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# Fluorescent Imprinted Polymers Prepared with 2-Acrylamidoquinoline as a Signaling Monomer

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## ABSTRACT


**A cyclobarbital-selective molecularly imprinted polymer was prepared using a fluorescent functional monomer, 2-acrylamidoquinoline. This monomer was designed to increase in fluorescence intensity upon hydrogen bonding to the cyclobarbital guest. The resultant imprinted polymers exhibited the enhancement of the fluorescence intensity when cyclobarbital was bound. Our results show that this fluorescent responsive imprinting method could be useful in the development of sensors for quantification of nonfluorescent compounds.**

Biomolecules, such as antibodies, receptors and enzymes, have specific molecular recognition abilities, which are useful in diagnostic biological assays. However, they are often produced via complex protocols and require specific handling conditions because of their poor stability. Therefore, artificial receptors/antibodies are desired.[1]

The synthesis of artificial receptors with high specificity is often also a tedious and time-consuming process. Specifically, it is difficult to arrange functional groups with the proper complimentary the distances and orientations to the target compound, using traditional organic synthesis techniques. Molecular imprinting is one of the strategies that offer a synthetically efficient route to artificial receptors.[2] This method is a template polymerization process using self-assembly of a target molecule with functional monomers followed by copolymerization with a cross-linker. After removal of the template molecule, the resulting polymers contain binding cavities that exhibit specific binding characteristics to the template and structurally related compounds. The method is versatile and can be used to prepare tailor-made polymeric receptors without the need for precise molecular design or complicated synthetic procedures. Thus, molecularly imprinted polymers have been extensively studied for use in a wide range of applications in the life, pharmaceutical, and environmental sciences.

Recently, imprinted polymers have been designed not only as molecular recognition materials but also as sensors that

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(2) (a) Sellergren, B. *Moleculary Imprinted Polymers*; Elsevier: Amsterdam, 2001. (b) Bartsch, R. A.; Maeda, M. *Molecular and Ionic Recognition with Imprinted Polymers*, ACS Symposium Series 703; American Chemical Society: Washington, DC, 1997. (c) Wulff, G. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1812. (d) Kempe, M.; Mosbach, K. *J. Chromatogr. A* **1995**, *694*, 3. (e) Takeuchi, T.; Haginaka, J. *J. Chromatogr. B* **1999**, *728*, 1. (f) Zimmerman, S. C.; Lemcoff, N. G. *Chem. Commun.* **2004**, 5.

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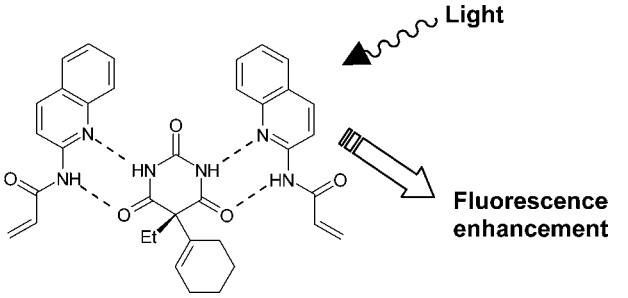
contain transduction elements that couple a readable signal to a binding event.[3] These types of molecularly imprinted materials can be used to directly detect and quantify the target analyte and could be widely applicable in assays and as sensors. In this paper, we report the development of a new fluorescent imprinted polymer that responds to a binding event with a significant enhancement in fluorescence intensity. We designed and synthesized 2-acrylamidoquinoline **1** as a new fluorescent functional monomer that has a polymerizable acrylate moiety and a fluorescent hydrogen-bonding moiety. The quinoline fluorophore of monomer **1** shows enhancement in fluorescence upon formation of two hydrogen bonds to the amide NH and the quinoline nitrogen. Cyclobarbital **2** was used as a model ligand, and we examined the utility of **1** as fluorescent functional monomer in constructing cyclobarbital-specific binding sites that emit a spectroscopic signal on binding.

To ensure the formation of complementary binding sites in the molecular imprinting process, functional monomers should bind to the template molecule during the polymerization process. Thus the interactions of the unpolymerized functional monomer play an important role in molecular imprinting process. Prior to the preparation of imprinted polymers, the utility of the fluorescent functional monomer **1** was tested. Barbital **2** presents two edges that present two-point hydrogen-bonding groups that are complementary to the functional monomer **1** (Scheme 1). The formation of

**Scheme 1**




hydrogen bonds between **1** and **2** in the pre-polymerization stage was independently verified by $^1$H NMR titrations.

In this system, the nitrogen of the quinoline ring and the NH of amide group of **1** presumably interact with the malonylurea structure of **2**. As expected, this was indicated by downfield shift of the amide proton of **1** when the concentration of **2** in $CDCl_3$ was increased. Further, the addition of **1** to **2** also resulted in a downfield shift of the

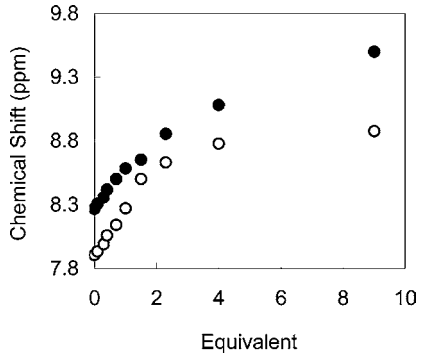



**Figure 1.** Chemical shift of NH proton of the amide group of **1** (10 mM) with the addition of **2** (●) and the NH protons of malonylurea of **2** (10 mM) with addition of **1** (○) on $^1$H NMR in $CDCl_3$.

two NH resonances of **2** (Figure 1). These observations indicate that the NH of the amide group of **1** and the NH of malonylurea structure of **2** are involved in a mutual two-point hydrogen-bonding interaction.

Next fluorescence titrations were carried out, where fluorescence spectra of **1** were measured in the presence of various concentrations of **2**, resulting in an increase in the fluorescence intensity of **1**. Figure 2 shows the emission

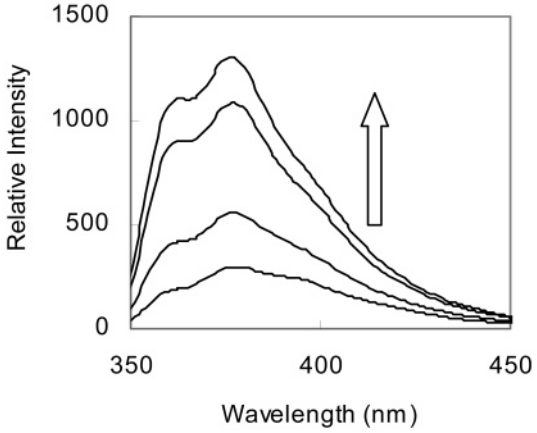



**Figure 2.** Fluorescence spectral changes of **1** (12.5 $\mu$M) in various concentrations of **2** (0, 1, 5, and 10 equiv) in $CDCl_3$. $\lambda_{Ex} = 330$ nm.

spectra of **1** at increasing concentration of **2** up to 10 equiv. A 3-fold increase in fluorescent intensity at the emission maxima of 376 nm was observed with the addition of **2**. A possible mechanism of the fluorescence enhancement is that the formation of a hydrogen bond to the quinoline nitrogen is similar to the effect of protonating the quinoline nitrogen, which is known to yield fluorescent enhancements.[4] We have reported a similar system, in which 2,6-bis(acrylamido)-pyridine was used as a fluorescent monomer that was excited by 270 nm.[3j] In the present system, the excitation wavelength

(3) (a) Haupt, K.; Mosbach, K. *Chem. Rev.* **2000**, *100*, 2495. (b) Kriz, D.; Ramström, O.; Mosbach, K. *Anal. Chem.* **1997**, *69*, 9, A345. (c) Turkewitsch, P.; Wandelt, B.; Darling, G. D.; Powell, W. S. *Anal. Chem.* **1998**, *70*, 2025. (d) Jenkins, A. L.; Uy, O. M.; Murray, G. M. *Anal. Chem.* **1999**, *71*, 373. (e) Matsui, J.; Higashi, M.; Takeuchi, T. *J. Am. Chem. Soc.* **2000**, *122*, 5218. (f) Leung, M. K.-P.; Chow, C.-F.; Lam, M. H.-W. *J. Mater. Chem.* **2001**, *11*, 2985. (g) Gao, S.; Wang, W.; Wang, B. *Bioorg. Chem.* **2001**, *29*, 308. (h) Takeuchi, T.; Mukawa, T.; Matsui, J.; Higashi, M.; Shimizu, K. D. *Anal. Chem.* **2001**, *73*, 3869. (i) Graham, A. L.; Carlson, C. A.; Edmiston, P. L. *Anal. Chem.* **2002**, *74*, 458. (j) Kubo, H.; Nariai, H.; Takeuchi, T. *Chem. Commun.* **2003**, 2792.

is 330 nm and it may have merit to reduce background fluorescence.

A fluorescent cyclobarbital-imprinted polymer was prepared by copolymerizing ethylene glycol dimethacrylate and the fluorescent functional monomer **1** in the presence of template molecule **2**. The binding characteristics (affinity and selectivity) of the polymer were evaluated chromatographically, using the polymers as stationary phases.

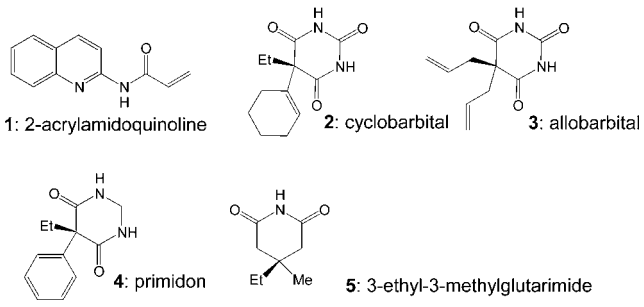
**Table 1.** Retention Factors of Cyclobarbital and Reference Compounds in the Cyclobarbital-Imprinted Polymer and the Nonimprinted Blank Polymer[a]

| | retention factor | | | |
|---|---|---|---|---|
| polymer | **2** | **3** | **4** | **5** |
| imprinted | 1.13 | 0.64 | 0.16 | 0.23 |
| blank | 0.32 | 0.23 | 0.11 | 0.17 |

[a] Eluent: acetonitrile (1.0 mL $min^{-1}$). The retention factors ($k'$) were calculated using the equation $k' = (t_R - t_0)/t_0$, where $t_R$ is the retention time of samples and $t_0$ is a time to elute acetone.

Table 1 shows the retention factors for cyclobarbital **2**, allobarbital **3**, primidon **4** and 3-ethyl-3-methylglutarimide **5** to the cyclobarbital-imprinted polymer and the nonimprinted blank polymer. Scheme 2 shows their structures. The

**Scheme 2**




imprinted polymer displayed strong affinity for **2** and weaker affinity for **3**. The difference in the retention behavior can be interpreted as arising from the imprinting effect, meaning that the polymer could recognize the differences between **2** and **3** in their substitution patterns at their 5-positions. The compounds **4** and **5**, which can form fewer hydrogen-bonding interactions with functional monomer **1,** show much lower affinities. In contrast the nonimprinted blank polymer, prepared without adding the template molecule, exhibited almost no affinity for any sample. Taken together, these results suggest that the recognition behavior of the imprinted polymer arise from the imprinting process.

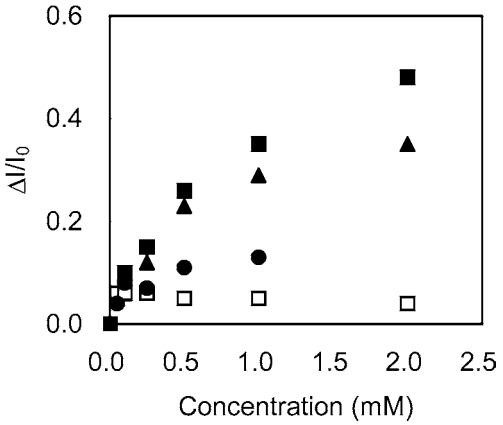



**Figure 3.** Fluorescent response of cyclobarbital-imprinted polymer in the presence of various concentrations of **2** (■) and reference compounds **3** (▲), **4** (●), and **5** (□) in $CDCl_3$. $\lambda_{ex}$ = 330 nm, $\lambda_{em}$ = 376 nm.

The fluorescent properties of the imprinted polymer were evaluated after incubation with various concentrations of **2**. As shown in Figure 3, when **2** was added to the polymer suspensions, the fluorescence intensity at 376 nm increased with the addition of increasing concentrations of **2**. This result demonstrates that the imprinted polymers containing functional monomer **1** possess the ability to signal the presence and concentration of an analyte.

To evaluate the fluorescent selectivity of imprinted polymer, the effects of **3**, **4**, and **5** on the fluorescent behavior of imprinted polymer were compared (Figure 3). The largest fluorescence enhancement was observed when **2** was added. Furthermore, only small fluorescence enhancements were observed on addition of **3** or **4**. The fluorescent measurement with 2 mM of **4** was not attempted because of the low solubility of **4**. Almost no enhancement in fluorescence was observed on addition of the structurally related **5**. These results are in agreement with the selectivity pattern measured in the chromatographic tests. Even though analytes **3** and **4** can in principle form 1:2 complexes having the same two-point hydrogen-bonding patterns to the functional monomer **1**, they exhibited much lower fluorescence responses, which may be due to their low affinities for the imprinted polymer (Table 1). In the case of **5**, only a 1:1 complex with **1** is possible, resulting in lower response compared to **2**. These results suggest that the fluorescence enhancement could result from the formation of selective hydrogen bonding in binding sites formed in the imprinting process. It should be mentioned that the order of retentions of **4** and **5** was not consistent with that of the fluorescence responses, suggesting that the fluorescence enhancement mechanism may not simple and further investigation would be necessary to clarify the mechanism.

To quantify the relationship between the fluorescent enhancement response and substrate uptake, the concentration of **2** in the supernatant after incubation with the imprinted and nonimprinted polymers were analyzed by flow injection

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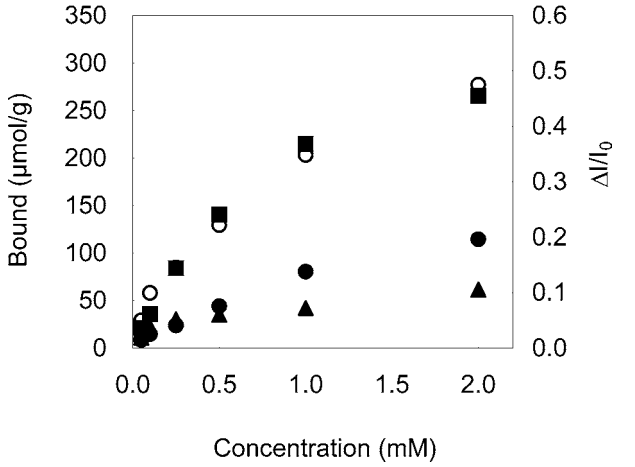


**Figure 4.** Binding isotherm of **2** for the imprinted polymers (■) and the nonimprinted blank polymer (●) and fluorescent response of imprinted polymer (○) and blank polymer (▲) in the presence of **2**. $\lambda_{ex}$ = 330 nm, $\lambda_{em}$ = 376 nm.

analysis. As shown in Figure 4, the fluorescence response followed almost identically with the binding of analyte **2** as independently measured by measuring the supernatants. This gives evidence for the fluorescence response of the imprinted polymer directly correlating to the binding of analyte **2** and enabling its use to measure concentrations. Therefore, it reveals that the fluorescence enhancement is caused by the hydrogen-bonding-based binding and the binding events can be transformed into the fluorescence change in this system.

The binding profile of the blank polymer did not fit the fluorescence enhancement profile at higher concentration range. This suggests that the nonspecific binding sites are not as responsive to the binding of **2**. The obtained results confirmed that the fluorescence response of the imprinted polymer was concentration-dependent, supporting the idea that the fluorescent enhancement is due to the uptake of **2** by the imprinted polymer and the binding events can be transformed into the readable fluorescence change.

In conclusion, the molecularly imprinted polymers that were prepared using fluorescent functional monomer **1** exhibited an enhancement in fluorescence intensity when target compounds were bound. This sensing method using fluorescent imprinted polymers as signaling receptors would be useful for the quantitation of nonfluorescent analytes. Development of the fluorescent functional monomers, which exhibit the ability to completely quench the fluorescence in free form and/or to shift the fluorescent maxima when target molecules are bound, will lead to the further development of this approach.

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