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The first fluorescent sensor for D-glucarate based on the cooperative action of boronic acid and guanidinium groups

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A new fluorescent sensor (1) with a recognition unit consisting of a boronic acid moiety and a guanidinium unit shows selective binding of D-glucarate in aqueous solution.

The design of compounds that are capable of selective recognition of molecules and ions is of great interest in bioorganic chemistry.[1] D-Glucarate is a biologically active carbohydrate that exists in human serum, vegetables, and fruits.[2] It has been used as a chemopreventive agent in certain cancers.[3] Moreover, the glucarate catabolic pathway is an attractive field that has been studied extensively.[4] The analysis of D-glucarate has been achieved by using enzymes.[5] Analysis using fluorescent sensors, on the other hand, offers the advantages of convenience and high sensitivity. Herein, we wish to report our efforts in developing the first fluorescent sensor for D-glucarate.

Our design uses a two binding site approach with a boronic acid and a guanidinium recognition moiety separated by a spacer (**1**). Such a design takes advantage of the reversible formation of cyclic esters of boronic acid with diols in aqueous media[6,7] and the known strong interactions between a guanidinium moiety and carboxylate.[8] The key in this design is the appropriate linker length and rigidity, and the relative orientations of the two recognition moieties. As for the fluorescent reporter group, an anthracene group was chosen because its fluorescence intensity can be regulated by a photoelectron transfer process.[6a,b] Molecular modelling studies seem to indicate that attachment of a boronic acid moiety and a guanidinium group to the 9,10 positions of the anthracene ring could afford a sensor compound with the appropriate linker length and functional group orientation for binding with glucarate (Fig. 1).

The sensor (**1**)† was synthesized starting with the conversion of aldehyde **4** to amine **5** through reductive amination (Scheme 1).[6k] Then the boronic acid moiety and guanidine group were attached to give **7**. Boc-deprotection using HCl gave **1** in about 10% overall yield, which was purified by precipitation from methylene chloride with the addition of ether. As controls for the binding studies, compounds **2** and **3** were also synthesized in similar fashions.

Fluorescence experiments were conducted to evaluate the binding of the sensor (**1**) and control compounds (**2**, **3**) for D-glucarate and other related saccharides in order to examine their selectivity and sensitivity. Upon addition of glucarate the fluorescence intensity of the sensor (**1**) solution indeed increased by about 4.5-fold at 20 mM (Fig. 2). On the other hand, control compound **3** did not exhibit any fluorescence intensity changes upon addition of glucarate (Fig. 3), indicating that interaction with the guanidinium group alone was not enough to affect the fluorescence intensity of the sensor. To examine whether sensor **1** works as designed, it is important to compare the affinity and selectivity of **1** with the monoboronic acid compound **2**. As expected, **2** also showed fluorescence intensity changes when glucarate was added. However, the magnitude of the changes was smaller (Fig. 3).

The stability constants of sensors **1** and **2** with different saccharides were determined in order to quantitatively measure the effect of the added guanidinium group on the selectivity and affinity of **1** for glucarate. From Table 1, it can be seen that

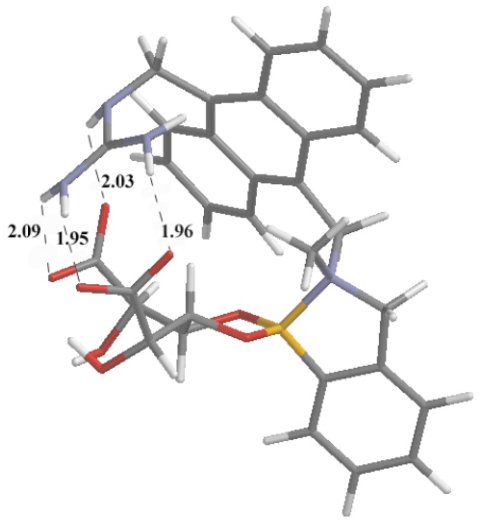
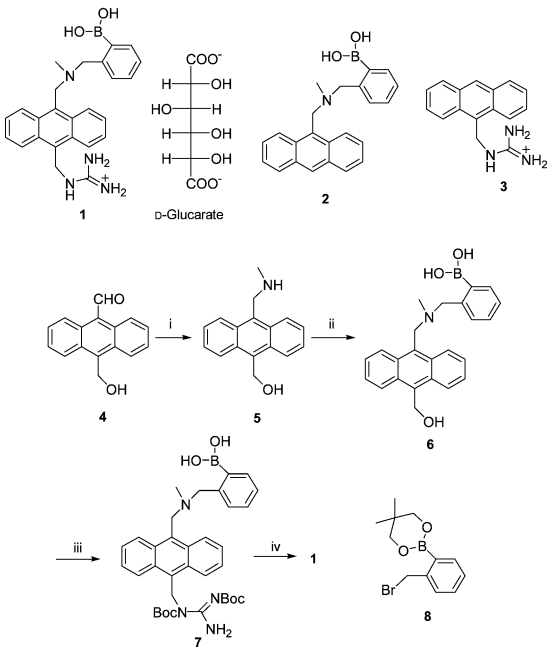


Fig. 1 Energy-minimized structure of **1**–glucarate complex. The calculation was carried out using Spartan '02. Conformational search was conducted using the MMFF molecular mechanics force field. Conformers within 10 kcal mol^{-1} of the lowest energy conformation were further optimized with AM1 semi-empirical calculation. The boronic acid was postulated to bind with 3,4-diol of D-glucarate.[9] The B–N bond length was set to 1.75 Å based on crystal structural evidence.[10] Hydrogen bonds are shown as broken lines.



Scheme 1 *Reagents and conditions:* i, MeOH, THF, $MeNH_2$ (40%, wt), then $NaBH_4$, 90%; ii, CH_3CN, **8**, K_2CO_3, 60%; iii, 1,3-bis(*tert*-butoxycarbonyl)guanidine, DEAD, Ph_3P, 33%; iv; 4 N HCl, dioxane, 55%.

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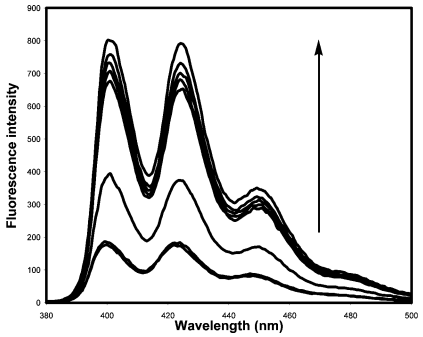

Fig. 2 Fluorescence spectra of **1** (1.0×10^{-5} M) upon addition of D-glucarate (0, 0.01, 0.1, 1, 2, 5, 10, 20 mM) at 25 °C in 50% MeOH/0.1 M aqueous HEPES buffer at pH 7.4, λ_{ex} = 370 nm.

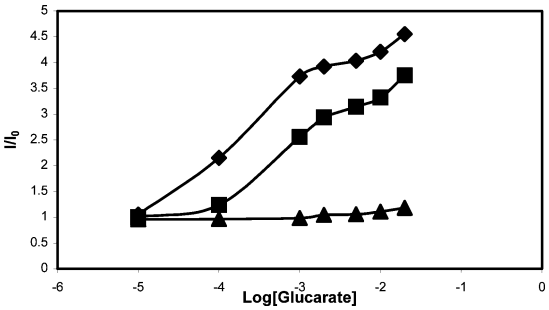

Fig. 3 Relative fluorescence intensity changes of (◆) **1**, (■) **2** and (▲) **3** as a function of D-glucarate concentration at 25 °C, 1.0×10^{-5} M of **1**, **2** and **3** in 50% MeOH/0.1 M aqueous HEPES buffer at pH 7.4, λ_{ex} = 370 nm, λ_{em} = 424 nm.

compound **2** with only the boronic acid binding site shows the strongest affinity with D-sorbitol, as would be expected with monoboronic acid compounds.[7] The order of selectivity for monoboronic acid **2** is: D-sorbitol > D-glucarate ≈ D-gluconate > D-glucuronic acid ≈ D-glucose, which reflects the intrinsic affinity of a monoboronic acid unit for various sugars. Compound **1** with two different binding sites, on the other hand, showed the highest affinity for D-glucarate, as designed, among all the structurally similar saccharides tested (Table 1). The binding constant of **1** with D-glucarate is increased by about 5-fold compared with that of the monoboronic acid compound (**2**), presumably due to the added guanidinium group in **1** for the recognition of the carboxylate groups of glucarate. Such results can only be attributed to the cooperative action of boronic acid and guanidinium units in affording the specific recognition of glucarate, and, therefore, indicate that **1** binds with glucarate in a two-point binding mode and the appropriate linker length and rigidity in **1** afford the selectivity for glucarate. Furthermore, the fact that glucarate binds more tightly than glucuronic acid indicates that both carboxylates of glucarate are involved in the binding, presumably through interaction with the guanidinium group. This is also consistent with the molecular modelling studies. As discussed earlier, computer molecular modelling results indicate that in the lowest energy conformation, the two carboxylate groups of glucarate can interact with the protonated nitrogens of the guanidinium group, assuming the boronic acid bind to glucarate *via* the 3,4-diol (Fig. 1).[9]

Table 1 Stability constants (M^{-1}) for the saccharides with **1** and **2**

Saccharide	**1**	**2**
D-Glucarate	5142 ± 267	846 ± 82
D-Gluconate	1452 ± 142	670 ± 39
D-Sorbitol	1300 ± 78	1647 ± 141
D-Glucuronic acid	46 ± 5	27 ± 3
D-Glucose	62 ± 9	18 ± 3

There are literature precedents that two identical binding units properly arranged in a synthetic receptor can be used for the selective recognition of various targets.[6d,k,l] Compared with using two identical binding units, the design and synthesis of a synthetic receptor with two different binding sites is more challenging. Only a few fluorescent sensors containing two different binding sites to capture a single species have been reported so far.[11] To the best of our knowledge, compound **1** is the first fluorescent receptor for D-glucarate, which features two-point interactions *via* boronic acid–diol complexation and guanidinium–carboxylate recognition. The receptor can discriminate well between D-glucarate and other structurally similar carbohydrates and the binding event results in significant fluorescence changes. Further work utilizing the concept of multi-point interactions for the design of sensors for various analytes is under way.

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Notes and references

† *Selected data* for **1**: Yield 55%. ^{1}H NMR (CD_3OD, 300 MHz) δ8.45–8.40 (m, 4H), 7.82–7.56 (m, 8H), 5.45 (s, 2H), 5.41 (s, 2H), 4.73 (s, 2H), 2.77 (s, 3H). MS-ESI: 427.3 (M^+ + H). HRMS (ESI) calcd. for $C_{25}H_{27}BN_4O_2$ (M^+ + H) 427.2305; found 427.2297. Anal. calcd. for $C_{25}H_{27}BN_4O_2 \cdot 1.4(C_4H_{10}O) \cdot 2.3HCl$: C, 59.78; H, 7.10; N, 9.11. Found: C, 59.82; H, 6.73; N, 9.09%.

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