, the contiguous *cis*-1,2-diols of DCI should allow it to bind two boronates quite readily.[17] In addition, while *myo*-inositol contains two hydroxyl groups that are in a *cis* relationship to the lone axial hydroxyl group, binding of a single boronate at this point of symmetry would leave a trans relationship among all of the remaining free hydroxyl groups, virtually precluding formation of a second boronate ester. This means a bis(δ-aminoboronate) receptor would not report the binding of a single boronate to *myo*-inositol due to PET quenching from the amine adjacent to the free boronate. Finally, initial molecular modeling results suggested that **3** could bind DCI while allowing both amines to coordinate their respective boronate esters.[18] In total, this would allow for selective detection of DCI in the presence of *myo*-inositol1.

Shinkai's receptor **3** was synthesized in racemic form using the modification described previously.[16] The fluorescent response of this compound to both DCI and *myo*-inositol was monitored and as the results in Figure 2 demonstrate, *rac*-**3** responds to the presence of DCI at millimolar concentrations (K_a = ca. 125 M^{-1}) while remaining 'silent' to *myo*-inositol at concentrations as high as 1 M. We propose that the aforementioned inability of *myo*-inositol to interact with both boronates of **3** in a 1:1 complex likely accounts for the lack of fluorescent response. While the

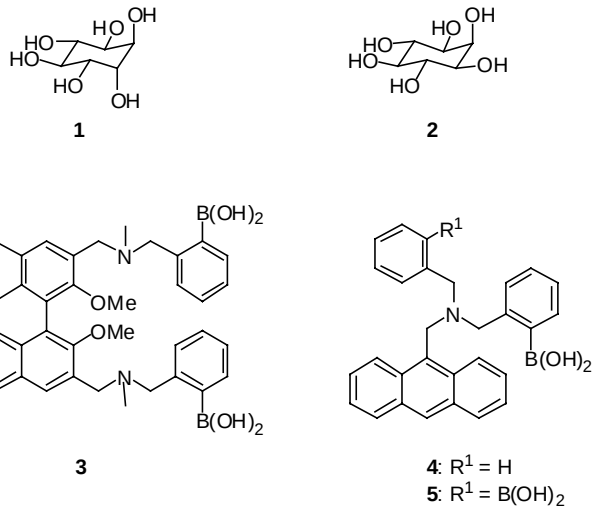

Figure 1. Inositol epimers and receptors used herein.

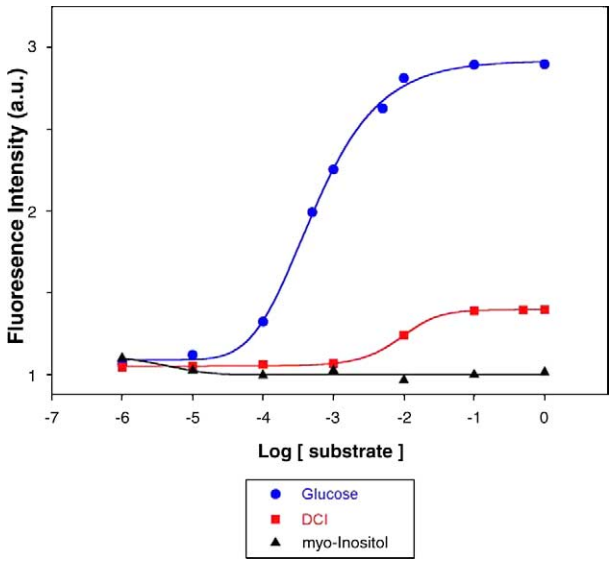

Figure 2. Fluorescent measurement of D-glucose, D-*chiro*-inositol (**1**), and *myo*-inositol (**2**) affinities for *rac*-**3**.[16]

affinity and fluorescent response of **3** are much larger toward glucose, samples could be treated enzymatically a priori to remove undesired sugars from a biological sample. Thus, the indicated specificity between inositol epimers is important as the proposed fluorescence assays have the potential for higher throughput of biological/clinical samples relative to time-intensive HPLC methods. What remains is to develop a bis(boronate) with a higher affinity for DCI.

Unlike **3**, receptors such as **4** and **5** can distinguish between **1** and **2** selectively, but not specifically. Once a single *cis*-diol binds to either of these receptors, PET quenching will occur. Monovalent ligands such as malic acid can generate a fluorescent response from **3** at high millimolar concentrations, but α-hydroxyacids generally have a higher affinity for boronates than diols do.[16] The interaction of *myo*-inositol with a single boronic acid was confirmed with the simplified receptor **4**. While it lacks some of the steric constraints of the bis(boronate), it maintains the δ-aminoboronate PET sensing arrangement. This compound was synthesized from 9-anthraldehyde using consecutive reductive amination reactions involving benzylamine (90%) and *o*-formylphenylboronic acid (79%).[16] The affinity of **4** for *myo*-inositol is larger than that observed for galactose and mannose, both of which are documented as having a higher affinity for benzeneboronic acid than glucose (Table 1).[12] In addition, α-galactose has the same contiguous *cis*-1,2-diol arrangement as DCI. This further supports the argument that the bis(boronate) **3**, while possessing the ability to form a 1:1 complex with *myo*-inositol by esterification of a single boronate, does not signal this interaction through an increase in fluorescent intensity since the second boronate cannot bind the cyclitol and shut down PET from its proximal amine. Such a feature makes **3**, and presumably other bis(aminoboronates), useful sensors for the detection of DCI in the presence of *myo*-inositol even though its affinity for both boronates in **3** is less than *myo*-inositol's

Table 1. Association constants with **4** in 99:1 10 mM aq phosphate (pH 7.8)/MeOH

Carbohydrate	K_a (10^2 M^{-1})	I/I_0
myo-Inositol	3.5	1.5
D-Galactose	2.3	1.7
D-Mannose	1.8	1.4

affinity for a monoboronate. Here, the mechanism of signaling takes precedent over raw affinity for particular guests.[19] While further understanding of the exact nature of the binding event is needed, this is the first report of epimer specific chemosensing with a synthetic receptor.[20]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.08.112.

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18. Predictions based on molecular mechanics minimizations (Merck Molecular Force Field) using Spartan (Wavefunction, Inc.) with the dative B–N bonds defined as covalent bonds.
19. Methyl glucosides have previously shown to be 'silent' toward glucose-binding bis(boronates) by a mechanism similar to what is occurring here (i.e., –methyl glucosides cannot bind two boronates at once although it presumably also has lower affinity for the receptor as well, unlike the present case): Takeuchi, M.; Yoda, S.; Imada, T.; Shinkai, S. *Tetrahedron* **1997**, *53*, 8335; Compounds such as **4** are specific sensors for glucose over glucosamine: Cooper, C. R.; James, T. D. *Chem. Commun.* **1997**, 1419.
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