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Molecular imprinted polymers binding low functionality templates

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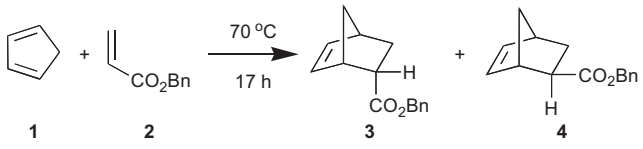
ABSTRACT

A series of highly specific molecular imprinted polymers (MIPs) for small, low functionality bicyclic Diels–Alder products was prepared as bulk polymers.

The technique of molecular imprinting, which is the creation of unique 3D cavities possessing 'lock and key' type fit for specific target molecules, has generated a great deal of interest,[1,2] due to the high degree of molecular specificity that is achievable, thermal and chemical stability and the ease with which such materials can be prepared.[3] This has resulted in MIPs being used in a wide range of applications including liquid chromatography,[4,5] solid phase extraction,[6] biosensors[7] and chemical synthesis.[8] In the area of organic chemistry, MIPs have been used as drug or lead-compound screening aids,[9] non-covalent protecting groups[10] and as catalysts.[11] Since the specific binding of template to imprinted site relies on non-covalent intermolecular forces, using templates that are small and/or have limited functionality remains a challenge.[12] Attempts to imprint small mono-functional molecules have been reported, and functional MIPs for pyridine[12] and propofol[13] have been prepared using conventional non-covalent and semi-covalent (sacrificial-spacer) approaches. For both of these simple template molecules, selectivities in general, although slightly better for MIPs prepared using the semi-covalent approach, were poor. The effect of template shape and functionality on the effectiveness of the MIP has previously been studied by Matsui et al.[14] who reported that template structures with complex 3D shapes tended to result in MIPs with higher selectivity. However, in this work the template possesses only one functional group containing two oxygen atoms (ester). We aimed to prepare MIPs for a mono-functional bicyclic template prepared as the *endo*-product of a Diels–Alder reaction in order to cast some light on the role of molecular shape in the imprinting process. It is notoriously difficult to characterise MIP selectivity on the basis of comparative template affinities for the imprinted and non-imprinted control polymer. To use such an approach requires that non-specific binding contributions are equivalent for the MIP and the control polymer and this is rarely, if ever, the case. Furthermore, to assess specificity effectively in this way typically involves MIP–control polymer comparative binding studies for a range of cross-reacting molecules. An alternative approach is to use a template as a single stereoisomer so that binding selectivity could be evaluated on the basis of chiral discrimination.[15] In this study selectivity was evaluated on the basis of *endo*/*exo* discrimination. The *endo* and *exo* products investigated herein are diastereomers containing three stereogenic centres where the configuration at only one carbon differs in the *endo* and in the *exo* product. For the demonstration of *endo* and *exo* selectivity, the MIP must discriminate on the basis of a change in configuration at a single stereocentre. Herein, we describe the preparation and evaluation of a MIP imprinted with the *endo*-product of a Diels–Alder reaction.

A Diels–Alder reaction between cyclopentadiene **1** and benzyl acrylate (**2**) at 70 °C for 17 h yielded the reaction products **3** (*endo*) and **4** (*exo*) with a combined yield of 80% in an *endo*/*exo* ratio of 3:1 as shown in Scheme 1.[16] Products **3** and **4** were then separated by medium-pressure liquid chromatography. The major isomer **3** was chosen as template.

In our preliminary studies using a related template (the Diels–Alder adduct of cyclopentadiene and methyl vinyl ketone) and acetamide (as mimic of the functional group monomer), it was found in ^{1}H NMR experiments that the interaction between those compounds did not correlate with subsequent equilibrium binding studies. Therefore, in order to identify a lead polymer composition, a number of MIPs containing different functional monomers were prepared and screened. This was carried out on a small scale using bulk polymerisation initiated at 60 °C using AIBN (Table 1).[17] Non-imprinted polymers (NIPs) were also made using the same reaction



Scheme 1. Synthesis of the template **3** via a Diels–Alder reaction between cyclopentadiene (**1**) and benzyl acrylate (**2**).

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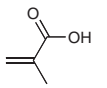
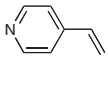
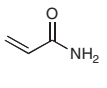
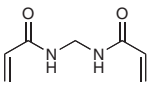
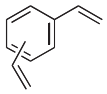
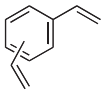
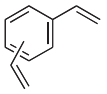
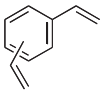
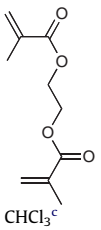
Table 1
Compositions of MIPs **P1**–**P5**[a]

P1	P2	P3	P4	P5[b]
				–
				
4 equiv.	4 equiv.	4 equiv.	4 equiv.	
CHCl3[c] (0.5 mL)	$CHCl_3$[c] (0.515 mL)	$CHCl_3$[c] (0.434 mL)	$CHCl_3$[c] (0.434 mL)	$CHCl_3$[c] (0.5 mL)

NIPs were prepared in the same manner, but in the absence of the template **3**.

[a] All polymers also contain 0.01 equiv of azobisisobutyronitrile (AIBN). MIPs also contain 0.25 equiv of template **3**. The reactants were placed in polymerisation vials and purged in nitrogen for 5 min and then heated at 60 °C for 17 h. Polymers were ground and washed prior to use.

[b] **P5** was prepared to investigate whether the non-specific binding could be reduced.

[c] Solvent volumes were varied so as to maintain a constant monomer: solvent (v/v) ratio.

conditions but in the absence of the template. The ability of each of these polymers to bind the template was evaluated using simple single point equilibrium binding assays (2 mg of polymer in 2 mL of MeCN; template concentration of 10–500 μM, mechanical shaking for 17 h) where equilibrium 'free' template concentrations were determined by HPLC. [HPLC-conditions: RP Kromasil C18 5 μm, 250 mm × 4.6 mm; 20 μL; 1 mL/ min; 215 and 235 nm; solvent: MeOH/H_2O (90/10)]. Preliminary studies demonstrated high non-specific binding (giving a poor imprinting affect) when assays were carried out in chloroform. This situation improved when acetonitrile was used. Initially, three different divinylbenzene (DVB) cross-linked MIPs were evaluated containing an acidic (methacrylic acid, **P1**), a basic (4-vinylpyridine, **P2**) and a neutral functional monomer (acrylamide, **P3**) at a monomer template ratio of (4:1) (Table 1). For **P1**, binding studies showed no difference between the amount of template binding to the MIP compared to the control. For polymers **P2** and **P3** the MIPs bound 6.6% and 7.7%, respectively, more template than did their controls. This was taken as an indication of an imprinting affect.

Although it is speculative to hypothesise as to the type and number of interactions responsible for binding between template and polymer it is interesting to note that for the methacrylic acid containing **P1**, there is no suggestion of an imprinting effect, whereas for both the 4-vinylpyridine- and acrylamide-containing polymers (**P2** and **P3**) binding is favoured to the MIP. Therefore, neither acid nor basic group appears to be a prerequisite for producing an imprinting affect. The most likely point of interaction for **P2** is between the nitrogen of 4-vinylpyridine and the carbonyl carbon of the template, whilst for **P3** a number of different hydrogen bonds are conceivable between the amide group of acrylamide and the template carbonyl group. Given the nature of the environment it is also possible that interfacial hydrogen bonding, between the acrylamide amide and template benzyl group, might also make a contribution.[18,19]

Following these initial observations, more extensive binding studies were undertaken in order to construct binding isotherms (not shown) for the binding of template **3** to **P2** and **P3** (MIP and control polymers). At starting concentrations >100 μM apparent specific binding (nmol bound per mg MIP—nmol bound per mg control polymer) was low suggesting a saturation of available MIP binding sites whilst at template concentrations <100 μM, significant apparent specific binding was observed. A predictable decrease in apparent specific binding was observed as initial template concentration increased.

A number of interesting observations arose when the percentage bound of **P2** and **P3** NIP and MIP were plotted against the initial template concentrations of 10, 25 and 50 μM (Fig. 1). Firstly, **P2** MIP binds a very similar percentage (~50%) of template over the range 10–50 μM, whilst for the **P2** control polymer there is a gradual increase in the percentage bound (11–42%) over the same template concentration range. For **P3** MIP, over the same concentration range, the percentage bound falls from 50% to ~40%, whilst for the control polymer the percentage bound remains relatively consistent. It can be misleading to over interpret such binding data since MIP binding site affinity is extremely polyclonal and estimations of available binding site concentration is fraught with difficulties. However, it was surprising, yet reassuring, that given the difference in monomer composition, **P2** and **P3** MIPs and control polymers behaved in a similar manner.

In an attempt to further reduce non-specific interaction between template and polymer, an alternative cross-linking monomer, ethylene glycol dimethacrylate (EGMA), was evaluated. Previously it had been reported that EGMA can result in reduced non-specific binding (in apolar solvents) and can provide the additional benefit of improving polymer flexibility and accessibility.[20] However, when the binding of **3** to a non-imprinted EGMA polymer **P5** was evaluated, non-specific binding was found to be greater than that of an equivalent divinylbenzene (DVB) cross-linked polymer when evaluated under the same conditions. As a result, DVB was favoured as the cross-linker. A further polymer modification was evaluated where the acrylamide in **P3** was replaced with an equimolar amount of *N*,*N*′-methylene bisacrylamide (MBA) in **P4**. The reason for this modification was to create conformational dependence between adjacent amide groups and increased rigidity in and around the imprinted site. In previous studies this had been shown to give rise to an improved MIP performance. However, by maintaining equimolar amounts of acrylamide and MBA the number of amide groups in **P4** was doubled compared with **P3**. Therefore, in order to make valid comparisons between acrylamide and MBA-containing polymers a further polymer (**P3–8**) was prepared containing 8 equiv of acrylamide. For completeness a polymer containing 8 equiv of 4-vinylpyridine was also synthesised (**P2–8**).

Interestingly, substitution of acrylamide in **P3** with an equimolar amount of MBA in **P4** resulted in a slight increase in MIP and NIP binding for low template concentrations (10 μM) and a slight decrease for higher template concentrations (25 and 50 μM). This is despite the fact that **P4** contained twice the number of amide residues compared to **P3**. However, the difference between MIP

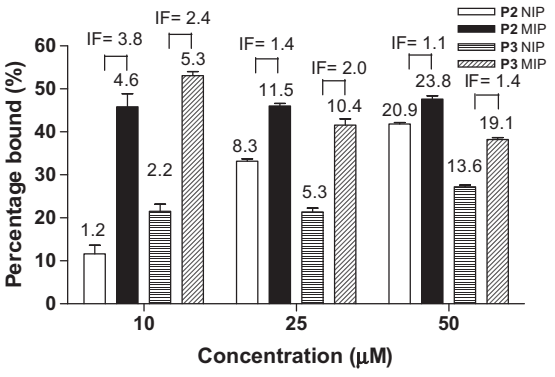


Figure 1. The percentage of **3** bound to **P2** and **P3** NIPs and MIPs. The amount of **3** per milligram of polymer is given at the top of each bar in nmol/mg. IF (imprinting factor): ratio between the amount of **3** bound to MIP and the amount of **3** bound to NIP.

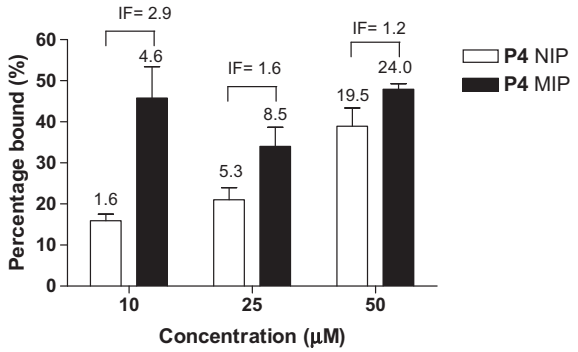


Figure 2. The percentage bound for **P4** NIPs and MIPs. The amount of **3** per milligram of polymer is given at the top of each bar in nmol/mg. IF (imprinting factor): ratio between the amount of **3** bound to MIP and the amount of **3** bound to NIP.

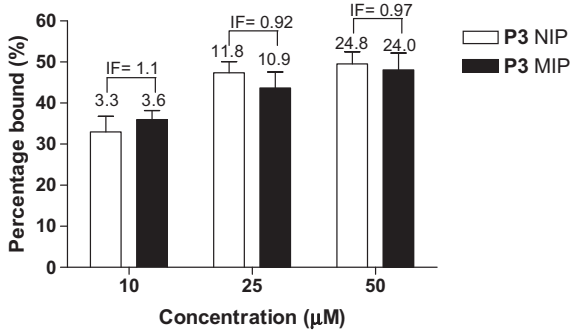


Figure 3. The percentage of **4** bound to **P3** NIPs and MIPs. The amount of **4** per milligram of polymer is given at the top of each bar in nmol/mg. IF (imprinting factor): ratio between the amount of **3** bound to MIP and the amount of **3** bound to NIP.

and NIP binding was similar (~14% at 25 μM) for **P3–8** (see Supplementary data) and **P4** (see Fig. 2).

At 25 μM, the imprinting factors (IF) for **P3**, **P4** and **P3–8** were 2, 1.6 and 1.7, respectively. This indicated that the imprinting effect was not affected by the number of acrylamide groups. The amounts of non-specific binding for **P3** and **P4** were the same (5.3 nmol/mg) even though **P4** had twice as many acrylamide groups. However, **P3–8** had lower non-specific binding compared to **P4** (3.2 nmol/mg for **P3–8** and 5.3 nmol/mg for **P4**) even though both polymers contained the same number of acrylamides.

From the different polymers, **P3** gave the largest amount of specific binding at higher concentrations (Fig. 1). In order to evaluate the *endo*/*exo* selectivity the equilibrium binding of **4** (*exo* form of the product) to **P3** (MIP imprinted with **3** and NIP) was evaluated. Figure 3 clearly shows that there was no difference between the amount of **4** binding to the MIP as compared to the NIP. It is interesting to note that, particularly at lower ligand concentrations, non-specific binding was significantly greater for the *exo* compound **4** as compared to the *endo* compound **3** [10 μM **3** to NIP = 1.2 nmol/mg; **4** to NIP = 3.3 nmol/mg].

In conclusion, a series of MIPs were prepared against a small, poorly functional template **3** and their specificity was evaluated using a simple equilibrium-binding assay. For a number of polymer systems, the amount of *endo* template **3** binding to the MIP was consistently greater than that binding to the NIP. When the *exo* form of the template **4** was evaluated, the amount binding MIP and NIP was similar.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.08.108.

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