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Highly efficient fluorescent sensing for α-hydroxy acids with C_3-symmetric boronic acid-based receptors

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ABSTRACT

Two boronic acid-based fluorescent chemosensors in C_3 symmetry have been prepared with a facial method. These compounds show remarkable ability to recognize α-hydroxycarboxy acids and sugar acids over most saccharides. The fluorescence intensity of the receptors decreased obviously upon adding the α-hydroxy acids in a pH 8.71 buffer of methanol–water, which can be explained with the internal charge-transfer (ICT) mechanism.

The molecular recognition for the saccharides has attracted much attention due to its potential application in biomedicine chemistry. Different from the hydrogen-bonding-interactions-based recognition between saccharides and their natural receptors (proteins) in the body, the main artificial hosts for saccharides' recognizing and sensing are boronic acid-based small molecules, which could react with 1,2- or 1,3-diols reversibly to form stable five- or six-membered cyclic esters.[1] In addition to *cis*-diols, boronic acid can also react with α-hydroxy acids,[2] which are present in the nature with large scale. The detection and quantification of tartrate and malic acid could be used in the beverage industry. The lactate in the body is the unique marker indicative of the capability of the muscle for athletic performance. D-Galacturonic acid is a component of plant gums and bacterial cell walls, and plays an equivalent role to the core oligosaccharide phosphate residues in establishing outer membrane integrity in *Escherichia coli* and *Salmonella*.[3] Therefore, the study of molecular recognition of α-hydroxy acids and sugar acids is important.

Fluorescence technique has high sensitivity, and boronic acid-based fluorescence molecules have been heavily investigated for sensing saccharides.[4] There are two main sensing mechanisms: the photo induced electron-transfer (PET) and the internal charge-transfer (ICT). Recently, the ground-state charge-transfer complex was also employed in the fluorescence sensing system.[5] Compared with the boronic acid-tertiary amine interaction in PET system, most of ICT systems are due to the change of electron density of anilinic nitrogen. James and co-workers have reported several ICT-type sensors for saccharides, which showed good selectivity on recognition.[6] However, there are still very few efficient fluorescent ICT systems for α-hydroxy acids or sugar acids. Herein, we describe the synthesis and fluorescent spectral studies of two new receptors **3a** and **3b**, which are C_3-symmetric with three binding sites. The experiment results have demonstrated that **3a** and **3b** are efficient sensors for α-hydroxy acids.

Starting from the commercial available materials, *o*- or *m*-nitroacetophenone, the compounds **3a** and **3b** were synthesized with a simple method, respectively[7] (Scheme 1). The condensation products **2a/b** reacted with 2-formylphenylboronic acid to form Schiff bases, which were reduced with $NaBH_4$ to afford the final products. The total yields for **3a**/**b** are 19% and 23%, respectively.[8]

Similar as what was reported in the previous publications, the fluorescent intensity of boronic acid **3a**/**b** was strongly pH-depended, which was mainly due to different boron ionization states[1] (Fig. 1). For example, **3a** displayed minimum fluorescent intensity at pH 8 and maximum changes with tartaric acid at pH 4.0 and 9.0. In this work, the fluorescence titration of **3a**/**b** with all guests was carried out in a pH 8.71 buffer (68.1 wt % methanol in water with KCl, 0.01000 mol dm^{-3}; KH_2PO_4, 0.002869 mol dm^{-3}; Na_2HPO_4, 0.002869 mol dm^{-3}).[9]

To our surprise neither **3a** nor **3b** showed obvious fluorescent changes for most saccharides under our conditions although some similar sensing molecules with ICT mechanism have shown good fluorescent responses in the presence of monosaccharides.[6] We

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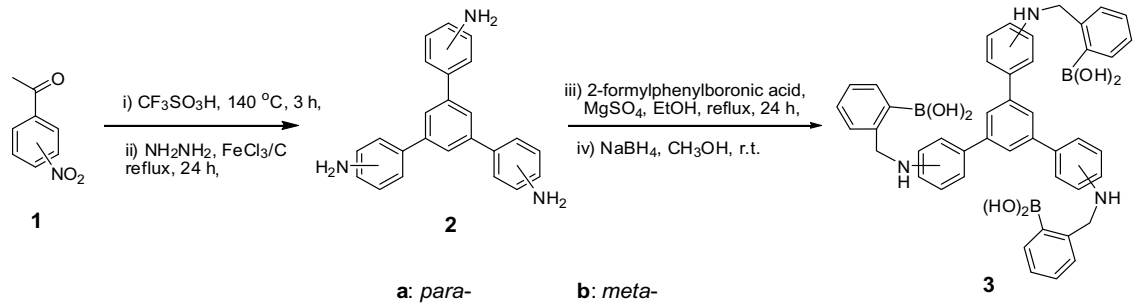

Scheme 1.

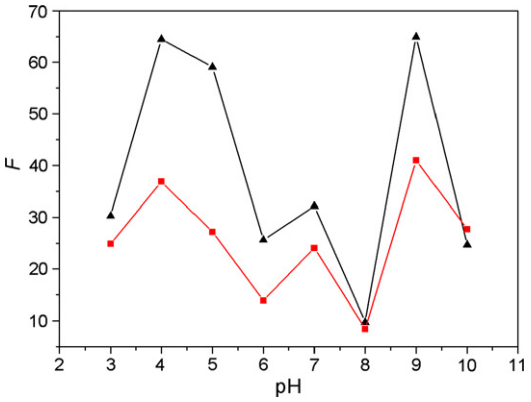

Figure 1. pH–fluorescence profiles of **3a**: (▲ free; ■ in the presence of tartaric acid; CH_3OH:H_2O = 52:48 wt/wt, using aqueous HCl and NaOH to control pH).

have tried D-arabinose, D-xylose, D-ribose, D-glucose, D-galactose, D-mannose, D-fructose, D-mannitol, methyl-D-glucopyranoside, maltose and α-lactose. Even adding large excess amount of these substrates, there are a few changes in the fluorescent density. However, the fluorescence of **3a**/**b** showed different properties on addition of α-hydroxy acids or sugar acids (Fig. 2). In the fluorescent spectra of **3a**, maximum emission wavelength was observed at 460 nm (λ_{ex} = 297 nm), and the intensity was stepwise decreased following the addition of malic acid. At the same time, the maximum emission wavelength has a little red-shift. The fluorescent spectra of **3b** were little different with **3a**. Upon adding α-hydroxy acids or sugar acids, there was a significant decrease of fluorescence intensity at 430 nm and an increase with a less magnitude at 358 nm (λ_{ex} = 257 nm). Different fluorescent changes of **3a** and **3b** may be due to their different conjugated states or conformation properties. Here, we used the buffer to control pH value of the solution during the titration. Moreover, adding the same or even larger amount of acetic acid upon the same conditions did not induce the fluorescent changes of **3a**/**b**. So this fluorescent sensing procedure did not come from the change of the pH of the solution but by forming new complexes.

The fluorescent sensors **3a** and **3b** not only remarkably differentiate α-hydroxy acids from saccharides, but also show good selectivity of sensing different common α-hydroxy acids. Figure 3 displays the fluorescent intensity of **3a** and **3b** upon addition of different α-hydroxy acids with the same concentration. The order of their selectivity was the same: tartaric acid > malic acid > lactic acid > galacturonic acid. From the Stern–Volmer plot for fluorescent quenching in a special concentration range,[10] we deduced that this plot fitted approximately the 1:1 stoichiometry of the complex and calculated the binding constants between the α-hydroxy acids and **3a**/**b** (Table 1). We find that there is an obvious cooperation between the three boronic acid moieties during binding. Both sensors **3a** and **3b** have the largest stability constant for binding tartaric acid. The selectivity may be attributed to the fact that tartaric acid containing two α-hydroxy carboxyls could form a bidentate complex with the sensors. The malic acid, lactic acid and galacturonic acid have only one α-hydroxy carboxyl, and their interaction with the receptor is weaker. Furthermore, the binding difference between **3a** and **3b** with same substrates was due to the distance and/or angle between two boronic acids located at two phenyl branches. So the precise placement of the recognition elements of the receptor in the proper position and orientation for optimal complementarity to the guest are very important to design sensors.

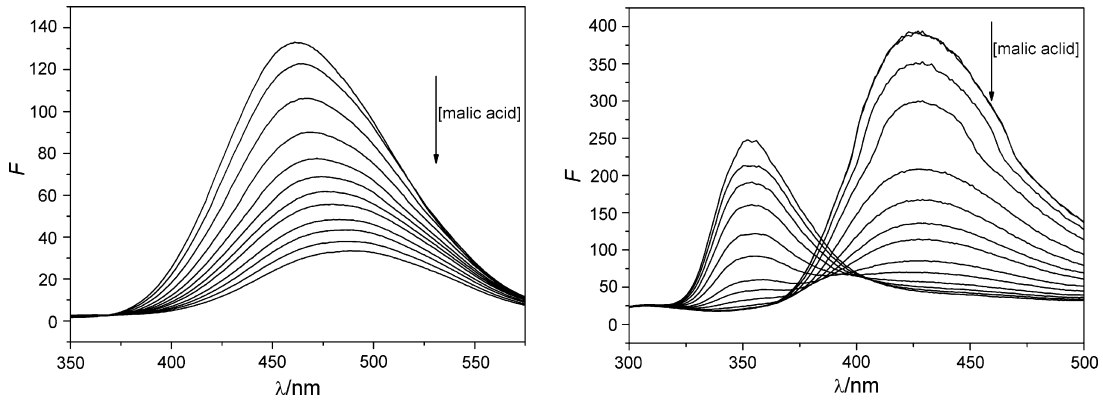

Figure 2. Fluorescence emission spectra of **3a** (1.4×10^{-2} mM) in the presence of malic acid (0, 0.93, 2.75, 3.65, 4.55, 5.43, 6.30, 7.17, 8.03, 9.72, 11.38, 13.81 mM), λ_{ex} = 297 nm, left; and fluorescence emission spectra of **3b** (1.6×10^{-2} mM) in the presence of malic acid (0, 0.74, 2.21, 2.94, 3.65, 4.37, 5.08, 5.78, 7.17, 8.54, 10.55, 12.52 mM), λ_{ex} = 257 nm, right.

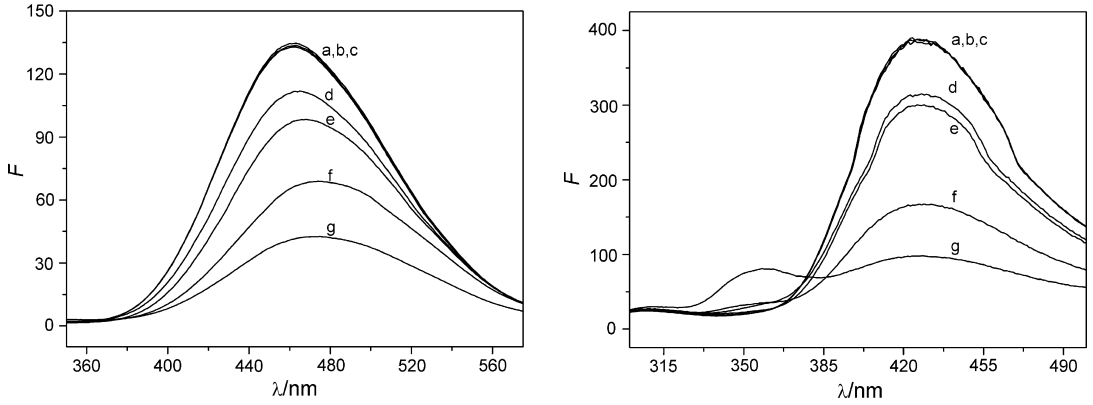

Figure 3. Fluorescence emission spectra of **3a** and **3b** in the presence of different substrates (a: free, b: D-glucose, c: D-galactose, d: galacturonic acid, e: lactic acid, f: malic acid, g: tartaric acid) with same concentration. Left: [**3a**] = 1.4×10^{-2} mM, [Substrate] = 5.0 mM, λ_{ex} = 297 nm; right: [**3b**] = 1.6×10^{-2} mM, [Substrate] = 5.0 mM, λ_{ex} = 257 nm.

Table 1
Stability constant K (M^{-1}) for α-hydroxy acids of fluorescent sensor **3a** and **3b**, in pH 8.71 buffer at λ_{ex} 297 nm (**3a**) and 257 nm (**3b**)

α-Hydroxy acids	3a	3b
Tartaric acid	440	2300
Malic acid	290	700
Lactic acid	100	270
Galacturonic acid	100	270

In this sensing system, fluorescence decrease may involve internal charge-transfer (ICT) mechanism because the amino groups are integrated into the fluorophore in the receptors.[11] The π- electron system of the receptors has very different dipole moments in their ground and lowest energy singlet excited states due to the internal charge-transfer. Upon complexing, the heteroatom will change the interaction state with the substrate and therefore affect the dipole moment of the fluorophore. It is known that the B–N bond can be broken to form boronate species **B** on addition of saccharide. But the stability constant for this reaction balance is low.[6] In our system, the two hydroxyl groups of α-hydroxyl acid could also form the less stable five-membered cyclic esters with boronic acid. However, the additional carbonyl group of the α-hydroxyl acid may form a new hydrogen bond with the NH group of aniline branch, which could play an important role for the high selectivity of the recognition (Scheme 2).

In summary, the two new fluorescent ICT sensors **3a** and **3b** displayed large decrease of fluorescence intensity upon binding with α-hydroxy acids. The different position of the amino-group at the phenyl has a significant effect on the fluorescence properties of these sensors. We believe that the high selectivity on recognition for α-hydroxy acids over most saccharides is due to the additional hydrogen bonds between the carbonyl groups and NH groups.

Acknowledgements

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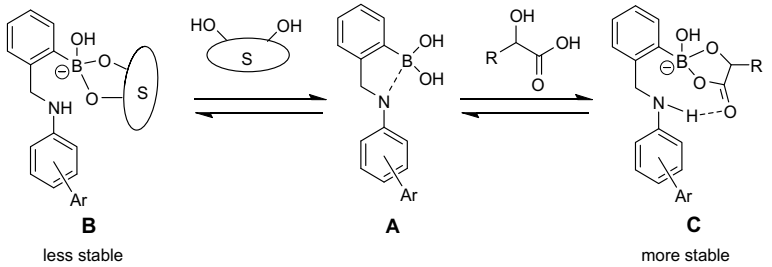
8. Compound **3a**: Yield: 19%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.48 (s, 3H), 7.89 (d, 3H), 7.80–7.84 (m, 9H), 7.69–7.74 (m, 6H), 7.45–7.48 (m, 6H), 7.34 (t, 3H), 4.62 (s, 6H); ^{13}C NMR (75.5 MHz, DMSO-d_6): 148.5, 145.2, 141.4, 132.2, 130.1, 129.7, 127.4, 126.3, 122.4, 117.8, 52.5; IR (KBr): ν 3384, 2923, 2854, 1610, 1515, 1460; ESI/MS (negative): m/z 754.23 ([M+1]$^-$). Compound **3b**: Yield: 23%. ^{1}H NMR: δ



Scheme 2. Proposed reaction balance in the buffer.

9.48 (s, 3H), 8.02 (s, 3H), 7.95 (s, 3H), 7.89 (d, 3H), 7.70 (d, 3H), 7.38–7.48 (m, 12H), 7.31 (t, 3H), 4.67 (s, 6H); ^{13}C NMR: 149.0, 146.7, 142.9, 141.5, 135.7, 130.6, 130.1, 129.9, 126.7, 125.0, 122.9, 119.6, 117.5, 116.8, 53.0; IR (KBr): *v* 3406, 2923, 2852, 1602, 1486; ESI/MS (negative): *m*/*z* 752.41 ($[M-H]^-$).

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