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Simultaneous selection, amplification and isolation of a pseudo-peptide receptor by an immobilised *N*-methyl ammonium ion template

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Immobilised *N*-methyl ammonium ions have been used to simultaneously select, amplify and isolate a pseudo-peptide receptor from a dynamic library of hydrazones.

In recent years dynamic combinatorial chemistry[1] (DCC) has emerged as a new strategy for the discovery of host–guest systems with the potential for catalysis and drug activity.[1,2] The unique feature of DCC is the dynamic combinatorial library (DCL), the members of which are comprised of building blocks connected by reversible bonds. Reversibility ensures that all the members of the library are interconverting, and that the library composition is thermodynamically controlled; thus its composition is adaptive and can respond to the presence of a template molecule that selectively binds and stabilizes one particular member of the library, resulting in amplification of the best binder.

We have recently described the preparation of DCLs using hydrazone exchange as the reversible reaction[3] and have shown that amplification can be achieved by non-covalent recognition,[4] to a degree that correlates with the affinity for the template.[5] So far the libraries generated have been of limited size and extension to larger and more diverse libraries is desirable. However, conventional methods of isolation for selected receptors are likely to prove ineffective in such complex mixtures. To overcome this problem we have proposed the use of immobilised templates.[6]

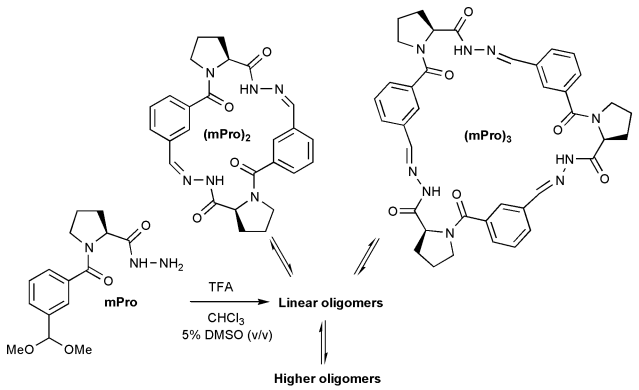
Attachment of the template to a solid support will result in a significant fraction of the amplified compound being bound to it and non-covalently immobilised. This will permit the separation of the undesired members of the library by filtration and subsequently, the best binder can be removed from the resin (ideally pure) by use of a solvent that disrupts the non-covalent interaction between the receptor and the template.

Here we describe the first example of simultaneous selection and efficient amplification of a receptor by an immobilised template, and show how this strategy allows the straightforward isolation of the best binder from a mixture of 12 very similar compounds.

The implementation of this procedure has been previously attempted. Klekota and Miller have shown a decrease in the concentration of the selected product from a DCL in contact with an immobilised template.[7] Unfortunately, the selected compound could not be removed from the solid support, making it difficult to evaluate whether amplification had taken place. In addition, Ramström and Lehn have observed selective binding of four members of a dynamic library using an immobilised template.[8] Although the selected compounds could be recovered from the solid support, it remains unclear whether the immobilised template induced any amplification.

Using a slightly different approach Eliseev and Nelen have separated the equilibration process from the selection process.[9] Generation of the library was carried out in one pot and selection *via* an immobilised receptor in another, followed by reinitiation of the equilibrium process. Each equilibration-selection cycle led to the retention of the strongest binding library member and hence, its overall enrichment. However, this system cannot take full advantage of the adaptive properties of DCLs, which require the coexistence of selection and amplification.

We have focused on libraries generated from the pseudo-peptide building block **mPro** (Scheme 1).[4b] Upon addition of ammonium ion templates the **mPro** library undergoes molecular amplification to form mainly cyclic trimer **(mPro)$_3$**. Using this system, we have investigated the effects of immobilisation of the template on amplification and assessed the practicality of isolation.



Scheme 1

Cyclisation of the **mPro** monomer was performed by the addition of TFA to a 5 mM solution of monomer in $CHCl_3$, containing 5% DMSO (v/v).† At equilibrium, HPLC analysis‡ showed the library to be comprised mainly of cyclic dimer **(mPro)$_2$** (85%),§ with small amounts of cyclic trimer **(mPro)$_3$** (7%), and higher oligomers (8%) present (Fig. 1a). Upon cyclisation under the same conditions in the presence of 5 equiv. of benzyltrimethyl ammonium iodide (BTA), a different library composition was observed, significantly reduced in dimer (47%) and higher oligomers (3%), and enhanced in trimer (50%) (Fig. 1b). A similar response was seen in earlier

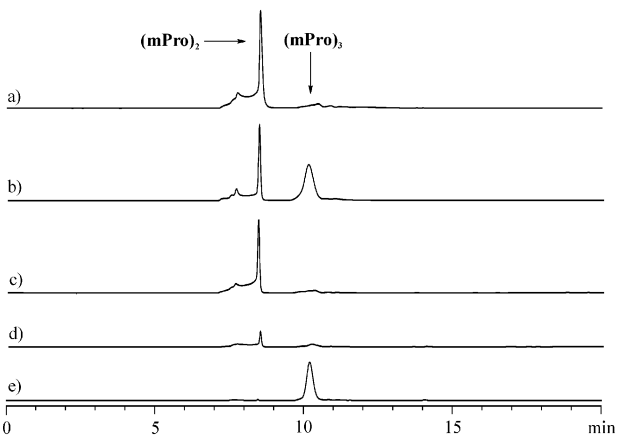


Fig. 1 HPLC traces of a **mPro** library (a) in the absence of template, (b) in the presence of BTA, (c) solution phase after 24 h exposure to **A27**, (d) $CHCl_3$ wash of **A27**, (e) MeOH wash of **A27**.

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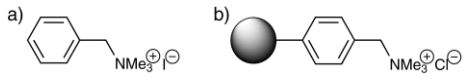
work.[5]

The commercial Amberlyst-27 resin (**A27**) contains immobilised cations that can be considered to be as solid phase analogues of BTA (Scheme 2). Cyclisation of **mPro** in the presence of **A27** was carried out in the hope that it would lead to the selective removal and amplification of **(mPro)$_3$** from the equilibrating mixture.¶ HPLC analysis of the solution phase after 24 h showed the presence of cyclic dimer **(mPro)$_2$** at a reduced concentration compared to that for the same system in the absence of the immobilised receptor (Fig. 1c). In addition, no significant **(mPro)$_3$** peak was observed (Fig. 1b,c). Filtration of the **A27** beads, followed by exhaustive washing with $CHCl_3$ yielded a small amount of dimer and higher oligomers (Fig. 1d), whilst further washing with MeOH, a solvent known to disrupt the interactions between template and receptor, afforded **(mPro)$_3$** in 40% yield (Fig. 1e).



Scheme 2 (a) Benzyltrimethyl ammonium iodide (BTA), (b) functional group of the Amberlyst-27 resin (**A27**).

These results show that immobilisation of the template (i) does not appear to have a significant effect on the extent of templating observed; (ii) can be used to isolate the amplified receptor. However, the mixture used here is of low diversity, consisting of mainly dimer and trimer and containing hardly any of the higher oligomers. In order to test the effectiveness in a more challenging system, and to prove that recognition is specific for the trimer, we have used a static library‖ containing **(mPro)$_3$** and many higher oligomers and exposed it to **A27**. As this is not a dynamic system, no changes in relative concentrations occur overall. HPLC analysis of the solution phase after 24 h showed a significant reduction of the **(mPro)$_3$** peak, whereas the other peaks were not affected. Filtration and washing of the **A27** beads with $CHCl_3$ yielded a small amount of trimer, whilst further washing with MeOH led to a recovery of 98% of the trimer that was initially present (Fig. 2).

For the practical use of such immobilised systems it is important to be able to move beyond the analytical scale. Therefore, the thermodynamically controlled cyclisation of **mPro** in the presence of **A27** beads was also carried out on a preparative scale (150 mg). Purification *via* preparative TLC of the trimer recovered in the MeOH wash was necessary, in order to remove impurities leached from the polystyrene resin. This afforded 58 mg (38% yield) of **(mPro)$_3$** with a purity in excess of 95%, which is almost identical to the results obtained previously on an analytical scale. Thus, scale up appears straightforward without significant loss in yield.

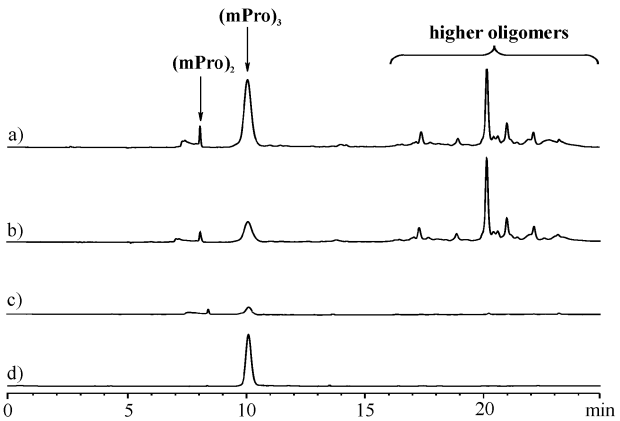


Fig. 2 HPLC traces of the static **mPro** library (a) library before exposure to **A27**, (b) solution phase after 24 h exposure to **A27**, (c) $CHCl_3$ wash of **A27**, (d) MeOH wash of **A27**.

In summary we have demonstrated that an immobilised *N*-methyl ammonium ion template can select and amplify a cyclic trimer species **(mPro)$_3$** from a dynamic library of interconverting species. In addition, it has been shown that template functionalised resin can also be used for the isolation and purification of this pseudo-peptide receptor. To the best of our knowledge, this is the first example of simultaneous selection, amplification and isolation of a receptor by an immobilised template, using a process which in effect combines chemistry and affinity chromatography into one single step. We believe that this method will be of critical importance to the future development of DCC, as it will allow the rapid isolation of selected species from DCLs containing many very similar compounds.

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Notes and references

† The general procedure for cyclisation experiments entailed the dissolution of **mPro** monomer in freshly distilled $CHCl_3$ containing 5% DMSO (v/v) and the addition of 5 equiv. of TFA. All analytical scale reactions were carried out on a 5 mg scale at a concentration of 5 mM. The reactions were stirred at rt for 24 h before HPLC analysis. The preparative scale reactions (150 mg) were carried out using the same concentration of **mPro** monomer. The procedure for cyclisation experiments in the presence of BTA was similar, with 5 equiv. of BTA being added immediately after addition of TFA.

‡ HPLC analysis was carried out using a Hewlett-Packard 1050 instrument, coupled to a HP 1050 DAD; data was analysed using a HP ChemStation. Separations were achieved in the reverse phase using a Supelcosil ABZ+Plus column (15.0 cm × 4.6 mm), a C16 alkyl based phase of 3 μm particle size.

§ Isolation and mass spectrometric analysis of both the sharp peak and the broad preceding peak have shown that both are due to the **mPro** dimer, probably existing as a mixture of *cis–trans* isomers.

¶ The general procedure for cyclisation experiments carried out in the presence of the immobilised template **A27** uses the conditions described above with the addition of 300 mg of the resin (dry weight), immediately after addition of TFA. Before use, the resin was crushed with a mortar and pestle and exhaustively washed with H_2O, MeOH and $CHCl_3$.

‖ The static library was obtained by stopping the reversible reactions, by neutralisation of TFA, before thermodynamic equilibrium was achieved.

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