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Fluorescence Sensing of a Ribonucleoside 5'-Triphosphate

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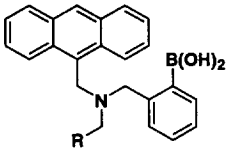
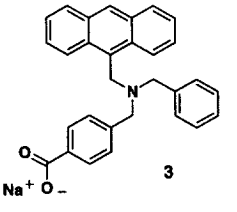
Abstract: Conjugation of a fluorescent saccharide sensor to poly(allylamine) produces a sensing compound with selectivity for ribonucleoside 5'-triphosphate. An association constant (log K_a) of 5.6 was determined for uridine 5'-triphosphate in pH 7.3 phosphate solution (15 mM, 298 K), whereas log K_a was less than 2 for uridine, uridine 5'-monophosphate, and uridine 5'-diphosphate.

A primary aim in abiotic supramolecular chemistry is to invent molecular devices with specific functions such as recognition and catalysis. The majority of cases reported in the literature describe a compound that has promising attributes but there is a need to convert the lead candidate into a second-generation version with improved binding affinity and/or binding selectivity. At present there are few ways to address this problem.[1] We have initiated a program to develop general methods of improving receptor binding through the incorporation of a tunable secondary recognition domain. As a starting point, we have chosen to explore methods that expand upon the binding characteristics of boronic acids which are known to form covalent complexes with diol-containing molecules such as carbohydrates.[2,3]

We have modified the carbohydrate receptor, **1**, invented by Shinkai and coworkers.[2,4] This receptor design was chosen for a number of reasons. It binds neutral monosaccharides in aqueous methanol solution with a selectivity for fructose ($K_a = 10^3\ M^{-1}$).[2] Compound **1** is fluorescent and is thought to signal binding via modulation of a photoinduced electron transfer quenching mechanism.[2] Although fluorescence provides limited information about the molecular details of binding, it is nonetheless a straightforward way of measuring association. This is an important technical point with sugar receptors because most carbohydrates lack an easily observable chromophore.



1 R = H
2 R = 4-$C_6H_4CO_2H$

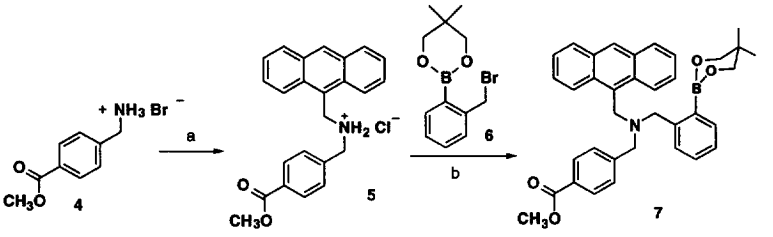


3

In this report we describe the versatile derivative **2**, which contains a benzoic acid linker group that allows conjugation to a range of secondary binding functionality. As a demonstration of its versatility, we have attached **2** to a cationic polyamine and produced a fluorescent sensor with greatly enhanced affinity for a ribonucleoside 5'-triphosphate. Nucleotides are important biological compounds and there is a need to develop fluorescent nucleotide sensors.[5] To date, only a few attempts have been reported.[6,7]

Compound **2** was prepared by the sequence shown in Scheme 1. The secondary ammonium **5** was treated with the known organoboron **6**[2] to provide **7**,[8] which was easily hydrolysized to **2** with aqueous lithium hydroxide. A control version, **3**, which does not contain a boronic acid was prepared by a similar sequence.

Scheme 1



(a) 9-anthraldehyde, CH_3OH, sieves; $NaBH_4$; HCl; (71 %); (b) **6**, K_2CO_3; (48 %).

Initially, the binding abilities of the free receptors, **1** and **2**, were determined in aqueous solution (pH 7.3, 15 mM phosphate buffer) by fluorescence titration methods. Compound **2** (log K_a = 4.6) binds fructose more tightly than parent **1** (log K_a = 3.7),[9] but the fluorescence enhancement at sensor saturation is smaller (two-fold fluorescence increase for **2** compared to a three-fold enhancement for **1**). Compounds **1** and **2** were also assayed for association with nucleosides and nucelotides. In all cases there was little or no change in receptor fluorescence suggesting very weak binding. To enhance nucleotide affinity, receptor **2** was attached to poly(allylamine) **PA** (MW 50,000 - 65,000) via an amide linkage.[10] A loading level of 10 % was employed (*i.e.*, the ratio of sensor **2** to allylamine monomer was 0.1). A control polymer was also prepared by functionalizing 10 % of **PA** with fluorophore **3**.

The **PA-2** conjugate was titrated with fructose, uridine and various uridine 5'-phosphate derivatives in aqueous solution (pH 7.3, 15 mM phosphate buffer). The resulting changes in fluorescence are shown in Figure 1. Titration of **PA-2** with fructose showed weakened affinity (log K_a = 2.7) compared to boronic acid receptor **2** alone. It is possible this is due to aggregation of the hydrophobic sensing groups. However, the fluorescence spectrum for **PA-2** in aqueous phosphate solution shows no excimer emission, indicating that the sensing groups are dispersed along the **PA** chain. Therefore, it appears that the cationic **PA** and its solvation shell of phosphate ions sterically encumber fructose binding to the sensing boronic acid groups.

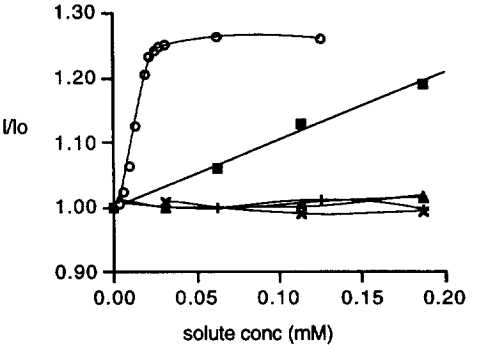
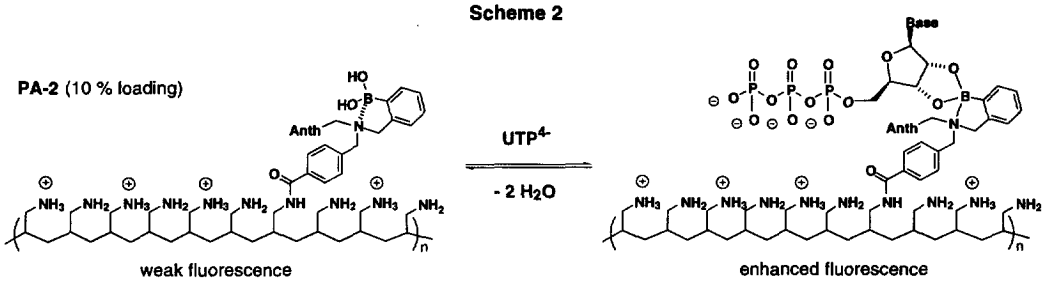


Figure 1. Change in fluorescence emission (λ_{ex} 389 nm, λ_{em} 423 nm) for **PA-2** upon titration with: O 5'-UTP^{4-}; ■ fructose; ▲ 2'-deoxy-5'-UTP^{4-}, + 5'-UMP^{2-}, and x 5'-UDP^{3-}, in phosphate buffer solution (pH 7.3, 15 mM), T = 298 K.

Titration of **PA-2** with uridine, 5'-UMP^{2-} and 5'-UDP^{3-} produced negligible changes in fluorescence. However, 5'-UTP^{4-} was found to bind very strongly (log K_a = 5.6).[9] Although the change in fluorescence is small, the high association constant for 5'-UTP^{4-} is particularly impressive since binding occurs in the presence of competing phosphate buffer. The association constant is comparable to the best affinities observed with Lehn's bis(intercaland) and tris-acridine cryptand.[6] In addition, **PA-2** exhibits a binding selectivity that is quite different to Lehn's receptors. The lack of response to 2'-deoxy-5'-UTP, a compound incapable of chelating with the boronic acid, is evidence in favor of the binding mode shown in Scheme 2. Further evidence that the boronic acid is crucial for sensing is the observation that in phosphate buffer the control polymer **PA-3** is unresponsive to 5'-UTP^{4-} or any of the compounds described in Figure 1.

Scheme 2



Competitive titration experiments provided a further demonstration of the sensing selectivity for nucleoside triphosphate. Titration of **PA-2** with 5'-UTP^{4-} in the presence of excess 5'-UMP^{2-} (1 mM) produced a titration curve that was essentially identical to that seen in Figure 1. The high selectivity for 5'-UTP^{4-} over 5'-UDP^{3-} and 5'-UMP^{2-} is rationalized in terms of a competition between the analyte and phosphate buffer for the cationic polymer **PA-2**. It appears that only when the analyte charge reaches -4 does binding to the cationic **PA-2** become highly favorable. When the titrations are repeated in HEPES (N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid) buffer all of the nucleotides induce a modest fluorescent response in the order 5'-UTP^{4-} > 5'-UDP^{3-} > 5'-UMP^{2-} > fructose. In this case, the anionic analytes can more readily displace the zwitterionic HEPES from the cationic **PA-2** chain and gain access to the sensing groups. A similar result was very recently reported by Anslyn.[11] In HEPES buffer, the control polymer **PA-3** also showed weak fluorescent responses to the different nucleotides, suggesting that in HEPES there are more signaling pathways available to **PA-2** than the boron-mediated mechanism shown in Scheme 2.

The fluorescence enhancement for **PA-2** saturated with 5'-UTP^{4-} is lower than that observed with fructose. This suggests that the anthracene fluorescence is partially quenched by the pyrimidine base, in agreement with the known fluorescence quencing properties of nucleobases.[6,12] Thus the observed fluorescence increase in phosphate buffer is a compromise between the enhancement due to boronic acid chelation, and quenching due to interaction between the anthracene and nucleobase.

In summary, conjugation of a fluorescent diol-sensor to a cationic polymer chain produces a sensing compound with selectivity for ribonucleoside 5'-triphosphate. Thus, we have demonstrated an application of a general concept where the introduction of secondary interaction enhances the affinity and selectivity inherent to a molecular receptor. Studies in progress are attempting to further improve these properties through the use of more highly functionalized secondary recognition domains.

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8. Characterization data for **7**: mp 157-159 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.92 (s, 6H), 3.37 (s, 4H), 3.59 (s, 2H), 3.89 (s, 3H), 3.96 (s, 2H), 4.66 (s, 2H), 7.22 (app. t, J = 7.5 Hz, 1H), 7.30-7.45 (m, 8H), 7.64 (d, J = 7.5 Hz, 1H), 7.90 (d, J = 8 Hz, 2H), 7.94-7.96 (m, 2H), 8.25-8.27 (m, 2H), 8.35 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.87, 31.42, 50.77, 51.98, 58.63, 58.70, 71.74, 124.72, 125.28, 125.35, 126.36, 127.39, 128.60, 128.89, 129.20, 129.43, 129.47, 130.30, 131.39, 131. 59, 134.04, 143.95, 145.70, 167.14; MS (FAB+) *m/z:* 557 (M^+, 12), 366 (13), 191 (100); HRMS 557.2788 (calcd for C36H36BNO4: 557.2744).
9. Association constants were determined by the Benesi-Hildebrand method or nonlinear curve fitting of the equation for 1:1 binding. Tsukube, H.; Furata, H.; Odani, A.; Takeda, Y.; Kudo, Y.; Inoue, Y.; Liu, Y.; Sakamoto, H.; Kimura, K. in *Comprehensive Supramolecular Chemistry*, Atwood, J. L., Davies, J. E. D., MacNicol, D. D., Vögtle, F., Eds.; Pergamon, New York, 1996, vol. 8, Ch. 10.
10. Menger, F. M.; Eliseev, A. V.; Migulin, V. A. *J. Org. Chem.* **1995**, *60*, 6666-6667. Poly(allylamine) hydrochloride (MW 50,00 - 65,000) was converted to its free amine form using a ten-fold excess of Dowex 1-X8 resin. An aqueous solution of poly(allylamine) (0.5 M of monomer units) was treated with solutions of **2** (0.1 molar equivalents of monomer units) in dimethylformamide and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.5 molar equivalents of monomer units) in water. The reaction was stirred at room temperature for 14 h before purification by gel filtration (Sephadex G15) and removal of the solvent. The residue (IR 1620 cm^{-1}) was redissolved in methanol/water to a make stock solution that was 20 μM in sensor unit. A 200 μL solution of this stock solution was diluted to 3.0 mL with phosphate buffer (15 mM, pH 7.3) to give a final sensor unit concentration of 1.3 μM.
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