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Tuning the Affinity of a Synthetic Sialic Acid Receptor Using Combinatorial Chemistry

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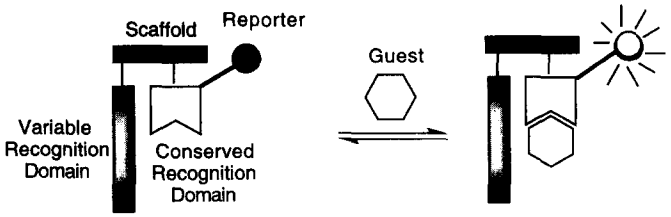
Abstract: Combinatorial libraries of carbohydrate receptors were produced by first derivatizing poly(allylamine) with the fluorescent boronic acid **1** followed by various mixtures of carboxylic acids. The libraries were screened for members that exhibited a larger response for sialic acid than glucose or fructose. The derivatized polymers appear to bind sialic acid by a cooperative mechanism. © 1998 Elsevier Science Ltd. All rights reserved.

The application of library methods to supramolecular chemistry is proving to be a useful way of improving the binding characteristics of synthetic receptors.[1-5] We are exploring synthetic receptors for biologically important ligands whose general design is shown in Scheme 1. A *molecular scaffold* connects two guest-binding domains, a *conserved recognition domain* which binds the guest with weak, non-selective affinity, and a *variable recognition domain*, which provides secondary interactions that increase binding affinity and binding selectivity. The general design also includes an optional *reporter group* which provides a way of determining association constants.

Scheme 1



Recently, we reported the synthesis of the conserved recognition domain, boronic acid **1**.[6] Compound **1** contains an anthracene fluorophore which exhibits an increase in fluorescence when the boronic acid condenses with a diol and becomes a boronate ester. The increase in fluorescence is attributed to a decrease in anthracene quenching by photoinduced electron transfer (PET) from the nearby tertiary amine.[7] After attachment to a poly(allylamine) scaffold, a series of grafted amide chains (R_1, R_2, etc.) act as the variable recognition domain (Scheme 2). In this communication we have used combinatorial methods to tune the selectivity of **1** for sialic acid, an important antigenic residue that is present on the surface of cells.[8] In particular we wanted a receptor that would bind sialic acid in the presence of a physiological concentration of glucose. It is known that uncharged boronic acids associate weakly with sialic acid, however, as recently shown by Shinkai, the introduction of cationic sites can improve sialic acid affinity.[7b]

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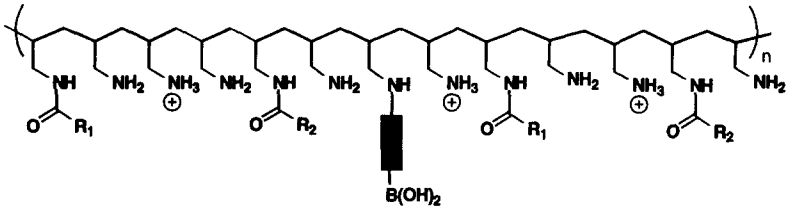
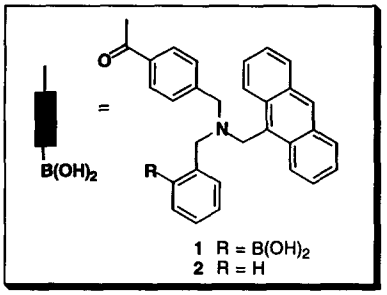
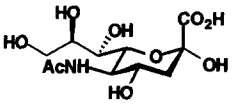
Scheme 2







Sialic Acid
(N-acetylneuraminic acid)

A library of grafted polymers was prepared and screened for fluorescence enhancement in the presence of glucose, fructose and sialic acid. The polymers were prepared in the following way.[3,6] A 2% loading of **1** was prepared by treating a solution of poly(allylamine) (**PAA**, MW 50,000-65,000) in H_2O/DMF with the carboxylic acid derivative of **1**[6] (one molar equivalent of **1** for every fifty free amine groups) and the coupling agent EDC (a five-fold excess based on **1**).[9] The **PAA-2%1** conjugate was further derivatized by condensing various amounts of 4-hydroxybenzoic acid (HOB), 4-imidazolacetic acid (IMA), octanoic acid (OCT) and/or succinic anhydride (SUC).

In the synthesis of our library a directed, iterative approach[10] was used where a number of small libraries containing 32 or 16 members were screened. Results of the primary screens were used to direct the effort on subsequent libraries. For example the initial screening uncovered the following trends: (1) Fluorescence enhancements were significantly reduced when the combined loading of amide chains was higher than 10 %; (2) Fluorescence enhancements were generally higher if HOB or IMA was one of the amide side chains. Additional libraries were therefore prepared where every member contained HOB or IMA and the combined loading of amide chains was less than 10%. The screening results shown in Figure 1 are a selection from a **PAA-2%1** library that contained at most two types of amide side chains each at 3% loading. The results show that underivatized **PAA-2%1** responds to sialic acid much better than glucose and even slightly better than fructose. The other library members shown in Figure 1 do not exhibit such a fructose selectivity, except for the member that has a loading of 3% IMA-3%OCT.

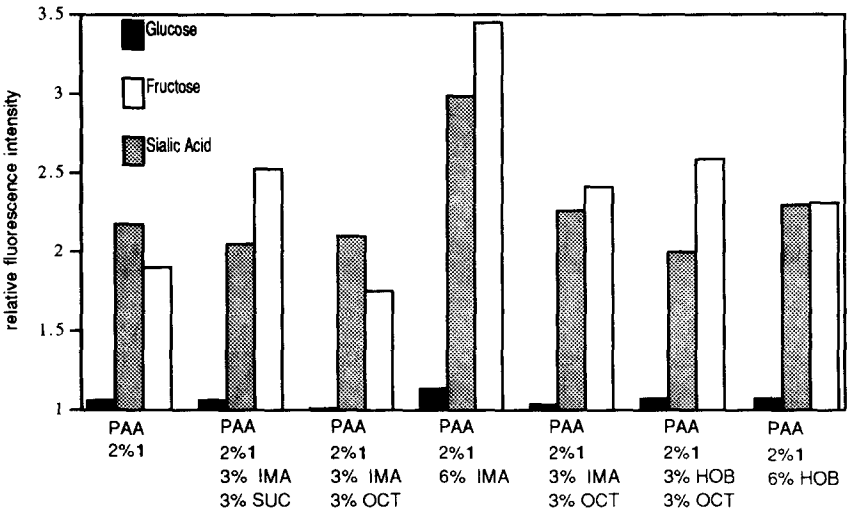


Figure 1. Fluorescence enhancement (λ_{ex} 389 nm, λ_{em} 423 nm) observed after treatment of combinatorially functionalized **PAA-2%1** polymers with 26 mM of glucose, sialic acid (sodium salt), or fructose in HEPES buffer (pH 7.3, 0.015M).

The sialic acid and fructose binding characteristics of these library members were further examined by titration methods, and the isotherms shown in Figure 2 are typical of the set. The step-like shape of the sialic acid titration curve indicates that the linear polymer binds by a cooperative mechanism.[11] Cooperative sialic acid binding curves were also obtained with other libraries including some that were prepared using the more branched poly(ethyleneimine) (**PEI-2%1**, data not shown). Cooperative binding curves have been reported before by Klotz and coworkers, who examined the association of methyl orange anion to cationic alkylated **PEI**.[2] In the case of the Klotz systems, the cooperativity was attributed to the initially bound apolar methyl orange inducing more favorable apolar sites that enhanced further binding. Conversely, the cooperativity seen here is more likely due to an induced fit mechanism with the functionalized cationic polymer undergoing a conformational change after binding a sialic acid anion. While this mechanism still has to be proved, it is worth noting that titration of the same polymers with neutral fructose produces curves (Figure 2) that indicate only slight cooperativity. The fluorescence enhancement observed upon sialic acid binding is thought to be induced by chelation of the boronic acid (the conserved recognition domain) by a diol on the sialic acid, with the functionalized polymer providing additional ionic and non-polar secondary interactions. Evidence in favor of this binding model is the observation that fluorescence enhancement is not observed with control polymers that use the non-boron containing **2** as a replacement fluorophore (as expected **PAA-2%2** does show an acid induced fluorescence enhancement).[7]

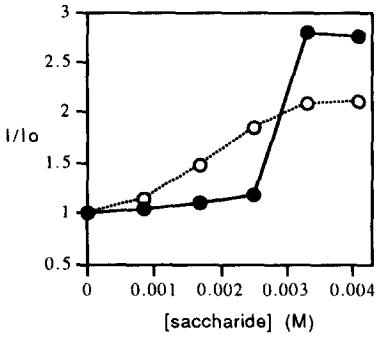


Figure 2. Change in fluorescence emission (λ_{ex} 389 nm, λ_{em} 423 nm) for **PAA-2%1-3%IMA-3%OCT** (1.6 μM) upon titration with: • sialic acid (sodium salt) and, O fructose in HEPES buffer (pH 7.3, 0.015M), T=298 K.

The polymer systems reported here are not likely to be useful as sialic acid sensors because of the cooperative binding curves. However, the results demonstrate our main design principle that recognition can be fine-tuned by screening libraries that contain a variable recognition domain. Currently there is renewed interest in using functionalized polymers as synthetic enzymes (referred to by some as *synzymes*[2b, 4, 12]) for a variety of neutral and charged reactants.[3,4] A reasonable conclusion drawn from our results is that the possibility of cooperative binding should be considered when interpreting synzyme library screens since substrate binding is the first step in a synzyme catalyzed reaction.

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9. The conjugated polymer **PAA-1** (2 % loading) was prepared and characterized using the same procedures described previously.[3,6] The fluorescence spectrum for **PAA-1** shows no excimer emission, indicating that the sensing groups are dispersed along the **PA** chain. The **PAA-1** was coupled with mixtures of the appropriate carboxylic acid(s) using the coupling agent EDC (a five-fold excess based on carboxylic acid) in a mixed solvent (H_2O/DMF). Note, if the **PAA-1** conjugates are not purified, then their ability to bind carbohydrates is lost after 3 days even when stored frozen. Dialysis does not solve this problem, but if the polymers are filtered through Sephadex G-15, then they retain their activity for a period of about 3 weeks.

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12. "In my long experience in the scientific world, I have learned that to attract attention it is more important to invent a catchy term than to discover a phenomenon." I. M. Klotz, ref. 2b, p. 176.