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Boronic Acid Porphyrin Receptor for Ginsenoside Sensing

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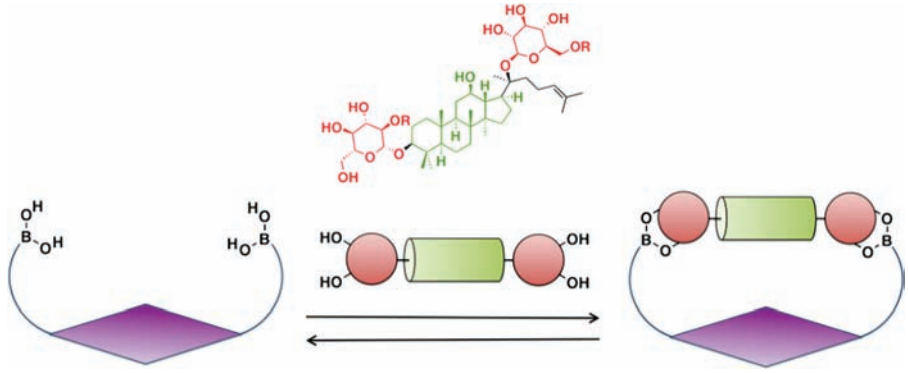
ABSTRACT



Ginsenoside detection was pursued through the design of a porphyrin receptor containing two boronic acid units. This receptor was found to undergo different degrees of fluorescence quenching with five ginsenoside guests and an acylated derivative. The trends in the 1:1 binding constants, as well as ESI−HRMS analysis, support a binding mode in which the ginsenoside sugar units are bound to the boronic acid groups, while the steroid core and porphyrin ring participate in hydrophobic interactions.

Ginsenosides represent a class of over 30 glycosylated steroids that are believed to be responsible for the physiological benefits of ginseng.[1] Ginseng has been used in medicinal practices for over 5000 years, particularly in China and Korea, and remains one of the most widely taken herbs in the world. However, the extent of its efficacy remains a subject of current study.[2] Nevertheless, ginsenosides have been implicated as treatments in a variety of diseases.[3] Ginsenosides contain a gonane steroid nucleus but differ in the degree, position, and type of sugar substitution (Figure 1). The difference in the biological activity of individual ginsenosides has been attributed to their respective glycosylation patterns.[1] This makes analysis of these patterns a topic of chemical interest.

It is recognized that the ginsenoside composition of ginseng is highly variable. It appears to depend on the species, plant age, the part of the plant from which the sample is taken, the season of the harvest, and the extraction method.[4] These variations represent a significant problem

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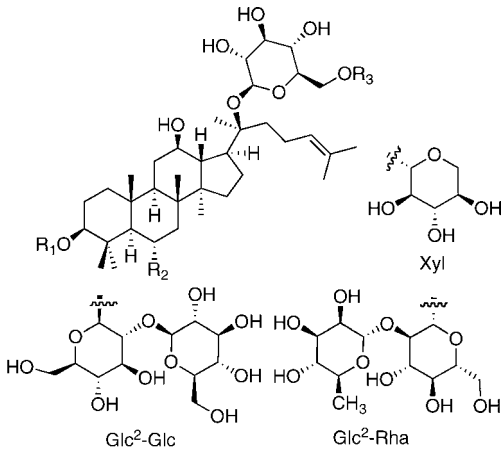

Protopanaxdiols
Rd: R_1 = Glc²-Glc, R_2 = H, R_3 = H
Rb_1: R_1 = Glc²-Glc, R_2 = H, R_3 = Glc
Rb_3: R_1 = Glc²-Glc, R_2 = H, R_3 = Xyl
Protopanaxtriols
Rg_1: R_1 = H, R_2 = Glc , R_3 = H
Re: R_1 = H, R_2 = Glc²-Rha , R_3 = H

Figure 1. Examples of ginsenoside structures. Glc = β-D-glucose; Xyl = β-D-xylose; Rha = α-L-rhamnose.

in terms of quality control.[5] Normally, ginseng products are analyzed through a time-consuming HPLC process.[6] The resulting inefficiency is providing a motivation to develop improved methods for the analysis of ginsenoside components. Despite recent efforts in this area,[7] a simple optical assay for ginsenosides has not been reported.

To address the need for improved ginsenoside sensing, we designed and synthesized the boronic acid appended porphyrin **8** (Scheme 1).[8] This hybrid system was expected to serve as a supramolecular receptor for ginsenosides. Specifically, it was expected that the hydrophobic porphyrin ring would interact with the steroid core of the ginsenosides while also providing a large scaffold for the appendage of sugar binding units. Boronic acids were chosen to serve this latter purpose. These groups have been used in some of the most successful synthetic saccharide receptors reported to date and are known to form reversible covalent bonds with 1,2- and 1,3-*cis*-diols.[9] The two boronic acid groups in receptor **8** were appended to the 5 and 15 porphyrin substitution positions with the goal of allowing the simultaneous binding of the saccharide substituents on both sides of the ginsenoside analyte (i.e., Figure 2).

The first step in the synthesis of receptor **8** involved the formation of zinc(II) porphyrin **3**. This was accomplished using the traditional Lindsey procedure[10] in three steps with an overall 19% yield (Scheme 1). Porphyrin **3** contains cyano groups as the latent amine functionalities. It also contains phenolic units, which were designed to serve as attachment sites for solubilizing groups. Substitution of the phenolic units of **3** with triethyleneglycol chains[11] to give **4**, followed by protection with tetrahydropyran, yielded intermediate **5** in 32% yield for two steps.[12] Reduction of the cyano units was accomplished with the use of LAH.[13] Subsequent heating in aqueous HCl served to oxidize any reduced macrocycle and cleave the protecting groups. This step also removed the zinc(II) ion from the porphyrin core. Recovered porphyrin **6**, obtained in 62% yield, was then used in the reductive amination of 2-formylphenylboronic acid (**7**).[14] The final product (**8**) was purified through reverse-phase chromatography and isolated in quantities of ca. 20 mg (44% yield for two steps).

The absorbance and fluorescence properties of **8** were found to be consistent with those previously reported for free base tetraphenylporphyrin macrocycles.[10] The absorbance intensity at the Soret band (λ_{max} = 420 nm) was found to be linear with the concentration up to 3.5 μM in a 9:1 DMSO: water solution buffered to pH 7.4. Efforts to study higher concentrations were precluded due to the high absorptivity of the receptor. The observed linearity was taken as evidence of minimal aggregation at concentrations $\leq$3.5 μM in this solvent mixture.

The effects of ginsenosides on the optical properties of **8** were then explored using a receptor concentration of 3 μM. While little change was observed in the absorption spectrum, the addition of ginsenosides led to significant quenching of the emission of receptor **8**. Fluorescence titration experiments revealed that the degree of quenching varied as a function of the ginsenoside structure (Figure 3).

The quenching curves were then fit to a 1:1 binding equation to estimate the association constant of the underlying interactions.[15] The method used was based on a previous report that allowed for the calculation of binding constants using the change in absorbance relative to concentration of

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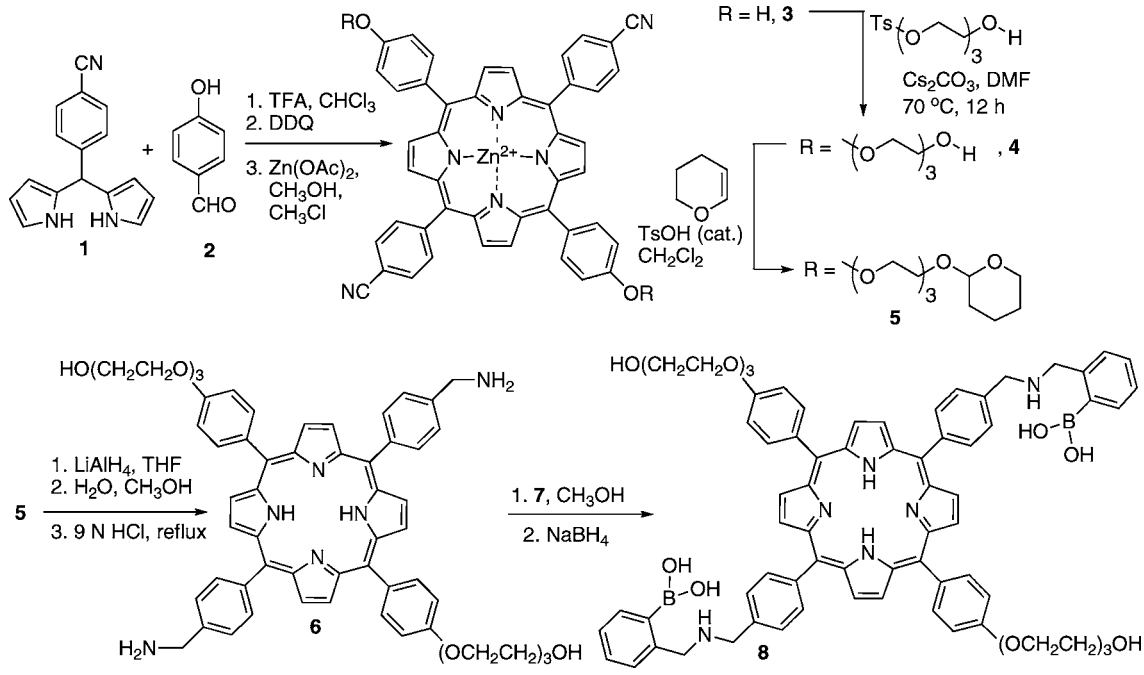
(15) We presumed a 1:1 binding stoichiometry for the purpose of estimating the overall strength of binding interactions. The low concentration of receptor **8** required to prevent aggregation, particularly as compared to the concentration of the ginsenoside guests, precluded efforts to carry out continuous variation (Job plot) experiments.

Scheme 1. Synthesis of Receptor **8**[a]



[a] Compound **7** = 2-formylphenyl boronic acid.

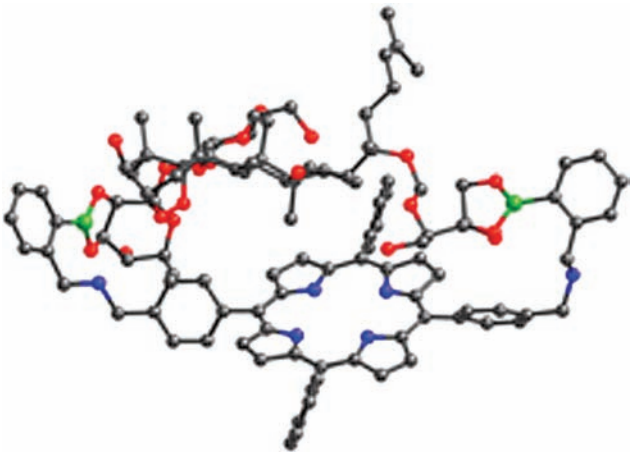


Figure 2. Proposed binding mode of ginsenosides to porphyrin **8** (the protopanaxtriol derivative Re is shown). This molecular model was built in HyperChem 8.0.3 and the geometry optimized using the MM+ force field (convergence at 0.002 kcal/(Å mol)). The planarity of the porphyrin ring was moderately constrained based on the reported crystal structures of similar compounds.[18]

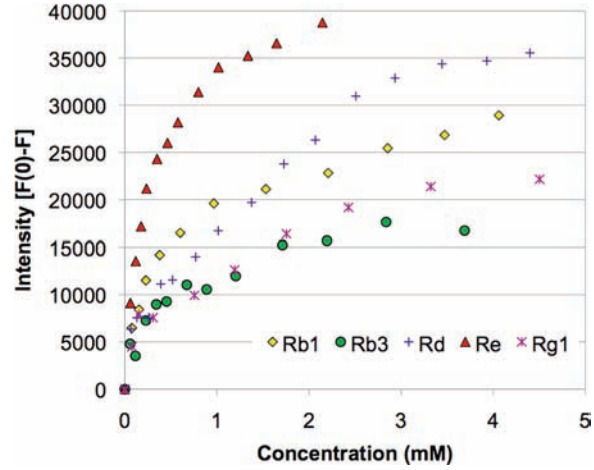


Figure 3. Emission intensity of a solution of porphyrin **8** (3 μM) following the addition of aliquots of solutions containing **8** (3 μM) and one of the following ginsenosides: Rb_1 (30.7 mM), Rb_3 (23.1 mM), Rd (26.4 mM), Re (26.3 mM), or Rg_1 (31.0 mM). The data are plotted as the difference in intensity (F) relative to the initial (0 mM) point of each titration ($F(0)$). Each point is the average of 4−5 measurements. All measurements were performed in 9:1 DMSO:50 mM HEPES pH 7.4 at 25 °C. Excitation wavelength: 420 nm. Emission wavelength: 655 nm.

the guest.[16,17] This method was applied to fluorescence data (see Supporting Information (SI)) with the change in emission ($F(0) - F$) relative to ginsenoside concentration being used in these analyses.

Within the series of five ginsenosides studied here (Figure 1), a number of correlations between the calculated association constants (Table 1) and the sugar substitution pattern can be proposed. The protopanaxdiol derivatives (see Figure 1) were considered first. Here, it was found that the tetrasaccharide dervatives Rb_1 and Rb_3 displayed significantly higher binding

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Table 1. Summary of Results Obtained from the Best Fit Curves of the Data Shown in Figure 3[a]

guest	K (1:1), M^{-1}	guest	K (1:1), M^{-1}
Rb1	2500 ± 350	Re	3900 ± 210
Rb3	2300 ± 470	Rg1	1200 ± 390
Rd	550 ± 98		

[a] The reported errors reflect the differences between the calculated curve fits and the experimental data.

affinities than the trisaccharide derivative Rd. This result led to the suggestion that the fourth sugar substituent of Rb_1 and Rb_3 plays a significant role in mediating the binding process, perhaps by allowing for simultaneous interactions with both boronic acid units (i.e., Figure 2).

The very similar binding affinities seen for Rb_1 and Rb_3 are consistent with a lack of preference for xylose over glucose in the case of **8**, at least for the protopanaxdiol derivatives. This result, which differs from the modest preference for xylose seen for simple phenylboronic acid in aqueous medium,[19] is thought to reflect the importance of ancillary interactions, including porphyrin−steroid associations.

The protopanaxtriol derivatives (Re and Rg_1) were also studied. As can be seen from Table 1, this class of ginsenosides exhibits stronger binding affinities than similarly substituted protopanaxdiol derivatives. We ascribe this finding to an increased geometric complementarity with the host porphyrin. The protopanaxtriol Re exhibited the strongest binding affinity of the ginsenosides studied; however, the general preference of phenylboronic acids for mannose over glucose may also contribute to the high affinity observed for this rhamnose (6-deoxy-L-mannose) derivative.[19]

To probe these binding interactions further, we sought to protect the hydroxyl units of ginsenoside Re with acyl groups. We expected the interaction of **8** with an acylated Re derivative (AcRe) would be significantly reduced as compared to the interaction with Re as the result of blocking the proposed boronic acid−diol binding interactions. Following the reaction of Re with acetic anhydride in the presence of pyridine, one major product fraction was isolated through column chromatography. Proton and ^{13}C NMR analyses were consistent with a mixture of heavily acylated derivatives. Low and high resolution ESI-MS revealed peaks for only undeca- and dodecaacylated Re derivatives. An average of the molecular weights of these two derivatives, which differ by less than 3%, was used to estimate a yield and prepare solutions of the AcRe mixture.

The fluorescence titration and binding constant determination with **8** and AcRe were then performed using the same procedure used for the ginsenoside derivatives described above. While greater quenching of receptor **8** emission was seen with AcRe than Re, further analysis revealed a significantly lower binding constant of 1600 ± 200 in the case of AcRe as compared to Re (3900 ± 210). This value represents ca. 40% of that seen for Re. Presumably, this reflects a reduction in the number of productive boronic acid−diol interactions. However, reduced binding due to the greater steric bulk of the AcRe derivative (relative to Re) cannot be definitely ruled out.

Additional support for the proposed binding mode was provided by high resolution ESI-MS experiments. A solution of receptor **8** in the presence of excess Re (1:1 chloroform:methanol) was found to display strong peaks for the predicted 1:1 **8**:Re complex containing one or two sodium adducts (see SI). Peaks corresponding to a 1:2 host:guest ratio were also observed. This latter result highlights the possibility of multiple host:guest stoichiometries, although it should be noted that data obtained through this method do not necessarily correspond to the compositions present in the solution.

In conclusion, we designed and synthesized bisboronic acid-functionalized porphyrin **8**, which was used to detect successfully five ginsenoside derivatives through fluorescence spectroscopy. To the best of our knowledge, receptor **8** represents the first small molecule optical sensor for ginsenosides. Further, different signals were obtained for each ginsenoside through fluorescence titrations with receptor **8**. The determination of 1:1 host:guest binding constants revealed that the strongest binding was obtained for ginsenosides with an increased number of saccharide units and also with the protopanaxtriol (as opposed to protopanaxdiol) substitution pattern. We propose a bifunctional binding mode in which the two boronic acid units of receptor **8** interact with the ginsenoside saccharide units, while the porphyrin ring participates in hydrophobic interactions with the steroid core of the guest. This binding mode was supported by the finding that receptor **8** interacts with an acylated derivative of Re (AcRe), albeit with a significantly reduced binding affinity compared to Re. It is expected that the dual binding mode of this receptor will allow for detection of ginsenosides in the presence of common interferents, such as oligosaccharides and starch found naturally in ginseng, as well adulterants, such as sawdust, that are sometimes found in commercial products.[7b] This study has served to demonstrate that hybrid bisboronic acid−monoporphyrin receptors can function as effective optical sensors for ginsenosides. This opens the door for the use of these or other supramolecular approaches to develop improved quality control methods for the ginseng industry.

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Supporting Information Available: Experimental procedures and spectral characterization for new compounds, optical spectra of **8**, best fit curves, AcRe titration results, and ESI-MS spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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