Polymer-supported cationic templates for molecular recognition of anionic hosts in water†

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Translocating solution-phase molecular recognition of oppositely charged hosts and guests to the solid phase represents a major challenge; we report a successful immobilisation strategy which allows selective host–guest interactions in water unencumbered by unwanted ion exchange-type interactions.

An increasing number of applications of supramolecular chemistry[1] rely on immobilisation of one of the partners engaged in molecular recognition, *e.g.* in sensing devices[2–7] and in molecularly imprinted polymers (MIPs).[8,9] Another area in which translocation of solution-phase molecular recognition to the solid phase is desirable is dynamic combinatorial chemistry. This technique relies on selective molecular recognition inducing a shift in the composition of complex equilibrium mixtures towards the host with the highest affinity for a particular guest (or *vice versa*).[10–12] Immobilisation of the guest[13–20] (template) or indeed the building blocks[21,22] simplifies analysis of the reactions and can also aid in the subsequent isolation of products.

It is now clear that molecular recognition of a species is not always unaffected by immobilisation, particularly when both host and guest are charged. For example, Anslyn *et al.* have reported the use of commercially available Tentagel resin[23] bound receptors immobilised on micro-machined silicon.[24,25] The authors acknowledge that differential ion-exchange chromatography is taking place, in addition to the desired selective molecular recognition events.[3] Our own early attempts at immobilising a cationic guest on an Argogel resin revealed binding that was completely dominated by unwanted ion exchange behaviour in which the most highly charged species are the most retained.[26] Undoubtedly, therefore, the influence of the polymer backbone on host–guest chemistry under heterogeneous conditions is not negligible, an aspect which we believe has not been given sufficient consideration. In our recent parallel investigations of the use of polymer-supported templates in dynamic combinatorial libraries (DCLs) in organic solvents, we demonstrated the importance of careful design of the resin for achieving highly selective and efficient molecular recognition.[20] We now report an extension of this work to what is probably the most intricate system of all: molecular recognition of a charged guest covalently linked to a polymer support by solution-based host molecules of various degrees of opposite charge in aqueous media, where supramolecular chemistry represents an exceptional challenge.[27] The goal of the present work was to design polymeric supports that are highly compatible with such conditions, while avoiding non-selective ion-exchange type interactions.

As a test system we selected one of our disulfide-based DCLs[28–34] made from anionic dithiol building blocks **1** and **2** (Fig. 1). In the presence of cationic guest **3** in solution, unambiguous amplification of receptors $(\mathbf{1})(\mathbf{2})_2$, and to a lesser extent $(\mathbf{1})_3$, is observed (Fig. 2). While we have previously reported amplification of $(\mathbf{1})(\mathbf{2})_2$ with other guests,[29,30,32] the amplification of $(\mathbf{1})_3$ has not been observed before. A successful resin would allow selective binding of these two receptors to an immobilised analogue of guest **3** without simultaneously binding some of the higher, more charged oligomers that would occur if an ion-exchange-type mechanism was taking place.

Polyacrylamide-type resins were expected to be highly compatible with molecular recognition in water. The general strategy adopted was to functionalise adamantylamine with a spacer, followed by coupling of the latter to a polymerisable acrylamide type vinyl monomer (Fig. 3). A control experiment using **4a** as a template in the DCL made from **1** and **2** gave essentially the same results as that shown in Fig. 2 for the parent template **3**, demonstrating that the introduction of the

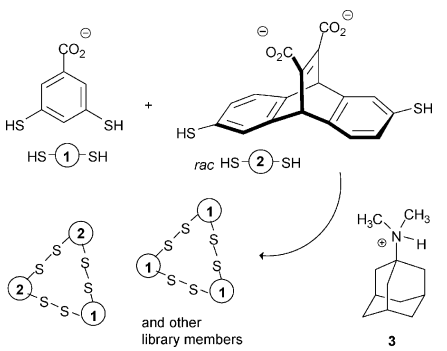

Fig. 1 Exposing a DCL composed of anionic building blocks **1** and *rac*-**2** to cationic guest **3** leads to the amplification of receptors $(\mathbf{1})(\mathbf{2})_2$ and $(\mathbf{1})_3$.

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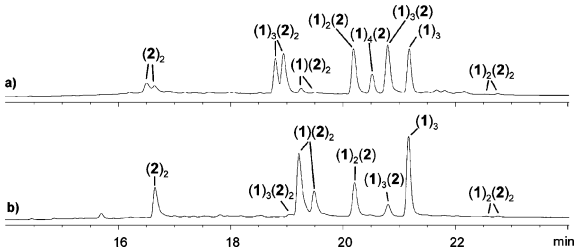

Fig. 2 HPLC analyses of a DCL made from building blocks **1** and *rac*-**2** (2 mM in total) (a) in absence of template, and (b) after 72 h exposure to template **3** (2 mM).

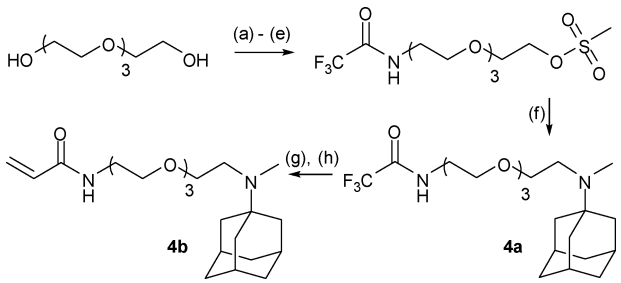

Fig. 3 Synthesis of polymerisable template **4b**: (a)[35] MsCl, Ag_2O, CH_2Cl_2, rt, 48 h, 48%; (b)[35] NaN_3, dry DMF, N_2, 120 °C, 2 h, 95%; (c)[35] (i) Ph_3P, dry THF, N_2, rt, 12 h; (ii) H_2O, rt, 10 h, 88%; (d)[36] ethyl trifluoroacetamide, Et_3N, MeOH, rt, 12 h, 90%; (e)[37] MsCl, Et_3N, CH_2Cl_2, rt, 12 h, 96%; (f)[38] *N*-(1-adamantyl)-*N*-methylamine, NaI, K_2CO_3, CH_3CN, reflux, 36 h, 82%; (g)[36] 6 M NaOH, rt, 12 h, 97%; (h)[36] acryloyl chloride, DMAP, K_2CO_3, CH_2Cl_2, rt, 12 h, 99%.

ethylene oxide spacer did not affect the host–guest interactions (*cf*. Fig. S1, ESI†).

The design and choice of polymer morphology (lightly crosslinked swellable gel-type *versus* more heavily crosslinked macroporous resins) and template loading was based on our previous work on polymer-supported templates in organic solvents.[20] These studies revealed that the more open gel-type resins with a relatively low guest loading gave the best results in templating combinatorial libraries of macrocyclic hydrazones. While it was unclear whether these observations would translate to a wholly non-related system (different solvent, different guest, different set of hosts and different polymer matrix), they appeared to provide a good starting point. Thus, monomer **4b** (20 wt%) was polymerised in an inverse-suspension polymerisation procedure to yield spherical gel-type (GT) resins **AM GT 4b** and **DMAM GT 4b** using acrylamide (**AM**) or dimethylacrylamide (**DMAM**) (76 wt%), respectively, as diluting/structural comonomer, and methylene bisacrylamide (**MBA**; 4 wt%) as crosslinker in ~6.5 fold excess of water (*cf*. Fig. S4 and S5, ESI†).

The first polymer-supported template examined was **AM GT 4b**. This was added to an equimolar mixture of building blocks **1** and **2** (2 mM in total) in 50 mM borate buffer (pH 8) to give an overall template concentration of 2 mM. After equilibration, the beads are filtered off, washed with borate buffer to remove any weakly bound species and eluted with ethanol to liberate the more strongly retained host molecules. Rather disappointingly, the HPLC trace of the filtered supernatant solution (Fig. 4b) showed little evidence for amplification of $(\mathbf{1})_3$ and $(\mathbf{1})_2(\mathbf{2})$. Indeed this trace looks very similar to that obtained in control reactions using resins **AM GT** and **DMAM GT** containing no template (*cf*. Fig. S2 and S3, ESI†). With the latter, of course, no templating effect is possible and the small changes seen in the library distribution may be due simply to minor differential partitioning of library members between the aqueous supernatant phase and the swollen polymer resin phase. Even more disappointingly, the HPLC traces of the buffer wash and the solution from the ethanol elution (Fig. 4c and d) indicate that none of the library members are strongly retained on **AM GT 4b**, confirming an absence of any significant involvement of the immobilised template. Perhaps understandably, therefore, we were not optimistic with regard to the likely behaviour of resin-bound template **DMAM GT 4b**. However, we were delighted to find that the results using this resin were quite different. Comparison of the HPLC traces in Fig. 5 with those in Fig. 2 confirms that the compounds which are selectively amplified by solution phase template **3** are also amplified by resin **DMAM GT 4b**, and *furthermore they are selectively retained by the resin and eventually emerge in the ethanol wash*. It is clear, therefore, that the **DMAM**-based gel-type polymer support is fully

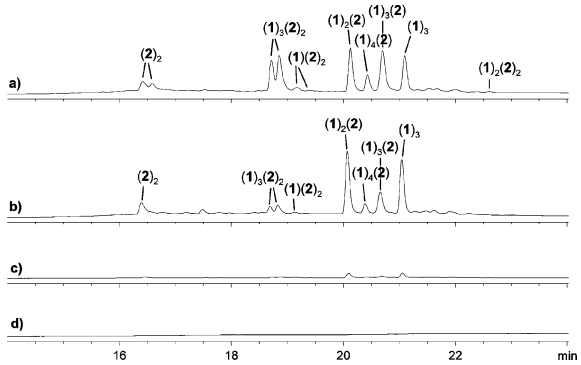

Fig. 4 HPLC analyses of a DCL made from building blocks **1** and *rac*-**2** (2 mM in total) (a) in absence of template, and (b) after 72 h exposure to **AM GT 4b** (4 mg ml^{-1}), (c) borate buffer wash of **AM GT 4b**, and (d) elution with ethanol.

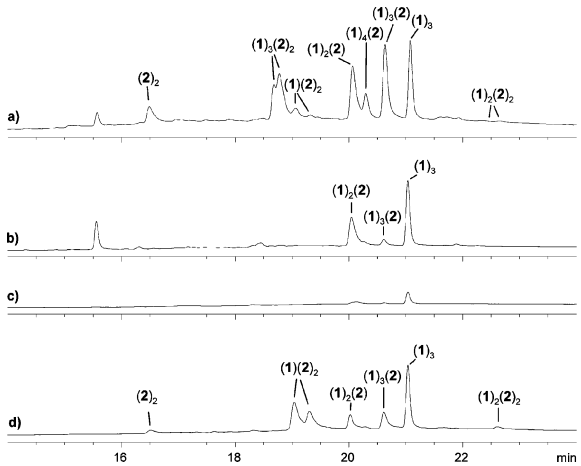

Fig. 5 HPLC analyses of a DCL made from building blocks **1** and *rac*-**2** (2 mM in total) (a) in absence of template, and (b) after 72 h exposure to **DMAM GT 4b** (4 mg ml^{-1}), (c) borate buffer wash of **DMAM GT 4b**, and (d) elution with ethanol.

compatible with the library conditions, giving rise to efficient host–guest interactions that parallel those in solution. When the HPLC traces from the filtrate, wash and elution are summed up, essentially the same library composition is obtained as under templating conditions in solution. Of particular significance is the fact that the same template to building block ratio (1 : 1) was used when working with the resin as compared to the solution-based libraries, and bearing in mind the highly water swollen state of the resin (*cf*. Fig. S5, ESI†), this suggests that most immobilised template in **DMAM GT 4b** is accessible to the hosts. The only difference between the **AM** and **DMAM** resins is the two methyl groups on the acrylamide monomer. We therefore tentatively ascribe the strikingly different templating behaviour to the strong hydrogen bonding donor capability of the acrylamide polymer segments. We speculate that extensive hydrogen bonding between amide hydrogens and carbonyl groups within the **AM GT 4b** resin may inhibit local access to the immobilised template or that the **DMAM GT** resin may provide a more favourable hydrophobic micro-environment around the template. Competitive interaction of the **AM** primary amide groups with reactants or products seems less likely since, as indicated above, control experiments involving unfunctionalised **AM GT** and **DMAM GT** show that neither of these resin matrices has any significant influence on the product distribution (*cf*. Fig. S2 and S3, ESI†).

In conclusion, a lightly crosslinked gel-type dimethylacrylamide resin has been developed that is suitable for immobilisation of a cationic guest. At a guest loading of 0.5 mmol g^{-1}, selective binding of anionic hosts in water has been demonstrated. Thus, a non-interfering template functionalisation strategy combined with careful design of the polymeric support allows solid-phase bound templates to mimic very closely solution-based host–guest interactions. Binding selectivity and efficiency can be preserved *with the important practical advantage of ease of separation of favoured library members*. This paves the way for more general exploitation of these materials in various applications of aqueous dynamic combinatorial chemistry, and in related host–guest binding studies.

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