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Molecular recognition by fluorescent imprinted polymers

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Abstract

New fluorescent imprinted polymers have been prepared for guest–host selectivity studies. Significant differences were found in the fluorescence response between the bound and unbound states.

Keywords: fluorescence; hydrogen bonding; molecular recognition; polymers.

As part of a programme of automated synthesis and biological screening we decided to investigate the phenomenon of molecular recognition in polymer networks with a view to constructing a high-throughput fluorescence-based assay. If a test compound hydrogen bonds to a fluorescent group in an imprinted polymer cavity then there should be a change in the fluorescence properties of the polymer which may be detected by fluorimetry. In this case the test compound presents a similar hydrogen bond donor/acceptor pharmacophore arrangement to that of the imprinting molecule. With an appropriate choice of imprinting molecule this may be used in an automated assay to screen libraries of potential drug candidates. Thus, compounds similar in size, shape and hydrogen-bonding profiles to that of a lead compound may be discovered. Other potential applications may be envisaged, for example, in screening out molecules which bind in the same manner as existing drugs having microbial resistance problems from libraries of active anti-microbial agents.

In recent years imprinted polymers have been developed having recognition sites that rely upon both the shape of the cavity and the presence of hydrogen bond donors and acceptors for their selectivity in guest–host interactions.[1–5] Imprinted polymers have been used successfully as stationary phases for HPLC enantiomeric separations and have been found to be an effective means for the selective removal of molecules from bulk materials such as in the decaffeination of coffee.[6] As a step on from this, molecular imprinting has been combined with fluorescence to produce a polymer-based fluorescent chemosensor for the detection of aqueous cyclic adenosine monophosphate.[7]

This paper describes our initial results from four fluorescent hydrogen-bonding monomers used in the preparation of new imprinted polymers which exhibit binding-dependent fluorescence.

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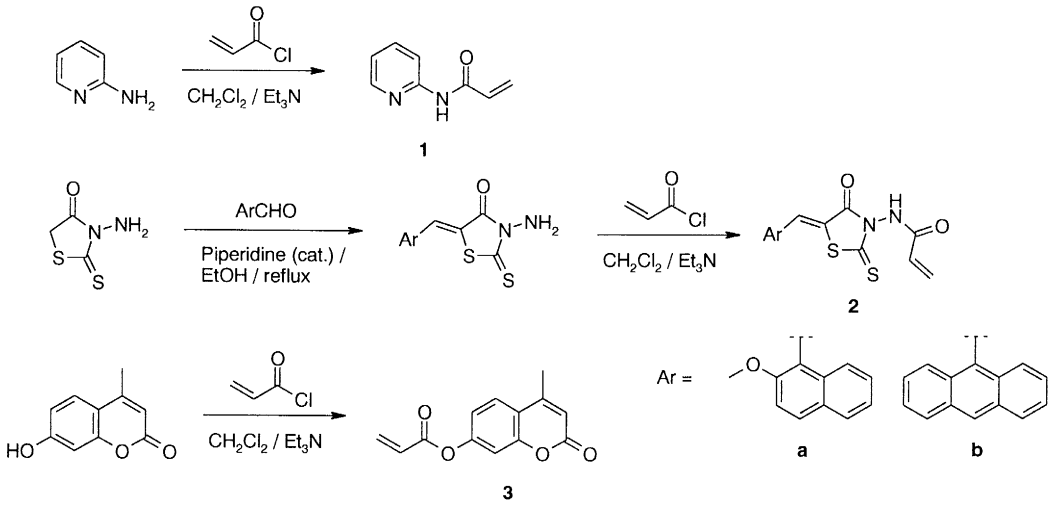
2-Aminopyridine, 7-hydroxy-4-methylcoumarin (themselves both fluorescent) and 3-aminorhodanine were chosen as suitable hydrogen bond donor/acceptor scaffolds from which to construct the desired monomers. Thus, 2-acrylamidopyridine (**1**), two fluorescent benzylidine 3-acrylamidorhodanines (**2a**,**b**; presumed to be the *Z*-isomers by analogy to *Z*-benzylidene rhodanines prepared in the same manner[8]) and 7-hydroxy-4-methylcoumarin acrylate (**3**) were prepared as in Scheme 1.



Scheme 1. Preparation of fluorescent monomers

Polymers were prepared from trimethylolpropane triacrylate[7] or ethylene dimethacrylate[1–3] containing 5 μmol g^{-1} each of cyclododecylidene pyridine-2-carboxamidrazone **4**[9] (the imprinting molecule, Fig. 1) and a fluorescent monomer ($CHCl_3$/AIBN/Ar/60°C/24 h). Compound **4** was chosen as the imprinting molecule by virtue of its obvious hydrogen bond donor/acceptor capabilities together with the fact that we have libraries of several hundred heteroarylcarboxamidrazones at our disposal with which to probe the selectivity of the imprinted polymers. The crude polymeric materials were washed free of starting materials, ground to a fine powder and extracted rigorously to remove the imprinting molecule. Each polymer was then re-exposed to the imprinting molecule and the fluorescence response of the polymer was obtained with and without the imprinting molecule using a Wallac Victor[2] 1420 multilabel counter in standard 96-well format. Three samples of each polymer were weighed into the 96-well plate, suspended in methanol and the fluorescence response was measured in quintuplet along with controls comprising methanol-filled and blank wells. The greatest differences in fluorescence between polymer-bound imprinting molecule and free polymer were found when trimethylolpropane triacrylate was used as the crosslinking agent rather than ethylene dimethacrylate. The results for the former are shown in Fig. 2 and are normalised for fluorescence counts/mg of polymer.

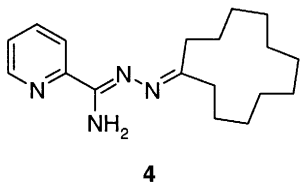


Fig. 1. Imprinting molecule

Of the four polymers, **1** and **2a** had the larger fluorescence output and the latter exhibited the greatest fluorescence count ratios between the bound and unbound states with an approximate two-

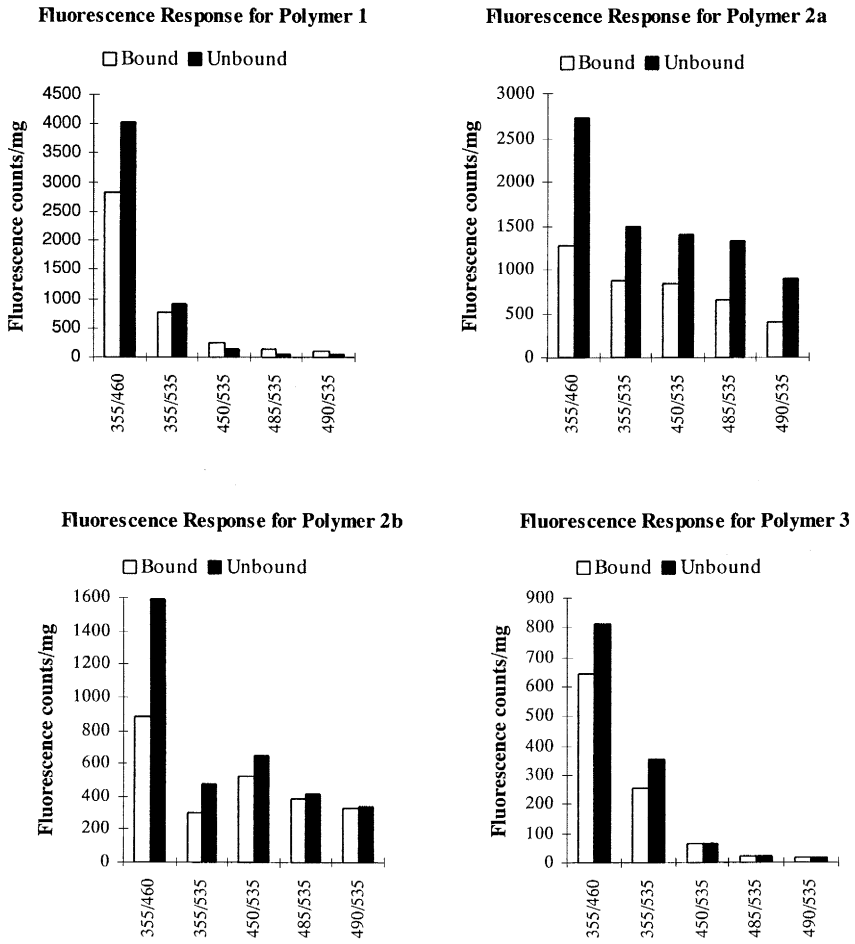


Fig. 2. Fluorescence response of the imprinted polymers in the presence and absence of bound imprinting molecule. The pairs of numbers on the horizontal axis denote the combinations of excitation and emission wavelengths used (nm)

fold difference across the entire wavelength range in this study. These results confirm the molecular recognition and fluorescence potential of these polymers. The selectivity of these polymers towards binding molecules other than the imprinting molecule is currently being investigated using libraries of benzylidene heteroarylcarboxamidrazones.

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