The Development of Photometric Sensors for Boronic Acids[1]

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Boronic acids bind certain 1,2- and 1,3-diols with high affinity through reversible formation of boronate esters. They have been utilized as the recognition moiety for artificial receptors, particularly receptors for carbohydrates that have *cis*-diol moieties. Therefore, sensors for boronic acids could serve as universal reporters for monitoring boronate formation. This paper reports the design and synthesis of a series of photometric chemosensors for phenylboronic acid using diethanolamine as the recognition moiety. Diethanolamine, which binds strongly to boronic acids, has been linked to three different types of optical reporters. A photoinduced electron transfer system based on the anthracene fluorophore has been used to create sensors that show up to a fivefold increase in fluorescent intensity in the presence of millimolar concentrations of phenylboronic acid. Sensor designs based on the restriction of free rotation of extended π systems and on the perturbed electronic properties of azo dyes are also included. This work demonstrates that sensors based on several different designs can be used for the detection of boronic acids.   © 2001 Academic Press

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INTRODUCTION

Boronic acids are capable of forming covalent linkages with diols to give boronate esters. Although the stabilities of these esters are dependent upon many factors, dissociation constants of boronates in aqueous solution have been reported in the micromolar range (*2*). Consequently, boronate formation has been widely used to recognize diol-containing molecules. This has led to the development of sensors for carbohydrates and amino acids (*3–7*) and selective transporters of nucleosides, saccharides, and nucleotides (*8–11*). Boronic acids are electron-deficient species that have also been used as organic reagents in Suzuki cross-coupling reactions (*12,13*), diol protection (*14*), Diels-Alder reactions (*15*), selective reduction of an aldehyde in the presence of a ketone (*16*), and asymmetric synthesis of amino acids (*17*). In

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addition, compounds containing boronic acid moieties have been used as inhibitors of proteases (*18–23*) and as therapeutic agents in boron neutron capture therapy (*24,25*).

Sensors for boronic acids could be used in a variety of applications (*26*). Sensors could monitor carbohydrate/boronic acid complexation by following the disappearance of free boronic acid, they might help elucidate the mechanisms of selective transport of biologically important molecules, or they may prove useful in mechanistic studies of reactions involving boronic acids or in monitoring reactions of boronic acids in combinatorial library synthesis. Of particular interest to us is the development of boronic acid sensors that may be used as universal reporters for monitoring boronate formation under near physiological conditions (*27*). As the range and utility of boronic acids increase in the fields of medicinal and organic chemistry, there is a growing need for the development of sensitive chemosensors for these compounds.

A chemosensor is generally composed of a recognition moiety that interacts with an analyte and a signaling unit that relays the recognition event. In designing our boronic acid sensors, we utilized diethanolamine as the recognition moiety. The diethanolamine structure displays high affinity binding with boronic acids through the formation of a boronate ester. This boronate is stabilized by the donation of the nitrogen lone pair electrons to the open shell of the boron atom (*28,29*), which allows for the formation of two fused five-membered rings (Scheme 1, **1b**). Such recognition has been used for the stabilization, purification, and characterization of boronic acids (*30*).

The second component of a chemosensor is the reporter. The reporter must provide a facile method for determining the binding between the boronic acid and the sensor. Photometric reporters allow sensitive analyte detection and are easy to monitor. To attain this type of reporting system, the attachment of a chromophore that is sensitive to the binding event is required. We have attempted to achieve this with three different designs: (i) photoinduced electron transfer based on a 9-(aminomethyl)anthracene system, (ii) rigidification of extended π system skeletons, and (iii) electronic perturbation of azo dyes. All systems show complexation with phenylboronic acid to varying degrees.

RESULTS AND DISCUSSION

Photoinduced Electron Transfer

It is well known that the nitrogen lone pair electrons of 9-(aminomethyl)anthracene can quench the fluorescence of an anthracene moiety through photoinduced electron transfer (PET) (*3,6,31,32*). Masking of the nitrogen lone-pair electrons (e.g., by protonation) causes a suppression of this fluorescence quenching and, therefore, results in fluorescence intensity increases (*4,6,33–35*). We envisioned that the diethanolamine recognition motif could be incorporated into an anthracene molecule so that its binding with boronic acid would lead to the formation of a boronate ester (Scheme 1, **1b–3b**), which has a boron atom ideally positioned to accept and, therefore, mask the nitrogen lone pair electrons. This masking of the nitrogen lone-pair electrons could then lead to an increase in fluorescence intensity of the anthracene moiety.

Three fluorescent sensors were designed, synthesized, and evaluated for their binding with phenylboronic acid (Scheme 1). In sensor **1a**, (*1*) a diethanolamine unit is
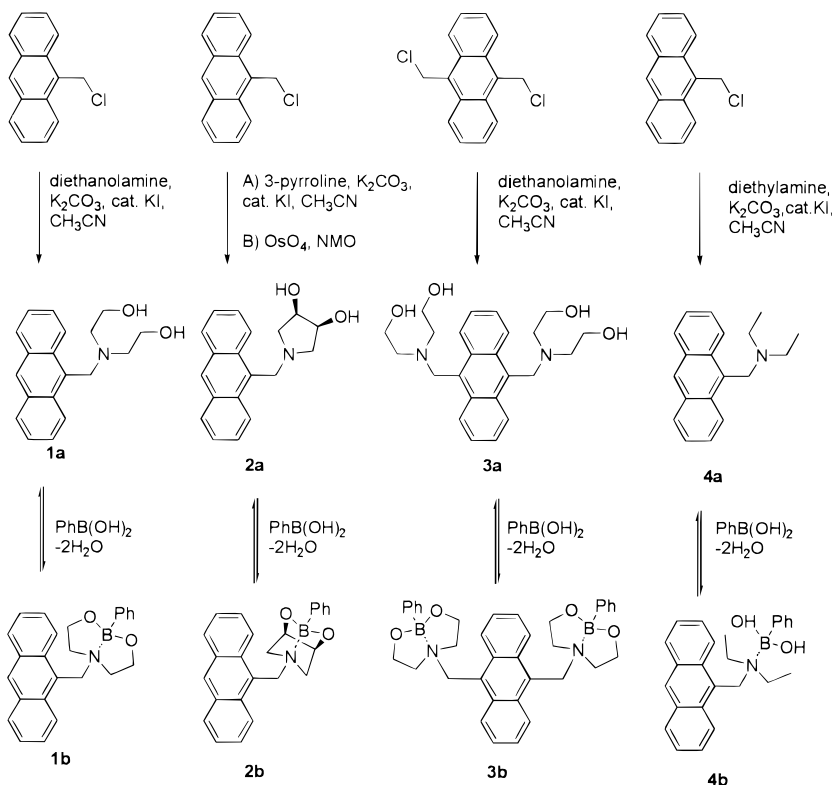
SCHEME 1. Photoinduced electron transfer sensors.

attached by a methylene linker to the 9 position of anthracene. Sensor **2a** is a constrained analog of **1a** and was designed to probe the effect of entropic factors upon the strength of binding. The bis(diethanolamine) sensor **3a** was designed to determine if two diethanolamine units would lead to more intense PET quenching in the absence of boronic acids, and therefore display a greater on/off effect. Sensors **1a** and **3a** and control compound **4a** were synthesized in one step from the appropriate chloromethylanthracene and diethanolamine or diethylamine following literature procedures (*34,36*). Sensor **2a** was synthesized in two steps by alkylation of pyrroline with 9-(chloromethyl)anthracene and then dihydroxylation with a catalytic amount of osmium tetroxide in the presence of *N*-methylmorpholine-*N*-oxide (NMO). The sensors were evaluated for binding strength by titrating phenylboronic acid into a 1.0×10^{-5} M solution of the sensor in methanol and measuring the increases in fluorescence intensity at an excitation wavelength of 370 nm and emission wavelength of 419 nm. Compound **4a** was included as a control to show that the changes in fluorescence intensity are not primarily related to changes in protonation state or due to nonspecific binding of phenylboronic acid with the sensor amine group. Sensors **1a** and **2a** showed the greatest activity compared with control **4a** (Fig. 1). Compound **2a** appeared to be more sensitive to low concentrations of phenylboronic acid, but the fluorescence
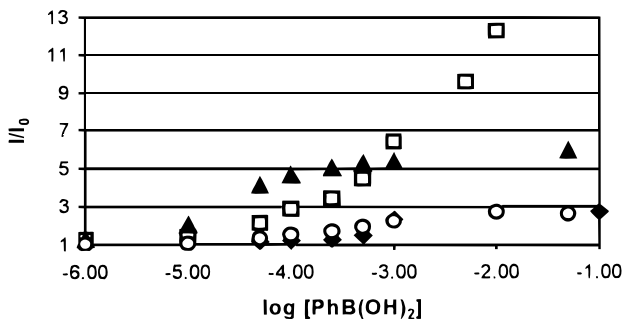
FIG. 1. Fluorescent response of PET sensors to phenylboronic acid. I_0, sensor **1a, 2a, 3a,** or **4a** at 1.0×10^{-5} M in MeOH. I, sensor **1a, 2a, 3a,** or **4a** at 1.0×10^{-5} M in MeOH with phenylboronic acid. □, Sensor, **1a;** ▲, sensor **2a;** ♦, sensor **3a;** ○, control **4a.**

reached a maximum at a lower intensity than that of **1a**. These results may be explained by the **2a**/phenylboronic acid complex having a greater binding strength, but lower overall fluorescent intensity than the corresponding **1a** complex. A stronger binding constant for **2a** may partially be explained by the constrained diol system leading to a decrease in the negative entropy component of the complex.

An NMR titration of sensor **1a** with phenylboronic acid was carried out to examine whether the sensor binds phenylboronic acid in a 1:1 ratio as designed. Mixtures of the sensor and phenylboronic acid with molar ratios of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.25, 2.0, and 10.0 in CDCl$_3$ were equilibrated for 45 min, and then ^{1}H NMR spectra were recorded. The pronounced chemical shifts of the CH_2O were observed. Without the binding to PhB(OH)$_2$, the chemical shift of the CH_2OH was about 3.5 ppm. However, with the binding, the chemical shift of the CH_2OB moved downfield to about 4.1 ppm. The ratio of [complex] to [complex + sensor] was calculated based on the ratio of integration of [complex] to that of [complex + sensor]. The molar ratio of [complex]/[complex + sensor] was plotted against [PhB(OH)$_2$]/[sensor] (Fig. 2), and shows a distinct 1:1 binding ratio.

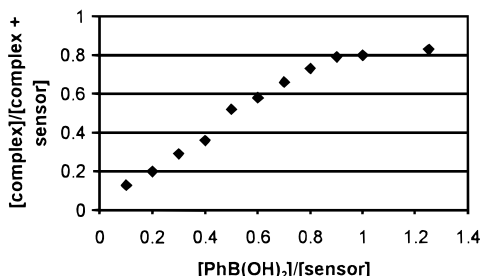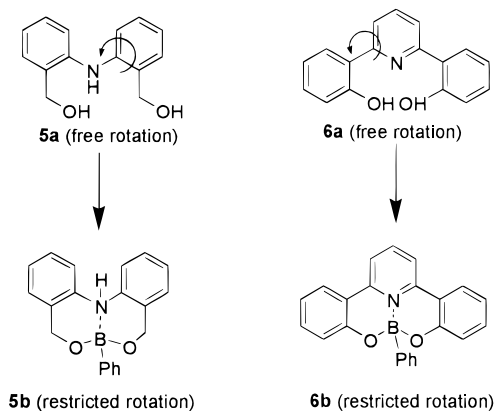Sensor **3a** appears to have a fluorescent profile similar to the control (Fig. 1). This

FIG. 2. Percentage of complexation as a function of the number of equivalents of phenylboronic acid. Calculated based on the ratio of ^{1}H NMR integration in CDCl$_3$.

SCHEME 2. Rigidification sensors.

may be caused by a lack of diester formation. A single free diethanolamine appears to have enough quenching ability to remove any fluorescent enhancement.

Rigidification

A second approach towards sensing boronate formation is the rigidification of a conjugated π system. Restricting the free motion of an extended π system can often deter nonradiative decay pathways of the excited state, and thereby promote fluorescence emission (*37*). We reasoned that properly positioned hydroxyls placed on adjacent aryl rings might present the correct architecture for boronate formation (Scheme 2, **5b** and **6b**). The covalent bonds of the ester would then hold the two rings in a planar, restricted conformation. Sensors **5a** (*38*) and **6a** (*35*) (Scheme 2) were synthesized according to literature procedures. Although the sensors have a dipropanolamine rather than a diethanolamine recognition moiety, we rationalized that the two six-membered rings might form a stable boronate ester (Scheme 2, **5b** and **6b**). The fluorescent increases shown by compound **5a** in dichloromethane (DCM) are displayed in Fig. 3. The fluorescent intensity increased by over threefold in the
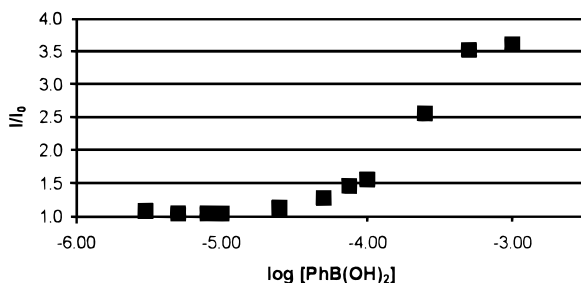

FIG. 3. Fluorescent response of 5a to phenylboronic acid. I_0 sensor **5a** at 1.0×10^{-4} M in DCM. I, sensor **5a** at 1.0×10^{-4} M in DCM titrated with indicated amount of phenylboronic acid.

presence of millimolar concentrations of phenylboronic acid. No intensity increases were seen in methanol. Compound **6a** showed no fluorescent intensity increases in either solvent. The lower electron density on the phenolic hydroxyls of **6a** may have led to a decreased stability of its boronate ester.

Azo Dye Electronics Perturbation

As a third approach toward sensing phenylboronic acid, we envisioned using an azo dye chromophore as an optical reporter. A diethanolamine moiety was attached to the azo system with the nitrogen in the aniline position of an aryl ring (Scheme 3). It is known that perturbation of the electronic properties of the aniline nitrogen can lead to quantifiable changes in the azo chromophore (*39*). We felt that boronate formation between phenylboronic acid and a diethanolamine moiety would change the electronic character of the aniline nitrogen (Scheme 3) and therefore shift the λ_{max} of its absorbance spectrum, resulting in a colorimetric response. By varying the substituents on the aromatic system we hypothesized that we could optimize the system to give large changes in the λ_{max}.

To verify that *N*-aryldiethanolamines bind boronic acids, the affinities of three *N*-aryldiethanolamine units (Fig. 4, **7**–**9** (*40,41*) for phenylboronic acid were determined by ^{1}H NMR studies (Table 1, **7**–**9**). The results showed that about 20–40% complexation occurred between **7** and **9** and the boronic acid. Given these promising results, a set of potential azo sensors was prepared by coupling an *N*-aryldiethanolamine with the diazonium salt of a substituted aniline (Sch. 4; Fig. 4). Commercially available anilines were used to synthesize compounds **10**–**16** (Fig. 4) (*42,43*)). Reduction of the respective nitro-substituted compounds gave the amine-substituted dyes **17**–**19** (*42*).

Initially ^{1}H NMR studies were used to verify the binding between the dyes **10**–**19** and phenylboronic acid (Table 1). As with the *N*-aryldiethanolamines **7**–**9**, the signal for the protons on the methylene bearing the hydroxyl group typically showed the greatest shift upon complexation (data not shown). Integrations of the signals for the free and complexed forms of the dye were used to determine the relative ratio of free to complexed dye. The sensors showed 20–50% complexation with excess phenylboronic acid (Table 1, compounds **10**–**19**).

Absorbance and fluorescence studies were also carried out on the target compounds.
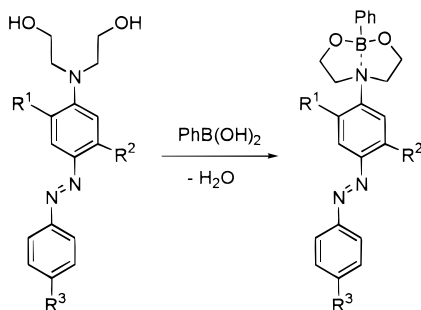
SCHEME 3. *N*-Aryldiethanolamine azo dyes designed as sensors for boronic acids.