Molecular Fluorescence Sensor for Saccharides Based on Amino Coumarin

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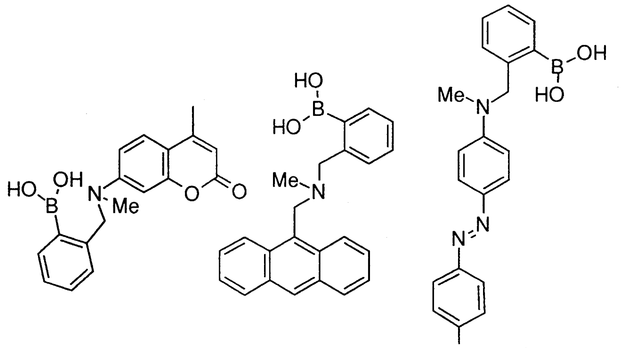
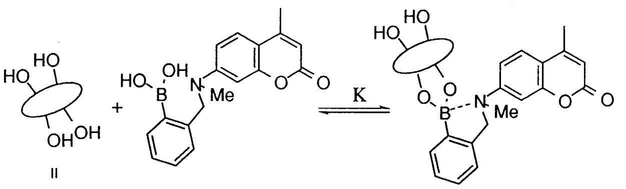
The intramolecular neighboring group participation of the amino group of 7-aminocoumarin moiety to phenylboronic acid gave fluorescence intensity and spectral changes upon saccharide binding which could be used in fluorescence mapping of saccharides in biological cells.

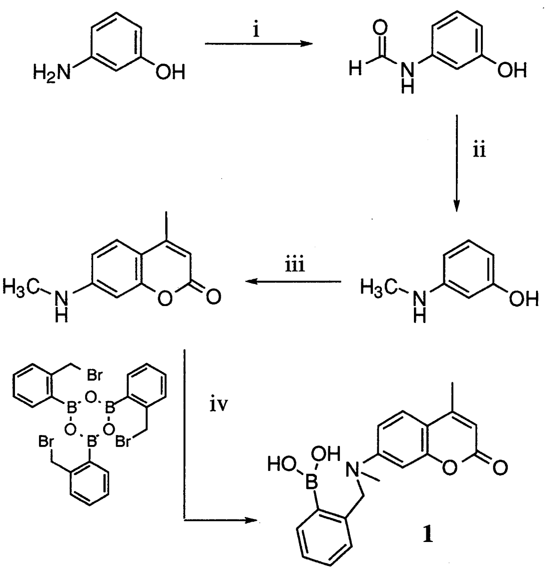
Molecular recognition and transduction of biologically important molecular species by synthetic molecular receptors are our current interest. Hydrogen bonding interaction has been the popular binding force in molecular receptor design for molecular species.[1] Recently, we and other researchers have demonstrated the importance of boronic acid in diol binding; hence in molecular sensor design for saccharides.[2-6] The intramolecular interaction of an amine group with the boronic acid moiety is found to be useful in increasing the acidity of the boronic acid moiety and creating an electron rich center around the boron atom. This enhanced binding site has been utilized in photoinduced electron transfer sensor design[4,7] and in molecular color sensors[5] and found to be extremely useful.



1 **2** **3**

Fluorescence molecular sensors are important in understanding the function of saccharides in biological systems. However, fluorescent photoinduced electron transfer sensors[4] do not give any spectral changes in terms of wavelength. This is a disadvantage in fluorescence mapping of saccharides in biological cells.[7] In order to achieve a fluorescence sensor with fluorescence color change we have designed coumarin based molecular fluorescence sensor **1**. 7-*N,N*-Dialkylamino coumarin is known to have good fluorescence quantum yields with an internal charge transfer chromophore. The intramolecular neighboring group participation of the aromatic amine to the boronic acid moiety in **1** could influence the donor site of the internal charge transfer chromophore hence, the fluorescence properties of the coumarin moiety.





Reagents (yields) : i. Ethyl formate, reflux, 11 days (90%); ii. BH_3•THF / THF (86%); iii. Ethyl acetoacetate, $ZnCl_2$, EtOH, reflux 18 hr (46%); iv. K_2CO_3, CH_3CN, rt (20%).

Scheme 1.

Molecular sensor **1** was readily synthesized according to the Scheme 1 in high yields. Saccharide titration of molecule **1** in 50% methanolic aqueous solution[9] gave fluorescence quenching together with fluorescence red shifts (Figures 1 and 2). The best fluorescence shift was found to be for fructose. This is in accord with the early finding of Czarnik *et. al.* [3] and our own results.[4,5,7] The stability constants (log *K*) for fructose of sensor molecules **2** and **3** were found to be 3.0[7] and 2.14[5], respectively. The lower basicity of the aromatic amine in **3** has resulted in lower stability constants for fructose. However, this may not

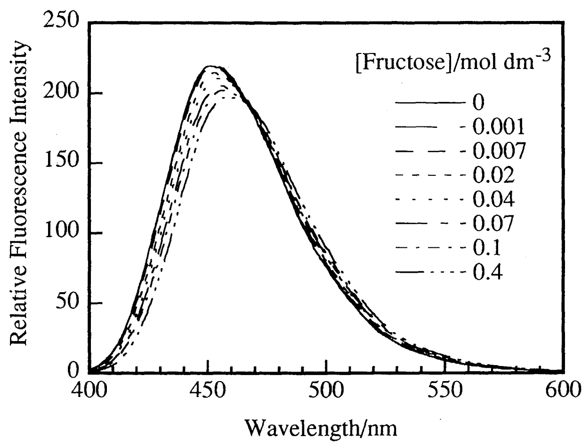


Figure 1. Fluorescence spectral changes of **1** at different fructose concentrations: 1×10^{-5} mol dm^{-3} of **1** in methanol-water mixture (1:1, v/v), excitation at 360 nm.

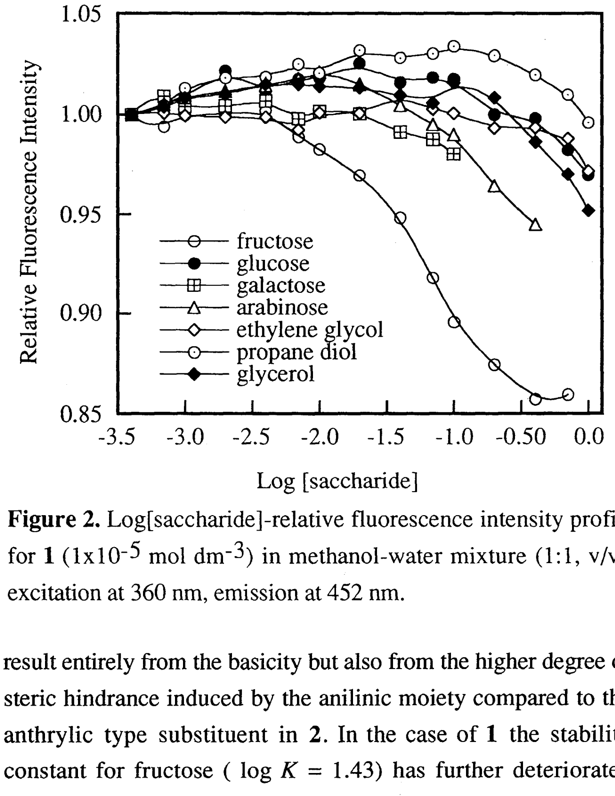


Figure 2. Log[saccharide]-relative fluorescence intensity profile for **1** (1×10^{-5} mol dm^{-3}) in methanol-water mixture (1:1, v/v): excitation at 360 nm, emission at 452 nm.

result entirely from the basicity but also from the higher degree of steric hindrance induced by the anilinic moiety compared to the anthrylic type substituent in **2**. In the case of **1** the stability constant for fructose (log K = 1.43) has further deteriorated probably due to lower basicity of the aromatic amine which is the donor site of a strong internal charge transfer system. 7-Dialkylamino coumarins are known to have a strong internal charge transfer system which leads to a large Stork shift.[10] A smaller contribution to the stability constant may arise from the fact that the stability constant for **1** has been measured in 50% aqueous methanolic solution.[7] The saccharide binding to **1** red shifts the UV absorption spectrum of **1**. The destabilization of the ground state of the internal charge transfer system, compared to the excited state, is responsible for this red shift. The stability constants calculated for fructose by UV absorption spectral shifts is in line with the fluorescence related values (log K = 1.44).

In conclusion we have designed, synthesized and evaluated a molecular fluorescence sensor which could be useful in fluorescence mapping of saccharides in biological cells. However, the sensitivity to the cell saccharide levels, selectivity towards a particular saccharide and larger spectral changes are important in selective mapping of saccharides. We believe that this molecular design opens the door to the selective mapping of saccharides in biological systems.

References and Notes

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