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4-(3-Aminopropylene)-7-nitrobenzofurazan: a new polymerisable monomer for use in homogeneous molecularly imprinted sorbent fluoroassays

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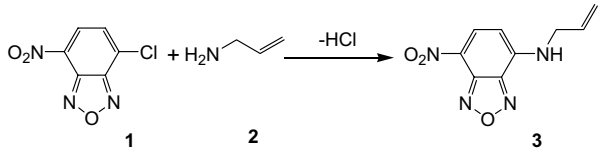
Abstract—The synthesis of a new polymerisable fluorescent monomer, 4-(3-aminopropylene)-7-nitrobenzofurazan, is described. This compound was further used to prepare a fluorescent atrazine imprinted polymer as a component of a homogeneous pseudo-fluoroassay.

Molecular imprinting involves the template-driven introduction of recognition sites into crosslinked polymers. In the traditional non-covalent approach, template molecules and functional monomers interact via weak interactions to form a pre-polymerisation complex. Polymerisation is initiated after addition of a crosslinker and in the presence of a porogenic solvent. Removal of the template reveals cavities with particular affinity and specificity towards the imprinted molecule. Rebinding of the analyte to the molecularly imprinted polymer (MIP) is similar to the antigen/antibody recognition process, and this has led to their use in molecularly imprinted sorbent assays.[1]

Use of a polymerisable fluorescent monomer provides molecularly imprinted polymers that can be used in homogeneous fluoroassays[2] where the native fluorescence of the imprinted polymer is attenuated, shifted or promoted by the specific interactions with the template molecule.[3] In the present study, a novel fluorescent polymerisable functional monomer was synthesised and used to prepare a fluorescent atrazine imprinted polymer.

4-(3-Aminopropylene)-7-nitrobenzofurazan (**3**) was synthesised as outlined in Scheme 1. 4-Chloro-7-nitrobenzofurazan (**1**) was selected as the fluorophore as it is a common fluorogenic compound that can react with amines to give a fluorescent derivative.[4] Furthermore, as the fluorescence of the nitrobenzofurazan moiety has been shown to be environmentally sensitive,[5] it was anticipated that rebinding of atrazine to the polymer would significantly modify the output signal of the material. In order to allow incorporation of the fluorophore into the polymer matrix, a vinyl group was introduced via nucleophilic substitution of the chlorine by allylamine (**2**). This type of substitution has been reported to proceed via a Meisenheimer complex.[6]



Scheme 1. Synthesis of **3**. Reagents and conditions: ethanol, pyridine, 45 °C, 24 h.

Reaction of **1** with 1.1 equiv of **2** was carried out in ethanol, in the presence of pyridine. The solution was stirred for 24 h under N_2 at 45 °C. The product was purified by silica flash column chromatography (toluene/EtOAc, 9:1) and isolated as a brown powder in a 30% yield. The purity of the compound was verified by TLC and elemental analysis. The structure of the compound was confirmed in ^1H and ^{13}C NMR spectroscopy. The presence of the nitro group was confirmed by IR, which showed peaks at 1350 and 1562 cm^{-1}.

Keywords: Fluorescent monomer; Imprinted polymer; Atrazine.
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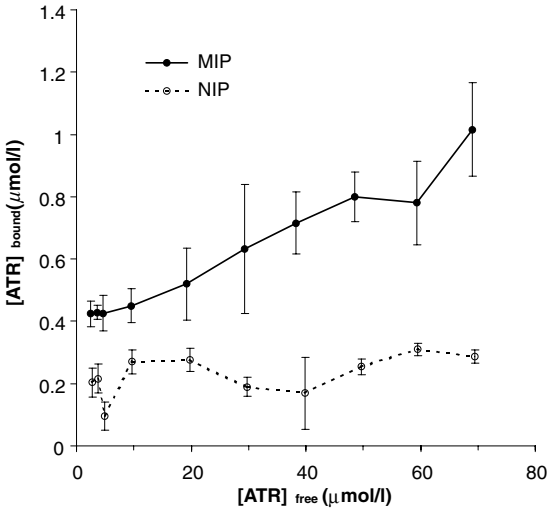

Figure 1. Binding isotherm of atrazine to imprinted and non-imprinted polymers (5 mg/mL). After incubation, bound and unbound fractions were separated by centrifugation, and the supernatant was analysed by RP-HPLC [ODS 2, acetonitrile/water (50:50), 1 mL/min, 260 nm]. Binding of atrazine to the MIP was significantly higher compared to the NIP, demonstrating that the polymer had been successfully imprinted (mean ± SD; $n = 3$).

A fluorescent MIP was prepared by radical polymerisation[7] using a mixture of methacrylic acid (MAA) and 4-(3-aminopropylene)-7-nitrobenzofurazan (**3**) as functional monomers, ethylene glycol dimethacrylate (EGMA) as crosslinker and chloroform as porogen. Atrazine (130 mg–0.6 mmol) was dissolved in chloroform (6.5 mL). MAA (153 μL–1.8 mmol), **3** (131.6 mg–0.6 mmol), EGMA (1.82 mL–9.65 mmol) and α,α′-azoisobutyronitrile (AIBN), as radical initiator (1% w/v), were added to the vial. The solution was degassed under vacuum for 5 min and purged with nitrogen for a further 5 min. Polymerisation was carried out in the dark at 60 °C for 24 h. The polymer was further ground with a pestle and mortar, wet sieved in acetone (45 μm) and template removal was achieved by soxhlet extraction with methanol for 7 days. Blank non-imprinted polymers (NIP) were prepared using the same protocol, but without atrazine.

The efficacy of the imprinting process was assessed by a batch rebinding experiment in acetonitrile (Fig. 1). The dissociation constant ($K_D = 4.75 \pm 1.98$ mol/L) and the total number of binding sites ($n_{max} = 0.17 \pm 0.01$ mol/g of dry polymer) were determined from the binding isotherm by fitting the curve to the single site Langmuir isotherm.[8,†] Similar ranges of capacity and affinity have been observed for other templates (diazepam and leu-enkephaline) and are associated with intermediate energy binding sites.[9]

To determine that the fluorescent monomer had been successfully incorporated into the polymer matrix, fluorescence emission and excitation spectra were recorded in several solvents (Fig. 2). These were in good agreement with the excitation and emission spectra of the functional monomer in solution. This polymer will be further used to develop a homogeneous sorbent fluoroassay for atrazine.

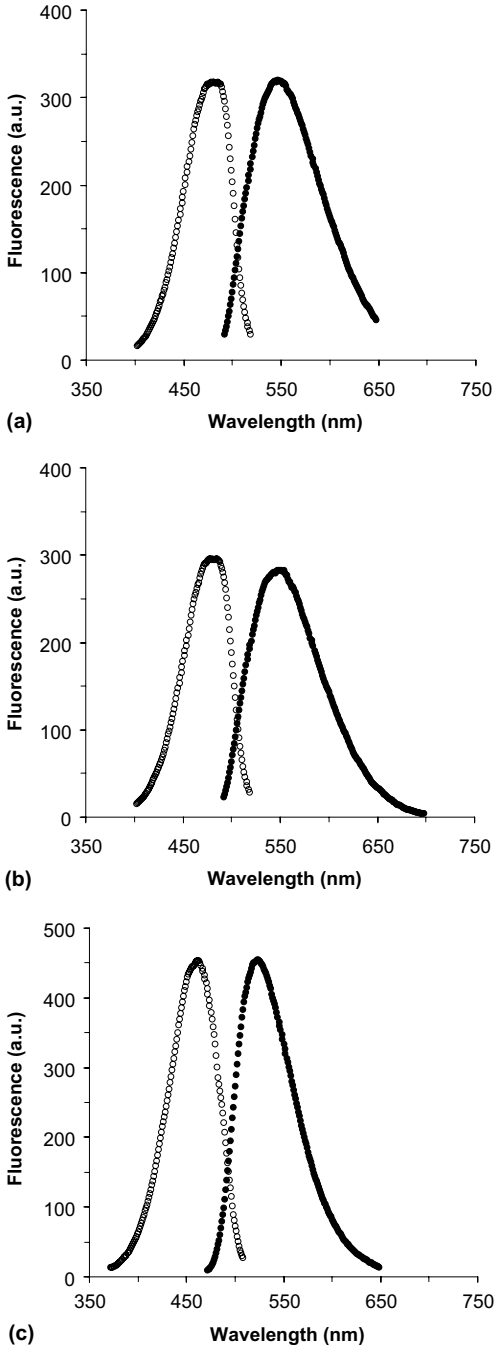

Figure 2. Excitation (○) and emission (●) fluorescence spectra of atrazine imprinted polymer (0.5 mg/mL) in several solvents. (a) H_2O or (b) PBS 0.1 M, pH 7.4: $\lambda_{exc} = 484$ nm; $\lambda_{em} = 552$ nm; (c) ACN (acetonitrile): $\lambda_{exc} = 464$ nm; $\lambda_{em} = 525$ nm.

† Curve fitting was carried out by nonlinear regression using DataFit software version 7.1.44 from Oakdale Engineering (Oakdale, PA, USA) on a PC microcomputer.

Characterisation of 3: Compound **3** was obtained in a yield of 30% (660 mg, 2.73 mmol) as a brown powder; mp 106–108 °C. Found: C, 49.15; H, 3.63; N, 25.30. Calcd: C, 49.09; H, 3.66; N, 25.43; $R_f = 0.58$ (ether/toluene, 1:1); δ_H (300 MHz, CD_3OD), 4.15 (s, 2H), 5.25–5.4 (m, 2H), 5.9–6.05 (m, 1H), 6.2 (d, 1H, $J = 8.9$ Hz), 8.4 (d, 1H, $J = 8.8$ Hz); δ_C (300 MHz, CD_3OD), 54.8, 114.9 (CH_2), 111.9, 123.1, 134.3 (CH), 119.5, 129.1, 132.3, 140.3 (C); ν (NO_2) 1350, 1562; m/z 221.18 $[M+H]^+$.

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