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Enantioselective molecularly imprinted polymers *via* ring-opening metathesis polymerisation

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Enantioselective molecularly imprinted polymers (MIPs) have been synthesised *via* ROMP for the first time.

The considerable interest in molecularly imprinted polymers (MIPs) derives from their potential to perform as artificial enzymes and catalysts.[1,2] Indeed for certain cases molecularly imprinted polymers (MIPs) have been shown to successfully mimic the recognition and catalysis behaviour associated with enzyme and antibody activity.[1] Ever increasing effort is being devoted to investigate MIPs for a wide variety of applications involving highly selective molecular recognition events (such as affinity chromatography (enantiopolishing) and sensing layers (ELISA assays)). However, the way in which these polymers are being synthesised has remained essentially unchanged since the introduction of the free radically cross-linked network approach by Takagishi and Klotz[3] and more ingeniously by Wulff *et al.* in the early 1970s.[3]

The process of forming a MIP is in many ways the nanoscale version of producing a plaster cast of a three-dimensional object (the template) (Fig. 1). MIPs are made *via* free radical polymerization in the presence of a template molecule. Removal of the template leaves behind cavities that are complementary to it in size, shape and electron density distributions. The functional monomers act as binding sites and self-assemble around the template prior to polymerisation. Once the template molecule has been removed these binding sites are arranged in a specific three-dimensional relationship very much in analogy to the specific arrangements of amino acid sidechains within the active site of an enzyme (Fig. 1).[1] It is clear from these considerations that MIPs possess the potential to be developed into synthetic enzymes and antibodies. To date a serious downside to the application of MIPs has been lack of synthetic control. The statistical, kinetically driven nature of the network forming process makes it impossible to achieve monoclonality. Instead polyclonal cavities are being formed exhibiting a wide range of sizes and shapes and as a consequence a wide range of selectivities and/or catalytic activities thereby limiting the potential of MIPs drastically.[1,2,4]

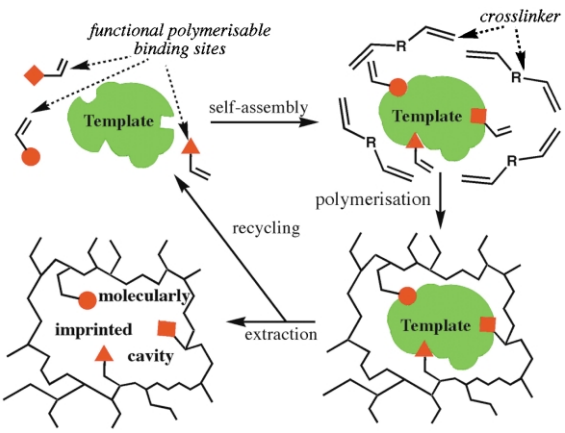


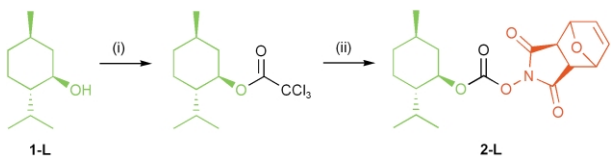
Fig. 1 Molecularly imprinted polymers (MIPs)—The concept.

A conceptual approach of addressing the issue of polyclonality in MIPs is to investigate the possibility of utilising thermodynamically controlled bond forming (polymerisation) reactions. The ability of silica (surfaces), which was used in early MIP syntheses as network forming material, to dynamically rearrange and reorganise itself in the presence of traces of water makes it eligible for such an approach.[1] Implicitly Morihara *et al.*, by allowing the surface of a silica network to form slowly around the template, elaborated successfully on this methodology some twenty years ago in a series of intriguing publications on 'footprint' catalysis.[5] It seems that the brittle nature of silica and its susceptibility to surface rearrangements might have been reasons why other groups have not pursued this direction any further.[1,3,6]

Recent advances in the reactivity and functional group tolerance of ruthenium alkylidene catalysts[7] and the concurrent development of the formation of highly crosslinked networks *via* ring-opening metathesis polymerisation with high conversion by Mühlebach *et al.*[8] and Buchmeiser *et al.*,[9] together with the incorporation of functional norbornene monomers by Feast *et al.*[10] made us decide to evaluate ROMP (incl. cross metathesis) as a thermodynamically controlled network forming strategy. A complementary 'small molecule' strategy has been developed by Sanders *et al.*, who recently applied ring-opening/ring-closing metathesis catalysis in the synthesis of templated supramolecular assemblies.[11]

In our case the template was covalently attached to a ROMP-polymerisable monomer, thereby ensuring maximum compatibility with the catalyst.[7] Upon hydrolysis the covalent interaction is replaced by reversible non-covalent ones, an approach pioneered by Whitcombe *et al.*[12] Thus monomer **2-L** was synthesized from the chiral template L-menthol, **1-L** (Scheme 1). Homopolymerisation, and copolymerisation of **2-L** with norbornene, both to high conversions using Grubbs' catalyst, provided us with the necessary evidence of monomer/catalyst compatibility. We also established that L-menthol could be hydrolysed from the copolymer almost quantitatively by using an excess of potassium trimethylsilanoate in THF.

Finally we embarked on the synthesis of the MIPs, substituting norbornene with the crosslinker dicyclopentadiene essentially following reaction conditions established by Mühlebach *et al.*[8] (Scheme 2). The polymer was obtained as a lightweight, brittle solid after 36 h at 80 °C in a sealed ampoule. The crosslinked network was crushed into small particles, extracted



Scheme 1 Synthesis of ROMP monomer **2-L**. (i) Triphosgene, py, DCM, −70 °C → rt, 12 h. (not isolated) (ii) *exo-N*-hydroxy-7-oxabicyclo-[2.2.1]hept-5-ene-2,3-dicarboximide, NEt_3, DMF, −30 °C → rt, 12 h 76%.

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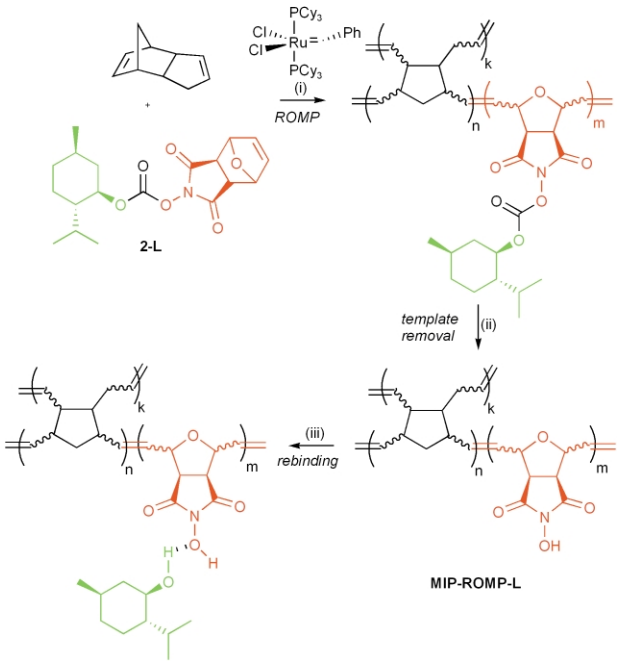
Table 1 Batch equilibration results of **MIP-ROMP-X** for an equimolar mixture of D- and L-menthol[a]

	Supernatant		MIP		D,L-Methanol		Sep. factor
	Menthol		Menthol		Supernat.	MIP	
Polymer	L (%)	D (%)	L (%)	D (%)	[mmol]	[mmol]	α-Value
MIP-ROMP-L	40.1	59.9	58.7	41.3	0.013	0.038	2.1
MIP-ROMP-D	58.7	41.3	40.8	59.2	0.012	0.039	2.1
MIP-ROMP-DL	49.6	50.4	49.4	50.6	0.013	0.038	1.0
Pre-equilibration	49.6	50.4	n/a	n/a	0.051	n/a	n/a

[a] 5.1×10^{-2} mmol of a 1:1 mixture of L- and D-menthol in 10 ml of hexane:chloroform (1:1 (v/v)).



Scheme 2 Synthesis of **MIP-ROMP-L**. (i) 5 mol% **2-L**, 95 mol% dicyclopentadiene, 0.1 mol% Grubbs' catalyst, DCM:2-propanol, 80 °C, 36 h, 99%, (ii) 10 mol eq. NEt_3, 10 mol eq. n-hexylamine, dioxane, rt, 12 h, 93%, (iii) for details see Table 1.

in a Soxhlet extractor and then dried (yield 99%). After lengthy optimisation a 10-fold excess of n-hexylamine and triethylamine allowed us to remove more than 90% (92–94%) of L-menthol (Scheme 2).

Molecular recognition studies were carried out as batch mode equilibrations. Preference was given to this analytical method as it measures the thermodynamic preference of the polymer for its template in line with the thrust of our investigation.[2] The MIP was suspended in a 1:1 mixture of hexane and chloroform containing equimolar amounts of L- and D-menthol. We established that 24 h were sufficient for the polymer to equilibrate fully. The supernatant was collected and the filtered polymer extracted exhaustively with chloroform to recover all menthol. The supernatant and the collected polymer extract were analysed by chiral GC (see Table 1).

It was satisfying to see that the template L-menthol was bound to its polymer preferentially. In fact the final composition on the polymer was 60% of L-menthol and 40% of D-menthol, which is equivalent to an enantiomeric excess (ee) of almost 20%. With about three quarters of the total amount of menthol absorbed by the MIP it is also possible to calculate the separation factor, which is a useful quantity when considering chromatographic investigations.[13] A value of 2.1 indicates that the MIP outperforms many chiral stationary phases.[13] Interestingly, one has to infer that recognition takes place as a result of a single accurately positioned alcohol functionality within a chirally imprinted cavity, since a three point binding model is usually invoked to rationalise chiral discrimination, though in this MIP only a single hydrogen bonding interaction can be present within each cavity.[13]

In earlier work imprinting with a single menthol enantiomer *via* the sol-gel process resulted in non-stereoselective MIPs.[14] Percival *et al.* on the other hand prepared non-covalently L-menthol imprinted MIPs as sensing layer for a quartz crystal microbalance. A three-fold preference for the template molecule was established through separate binding assays so that a direct comparison with our data at this stage is not possible.[15]

To add further proof that the observed chiral discrimination is the result of cavities chirally imprinted by the template, we synthesised two related MIPs, **MIP-ROMP-D** and **MIP-ROMP-DL**. In **MIP-ROMP-D** the template monomer **2-L** was replaced by its enantiomer, **2-D**, and subsequently polymerised in identical fashion to **MIP-ROMP-D**. The other MIP (**MIP-ROMP-DL**) was synthesised in the presence of an equimolar mixture of **2-L** and **2-D**. **MIP-ROMP-D** exhibited the same level of chiral discrimination for its template (D-menthol) had been found for **MIP-ROMP-L**. **MIP-ROMP-DL** on the other hand showed no preference for either L- or D-menthol (Table 1). Both findings are fully consistent with our expectations and demonstrate that chiral recognition is controlled entirely through the presence of chirally imprinted cavities.

We are now investigating reaction conditions and catalysts to influence the selectivity distribution of the cavities found in these MIPs with the thrust of our investigation aimed at reducing polyclonality.

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