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A Modular Fluorescence Intramolecular Energy Transfer Saccharide Sensor

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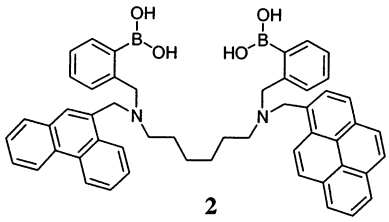
ABSTRACT



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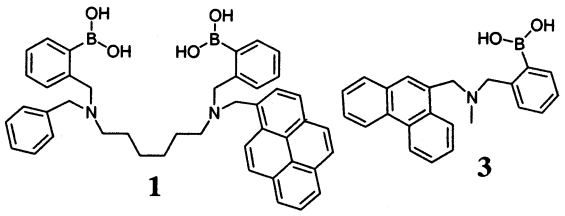
A modular fluorescence intramolecular energy transfer saccharide sensor 2 has been prepared with phenanthrene as the donor and pyrene as the acceptor.

A great amount of attention continues to be devoted to the development of synthetic molecular receptors with the ability to recognize neutral organic species, including saccharides.[1,2] The vast majority of these systems have relied upon hydrogen bonding interactions for the purposes of recognition and binding of guest species. However, there is still no designed, monomeric hydrogen bonding receptor that can compete effectively with bulk water for low concentrations of monosaccharide substrates.[3] The boronic acid−saccharide interaction can be utilized to overcome the problem of undesired solvent competition for the host. Boronic acids readily and reversibly form cyclic boronate esters with diols in aqueous basic media.[1,2] Saccharides contain a linked array of hydroxyl groups that provide an ideal structural framework for binding to boronic acids. The most common interaction is with 1,2- and 1,3-diols of saccharides to form five- or six-membered rings, respectively, via two covalent bonds.



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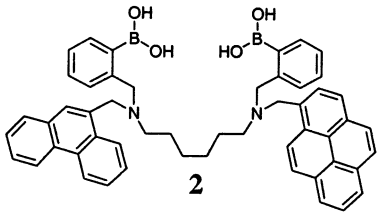

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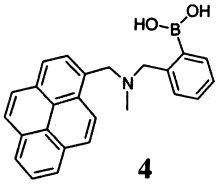

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Because of these properties the boronic acid is becoming the receptor of choice in the design of fluorescent sensors for saccharides.[1,2,4−13] Over the past few years we have been

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interested in developing new fluorescence sensors selective for saccharides employing a modular approach.[11,14,15] The basic idea was to break a sensor into three components: receptor units, linker units, and fluorophore units. The approach requires the selection and synthesis of a set of molecular binding blocks from which the selective fluorescent sensors can be easily constructed. The quick assembly of a diverse selection of fluorescent sensors will require that the receptor and fluorophore units are linked to core units using the minimum of synthetic linkage reactions. The use of common reactions means the synthetic routes toward the new sensors will be convergent. Our modular system **1** contains two phenylboronic acid groups (for selectivity), one pyrene group (for fluorophore), and hexamethylene (for linker).[11] The modular nature of **1** makes it easy to vary both the fluorophore and linker length. The choice of linker is very important because it determines the selectivity for a particular saccharide. Our research has demonstrated that hexamethylene was the best linker length to obtain D-glucose selectivity.[11,15]

Our aim with this research was to apply the modular design to prepare a saccharide sensing system using fluorescence energy transfer. Fluorescence energy transfer is the transfer of excited-state energy from a donor to an acceptor. The transfer occurs as a result of transition dipole−dipole interactions between the donor−acceptor pair.[16] In this paper we report on a new fluorescence sensor **2** that has two phenylboronic acid groups, hexamethylene linker, and two different fluorophore groups (phenanthrene and pyrene).

Our idea with this system was to investigate the efficiency of energy transfer (ET) from phenanthrene to pyrene as a function of saccharide binding. A similar concept has previously been employed in the construction of a fluorescent calix[4]arene sodium sensor.[17]

The excitation and emission wavelengths of phenanthrene **3** (donor) are 299 and 369 nm, respectively, while the excitation and emission wavelengths of pyrene **4** (acceptor) are 342 and 397 nm, respectively. The emission wavelength of phenanthrene **3** (369 nm) and excitation wavelength of pyrene **4** (342 nm) overlap. These observations suggest that intramolecular energy transfer from phenanthrene to pyrene can take place in modular sensor **2**. In addition it is also possible to observe the long wavelength excimer emission due to $\pi-\pi$ stacking of phenanthrene and pyrene.

To confirm that the $\pi-\pi$ stacking of sensor **2** is only intramolecular and not intermolecular we plotted the absorption versus concentration of **2** and **3** + **4** in pH 8.21 buffer (52.1 wt % methanol in water with KCl, 0.01000 mol dm^{-3}; KH_2PO_4, 0.002752 mol dm^{-3}; Na_2HPO_4, 0.002757 mol dm^{-3}).[18] The plots for sensor **2** and the mixture of sensor **3** + **4** are linear until 2.0×10^{-5}mol dm^{-3} ($\epsilon = 2.12 \times 10^4$ dm^3 mol^{-1} (λ_{max} 342 nm) for sensor **2** and 4.59×10^4 dm^3 mol^{-1} (λ_{max} 342 nm) for sensors **3** + **4**), clearly demonstrating that the $\pi-\pi$ stacking of sensor **2** is only intramolecular.

Fluorescence titrations of **2** (2.5×10^{-6} mol dm^{-3}) excited at λ_{ex} 299 nm (phenanthrene) and λ_{ex} 342 nm (pyrene) were performed with different saccharides in pH 8.21 buffer. The fluorescence intensity of sensor **2** at 417 nm increased with added saccharide when excited at 299 and 342 nm, while the excimer emission at 460 nm decreased with added saccharide. The excimer emission change indicates that the fluorophore stacking of phenanthrene and pyrene is broken on saccharide binding.

At an excitation wavelength of 299 nm (phenanthrene) no emission was observed at 369 nm (phenanthrene), but emission was observed at 417 nm (pyrene) (Figure 1). This result implies that the excited energy of phenanthrene (donor) was transferred to pyrene (acceptor), so that only the emission spectra of pyrene was observed.

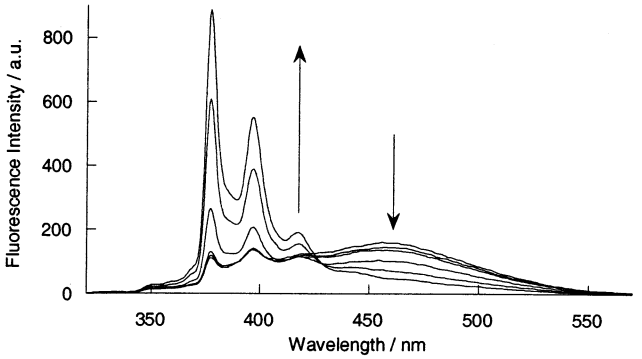

Figure 1. Fluorescence spectral change of **2** (2.5×10^{-6} mol dm^{-3}) with different concentrations of D-glucose in pH 8.21 buffer (λ_{ex} 299 nm).

The stability constants (K) of sensors **2**(λ_{ex} 299 nm), **2**(λ_{ex} 342 nm), **3**, and **4** were calculated by fitting the emission wavelengths at 417, 417, 367, and 397 nm versus concentration of saccharide curves and are given in Table 1.[14,19] The stability constants K for diboronic acid sensor **2** (λ_{ex} 299 and 342 nm) with D-glucose were enhanced relative to those of monoboronic acid sensors **3** and **4**, while the stability

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Table 1. Stability Constant K (coefficient of determination; r^2) for the Saccharide Complex of Diboronic Acid Sensor **2** and Monoboronic Acid Sensors **3** and **4**[a]

	2				**3**		**4**	
	λ_{ex} 299 nm, λ_{em} 417 nm		λ_{ex} 342 nm, λ_{em} 417 nm		λ_{ex} 299 nm, λ_{em} 369 nm		λ_{ex} 342 nm, λ_{em} 397 nm	
saccharide	K, dm^3 mol^{-1}	fluorescence enhancement	K, dm^3 mol^{-1}	fluorescence enhancement	K, dm^3 mol^{-1}	fluorescence enhancement	K, dm^3 mol^{-1}	fluorescence enhancement
D-glucose	142 ± 12 (0.99)	3.9	108 ± 10 (0.99)	2.4	30 ± 7 (0.98)	1.5	44 ± 3 (1.00)	4.5
D-galactose	74 ± 7 (0.99)	2.2	81 ± 8 (0.99)	2.6	77 ± 12 (0.98)	1.4	51 ± 2 (1.00)	4.2
D-fructose	76 ± 10 (0.98)	1.7	125 ± 11 (0.99)	3.5	548 ± 55 (0.99)	1.4	395 ± 11 (1.00)	3.6
D-mannose	—[b]	—[b]	8 ± 1 (1.00)	3.5	58 ± 8 (0.98)	1.4	36 ± 1 (1.00)	3.7

[a] [**2**] = 2.5 × 10^{-6} mol dm^{-3}, [**3**] = 5.0 × 10^{-7} mol dm^{-3}, [**4**] = 1.0 × 10^{-7} mol dm^{-3}, pH 8.21 buffer. [b] The K and fluorescence enhancement could not determined because of the small changes in fluorescence.

constants K for diboronic acid sensor **2** (λ_{ex} 299 and 342 nm) with D-fructose were reduced relative to those for monoboronic acid sensors **3** and **4**. These results were not surprising since it is well-known that D-glucose easily forms 1:1 cyclic complexes with diboronic acids, whereas D-fructose tends to form 2:1 acyclic complexes with diboronic acids.

Sensor **2** is particularly interesing in that the differences between the observed fluorescence enhancements obtained when excited at λ_{ex} 299 nm (phenanthrene) and λ_{ex} 342 nm (pyrene) (Table 1) can be correlated with the molecular structure of the saccharide−sensor complex.

The fluorescence enhancement of sensor **2** with D-glucose is 3.9 times greater when excited at 299 nm and 2.4 times greater when excited at 342 nm. Whereas, with D-fructose the enhancement was 1.9 times greater when excited at 299 nm and 3.2 times greater when excited at 342 nm.

These results indicate that the energy transfer from phenanthrene (donor) to pyrene (acceptor) in a rigid 1:1 cyclic D-glucose complex is more efficient than in a flexible 2:1 acyclic D-fructose complex. The more efficient energy transfer leads to an enhanced fluoresence response to D-glucose.

In conclusion, we have shown that it is possible to prepare a fluorescent energy transfer saccharide sensor, with two different fluorophores phenanthrene (donor) and pyrene (acceptor), using simple building blocks employing a modular approach. Our ongoing research is directed toward the development of other fluorescent sensors employing energy transfer as a method to enhance sensitivity and selectivity.

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Supporting Information Available: Selected data and synthetic scheme for the preparation of **2**; absorption versus concentration plots of **2** and **3** + **4**; fluorescent spectral changes of **2** (2.5 × 10^{-6} mol dm^{-3}) with different concentrations of D-glucose in 52.1 wt % methanol at pH 8.21 phosphate buffer (λ_{ex} 342 nm); saccharide concentration vs relative fluorescence intensity profiles of **2** (2.5 × 10^{-6} mol dm^{-3}) with added saccharides in pH 8.21 buffer (λ_{ex} 299, 342 nm and λ_{em} 417, 460 nm). This material is available free of charge via the Internet at http://pubs.acs.org.

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