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# First steps towards conformationally selective artificial lectins: the chair-boat discrimination by molecularly imprinted polymers†

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**A series of molecularly imprinted polymers (MIPs) were prepared in the presence of a synthetic galactoside locked in a $^{1,4}B$ boat conformation. This study demonstrates that, depending on the polymerisation technique, an organic material can selectively bind a carbohydrate in a biologically relevant boat conformation.**

Many biological processes are guided by the tremendous information capacity of carbohydrates expressed as mono- and oligosaccharides in living organisms.[1] Moreover, carbohydrates may adopt a range of different conformations that even increase their information-storage capacity. Thus, it is not surprising that carbohydrates play an important role in numerous chemical, biological and physiological processes, including diseases.[2] For instance, carbohydrates have been identified as biomarkers of key pathologies such as cancer for which diagnostic tools still need to be developed.[1] Therefore, the discovery of molecular or macromolecular tools that can specifically bind carbohydrates or oligosaccharides has become an exciting domain with potential applications in biomedicine.[3]

A promising technology for the synthesis of receptors is the imprinting technology. A Molecularly Imprinting Polymer (MIP) is a polymer possessing binding and/or active sites.[4] MIPs have been used as analytical tools, sensors and receptors,[4a,5] but also as catalysts.[6] More recently, MIPs have also been exploited as specific enzyme inhibitors[7] and as tools for proteins recognition and capture in physiological media.[8] Moreover, very recent polymerization techniques allow the preparation of clickable molecularly imprinted nanoparticles, opening new possibilities in chemical sensing.[9]

Wulff *et al.* pioneered the field of carbohydrate recognition by MIPs by developing polymers capable of differentiating carbohydrate epimers or enantiomers with direct applications in separation technologies.[10] Later on, MIPs have been used as carbohydrate sensors or drug delivery devices.[10,11] To date, the MIP technology has never been exploited for the recognition of a selected conformation of a single carbohydrate. The ground state conformation of pyranoses in mono- or oligosaccharides is usually a chair (C) conformation. However, some natural or synthetic glycosides adopt the less populated boat (B), skew-boat (S) or half-chair (H) conformations in their bioactive form. A striking example is the skew-boat conformation adopted by L-iduronic acids in natural or synthetic heparin fragments,[12] a conformation required for the antithrombotic activity. Moreover, numerous glycosyl processing enzymes preferentially bind glycosides in a boat or half-chair conformation.[13]

For all these reasons we decided to determine whether a molecularly imprinted polymer could be generated with a binding selectivity for a boat conformation. We specifically selected the $^{1,4}B$-boat conformation because this conformation is transiently adopted by saccharides during enzymatic reactions.[14,15]

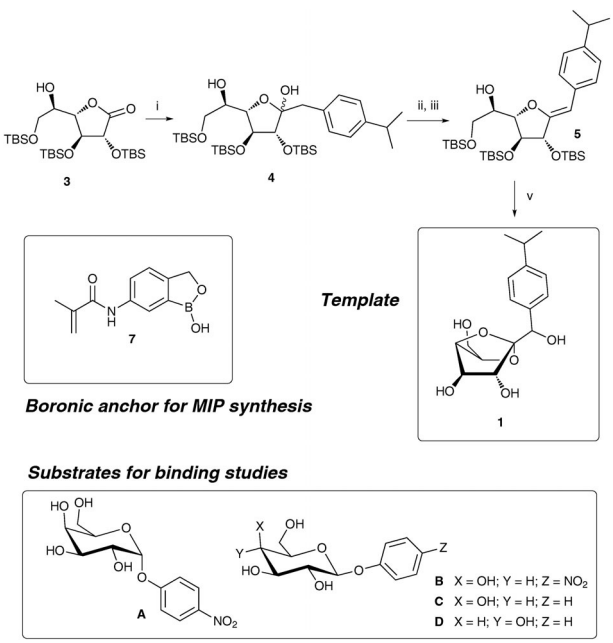


**Fig. 1** Reagents and conditions: (i) *p*-isopropylbenzyl magnesium bromide, $Et_2O$, 0 °C, 66%; (ii) pyridine, TEAA, THF, 0 °C then rt; (iii) $K_2CO_3$, MeOH–DCM, rt, 78%; (iv) *m*CPBA, CSA, DCM, rt, 77%; (v) TBAF, THF, rt, 81%.

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Design and synthesis of the template – for the imprinting process, we designed a specific template (molecule **1**, Fig. 1), possessing the two essential features for both the boat/chair selectivity and the binding measurements: (i) the [2.2.1] bicyclic structure imposes a *locked* $^{1,4}B$-boat structure, (ii) the UV-active phenyl ring was used to allow binding analyses by simple HPLC techniques. The *p*-isopropyl moiety was added on the phenyl ring of **1** because we planned to use *p*-nitrophenyl glycosides **A** and **B** (Fig. 1) for the MIPs' binding studies. The nitro functionality being known to inhibit polymerizations,[16] we opted to place an isopropyl which is isosteric of the nitro group.[17] The synthesis began from known lactone **3**[14b] that was condensed with *p*-isopropylbenzyl magnesium bromide to yield **4** in 66% yield. Intermediate **4** was then dehydrated to afford the expected (*Z*)-*exo*-glycal **5**[14b] which was then epoxidized with *m*CPBA. The presence of CSA in the reaction mixture catalysed the intramolecular 5-*exo*-trig cyclization of the intermediate epoxide, to afford the desired $^{1,4}B$ boat **1** after TBAF deprotection.

Choice of the boronic anchor – to obtain a polymeric material displaying recognition cavities for carbohydrates, it is necessary to copolymerize the template with cross-linking units and "anchors". The latter are monomers possessing functional group(s) allowing favourable and reversible covalent or non-covalent binding to the template. Since our boat-locked template **1** does not display *cis*-diols that can efficiently bind 4-vinylphenylboronic acid,[10] we chose to use the benzoboroxole anchor **7** (Fig. 1) which has already been used also for the impression of carbohydrates and monoalcohols.[10,18]

Synthesis of the imprinted polymers – usually, the first step of the synthesis of an imprinted polymer is the preparation of a pre-polymerisation complex which consists of mixing the template and the anchors in the appropriate solvent to promote a covalent or non-covalent association before starting the polymerization.[4a] The formation of boronic esters is a dynamic equilibrium that can be shifted towards the formation of the ester by azeotropic distillation or by addition of a desiccant. We chose the procedure developed by Mioskowski,[18a] which uses an equimolar amount of anchor per alcohol functionality in the presence of $CaH_2$ in acetonitrile. After filtration, the cross-linking reagent was added, and the mixture polymerized 16 h at 60 °C. The polymers were then washed by the water–acetonitrile (1/1) mixture, dried and filtrated through a 250 μm sieve. A series of polyenes were screened as cross-linking reagents: EGDMA and PETrA represented in Fig. 2 gave the best results. Binding studies were then realized to determine if the imprinting process was successful. As a control, non-imprinted polymers (NIPs) were systematically prepared.

Binding experiments – the binding studies were performed using standard techniques:[4a] the polymers were incubated, at a constant concentration, with increasing concentrations of

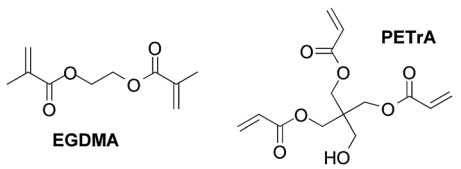


**Fig. 2** The two best cross-linking reagents found after screening.

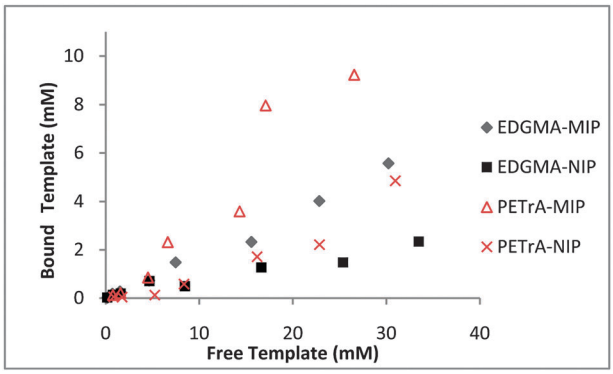


**Fig. 3** EGDMA-MIP and PETrA-MIP binding isotherms. Incubations were realized with 5 mg of polymer in 1 mL of template solutions (5 : 95 water : acetonitrile), and incubated 24 h at 22 °C. The vials were then centrifuged, and the resulting supernatants were analyzed by HPLC.

the template during a fixed time at a constant temperature. The resulting solutions were then analysed by HPLC to determine the resulting concentration of the free template (F). The difference with the initial concentration gave the bound template concentration (B). The binding isotherm B *vs.* F was then plotted.[4a] The MIP imprinting effect is graphically demonstrated when the MIP isotherm plot is higher than the NIP one (see for instance Fig. 3), which means that the MIP has a higher affinity and binding capacity for the template. Moreover, the imprinting efficiency can be quantified by calculating an imprinting factor (IF). The ratio between the bound template concentration in MIP and NIP directly gives the IF.[4a,19]

The incubations were performed in acetonitrile, for 24 h. After centrifugation, the supernatants were injected in HPLC. The EGDMA polymers gave binding isotherms with a significant imprinting effect (Fig. 3). The imprinting factor varies from 1.03 to 3.01 depending on the polymer and the template concentration. The two plots being linear, a reasonable average of the IF can be calculated as the ratio of the two slopes which gives a value of 2.61.

Interestingly, the best imprinting effects were observed at high template concentration (> 5 mM), as if the non-specific interactions dominate at low concentration. At these high concentrations, the linear shape of the isotherm shows that the MIP is not saturated. The PETrA polymers also gave imprinting effects (Fig. 3), with even higher binding capacities than EGDMA polymers. The imprinting factor measured from 1.91 to 6.75. The average IF is in this case 2.81. Moreover, in contrast to EGDMA MIP, the PETrA MIP had a better imprinting effect at moderate template concentration (between 1 and 10 mM).

Binding assays with the "chair" glycosides – to demonstrate the conformational selectivity, the EGDMA and the PETrA polymers (MIPs and NIPs) were also incubated with the *p*-nitrophenyl-α- and -β-galactopyranosides **A** and **B**. No specific binding could be measured with **A** and **B**, thus showing that 1-aryl-galactosides adopting a chair conformation do not bind the polymers that have been imprinted with a boat-locked galactose.

Effect of the nitro group – as an additional control experiment, we also found that phenyl-galactoside **C** and phenyl-glucoside **D** had no affinity for the MIP, thus showing that the lack of binding

**Table 1** Imprinting effect of the EGDMA MIP with the template as a function of the solvent

| Solvent | $CH_3CN$ | $H_2O/CH_3CN$ (1/1) | $H_2O$ |
|---|---|---|---|
| IF (EGDMA-MIP) | 2.6 | 3.1 | 1.3 |

is not related to the nitro group or the absolute configurations of the carbohydrate at position 4. These experiments clearly show that it is indeed possible to generate polymers finely tuned for a selective conformational recognition of D-galactose.

Further controls with other "non-imprinted" polymers – to strengthen our study, we also prepared NIPs with different "control" templates (for instance by using ethylene glycol as the template surrogate). In each case, we could always measure an imprinting effect for template **1**. As a second control, polymers were also prepared with EGDMA in a non-covalent fashion, without formation of pre-polymerization complexes, by just mixing the constituents, in the absence of drying agent. Interestingly, no significant imprinting effect was observed, in contrast to the covalent protocol described above. These experiments nicely illustrate the dramatic impact of the preorganization of the components before the polymerizations on the recognition capacity of the resulting binding cavities.

Solvent effect – to determine the scope of the binding recognition, we also measured the imprinting factors in pure $CH_3CN$, in a 1/1 mixture of $H_2O$–$CH_3CN$ and in pure $H_2O$ (see Table 1). These experiments showed that the level of binding was in the same range in a mixture of $H_2O$–$CH_3CN$ whereas a strong decrease in the IF factor was observed in pure water.

This study shows that it is possible to generate an imprinted polymer that can selectively bind a carbohydrate in a chosen conformation, without binding the same molecule in the ground-state chair conformation.

These results are in line with important questions recently addressed in glycosciences, for instance the development of artificial lectins sensing biologically relevant molecules with applications in the field of diagnostics. The huge advantage of using MIPs for such applications is their obvious robustness and recyclability as compared to biomolecules such as antibodies and DNA. Further improvements remain to be achieved in this MIP technology, in particular in obtaining polymers with full carbohydrate recognition capacities in pure water or in physiological media. Very recent advances in MIP technologies that enhanced template binding properties in aqueous media should allow us to develop a new generation of conformationally selective artificial lectins.[20,21]

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