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# Imprinted polymers: artificial molecular recognition materials with applications in synthesis and catalysis

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## 1. Introduction

Molecular imprinting, a means by which highly selective recognition sites can be generated in a synthetic polymer, was first formalised as a practical methodology by Wulff and co-workers in 1972[1] and is now an established research area.[2–20] In recent years there has been an almost exponential growth in publications resulting from imprinted polymer research (Fig. 1) which both target the fundamental aspects of these materials and their potential and 'real-world' applications.

This review addresses one specific area of this research, namely the use of imprinted materials in synthetic organic chemistry and considers the advantages that these polymers might offer to the synthetic chemist.

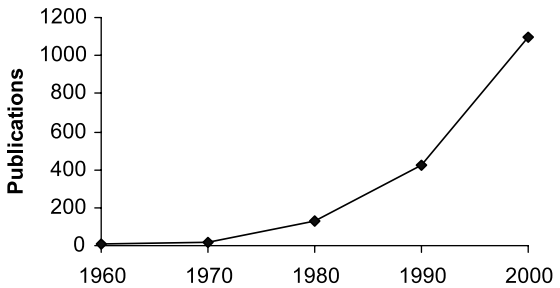


**Figure 1.** Graph to show the number of papers published in molecular imprinting since 1970.

The concept underlying molecular imprinting is the assembly of a cross-linked polymer matrix around templating moieties. Upon removal of the templates, cavities or recognition sites are created which are complementary both in terms of shape and functionality to the original template present in the sites. The binding of functional monomers to templates, and in the recognition sites following template removal, can be effected by either covalent or non-covalent interactions (or combinations of both), thereby allowing

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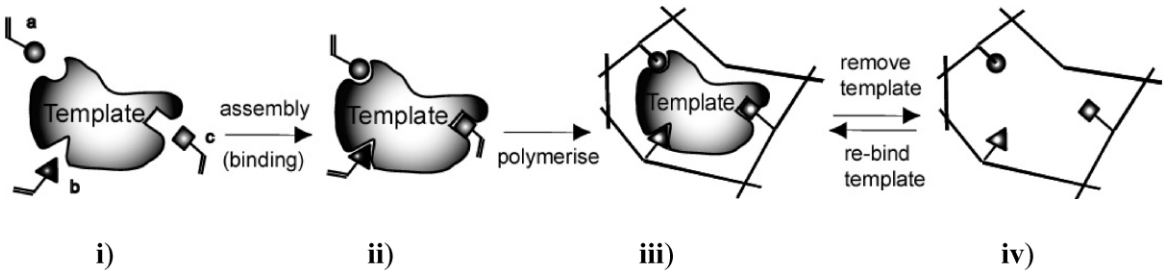



**Scheme 1.** Schematic diagram of the molecular imprinting process: (i) the template is mixed with vinyl monomers, selected to interact with specific functionality of the template, (ii) the template-monomer complex may be formed by covalent or non-covalent associations (or a mixture of both), (iii) the complex is co-polymerised with an excess of cross-linking monomer; ethylene glycol dimethacrylate (EGDMA) or divinylbenzene (DVB) typically being used whilst the inclusion of a small amount of solvent ensures that the polymer structure is porous, allowing access to the sites within the polymer monolith, and (iv) the polymer is usually ground to a powder for ease of handling and the template removed by solvent extraction or chemical treatment. The sites created in the polymer are complementary in shape to the template and bear the functionality originally involved in complex formation, precisely arranged to interact with the template on rebinding.

considerable flexibility in the choice of monomers and the types of templates that can be imprinted. To date, polymers have been produced for recognising templates as diverse as sugars,[21] peptides,[22,23] nucleotides,[24] proteins,[25] crystals[26] and even whole cells.[27,28]

The process by which molecularly imprinted polymers (MIPs) are prepared is shown in Scheme 1.

Imprinted materials can be considered as analogues to enzymes in that the binding sites are constrained by a three-dimensional scaffold, contain oriented functional groups and can only accommodate guest molecules that fit closely within the cavities. Additionally, MIPs can utilise the same types of molecular interactions present in biological systems such as ionic and hydrophobic interactions, directional hydrogen bonds or metal coordination further enhancing their desirable properties. There are, however, significant fundamental differences to natural macromolecular networks in that MIPs are highly cross-linked rigid matrices, and are extremely tolerant to conditions that denature most proteins and biopolymers, such as high temperatures, reactive chemicals and organic solvents. The flexibility inherent in synthetic chemistry further allows an element of 'design' to feature in the preparation of imprinted polymers, enabling materials with varying selectivities and formats to be produced.

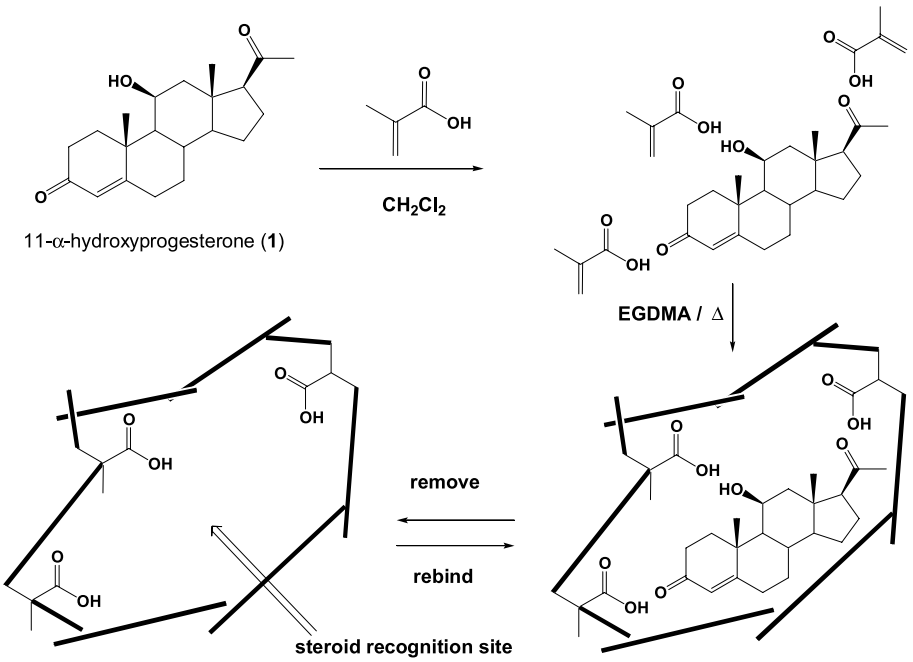
Whilst this field has, to date, been explored most extensively by academic research groups, an increasing number of fine chemicals and pharmaceutical-based industries are starting to address the potential of molecular imprinted polymers in commercial processes. Examples of industrial studies include the use of imprinted polymers as supports for chiral chromatographic separation[29–33] and as recognition elements capable of binding ligands with specificities close to those of natural polymers such as antibodies.[34,35] Imprinting methodologies are sufficiently versatile that new polymers are being developed for applications beyond 'traditional' uses such as chiral resolution and chromatographic separations which exploit other aspects of the polymeric materials in addition to the key molecular recognition elements. Of special note in preparative organic chemistry are the applications of these materials in synthesis, where the imprinted polymers can act as heterogeneous scavengers or clean-up aids, as passive or reactive supports, as 'protecting groups' and perhaps most importantly, as catalysts. This review features key examples from these aspects of molecular imprinting research, and considers recent developments that are exploiting imprinted polymers across the whole field of synthetic chemistry.

## 2. Imprinted polymers as synthetic aids

The enormous growth in combinatorial chemistry and parallel synthesis for drug discovery[36] and in academic laboratories[37] has led to an increased demand for rapid and efficient reaction clean-up methodologies, such that potential lead compounds can be separated from impurities or side-products prior to high-throughput screening.[38] In addition, the screening process has been subjected to intense development, as the number and complexity of the materials produced from combinatorial libraries has increased. Imprinted polymers offer significant potential for both separation and screening applications as a result of their tailored recognition specificity and robustness.

The first example in this area was the use of MIPs for screening a combinatorial steroid library,[39] wherein polymers were imprinted (see Scheme 2) with two androstene-3-one derivatives, 11-α-hydroxyprogesterone (**1**) and corticosterone (**2**).

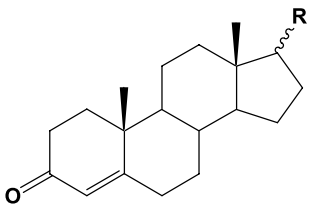
Methacrylic acid was used as a functional monomer and pre-assembly with the templates followed by cross-linking with ethylene glycol dimethacrylate (EGDMA) in dichloromethane afforded the imprinted polymers which were subsequently ground to a fine powder, sieved and washed with acetone to remove the templates. The polymers were then packed in HPLC columns and screened against a library of 12 closely related $\Delta^4$-androsten-3-one derivatives, (see Table 1) differing at positions 1, 11 and 17 (including the steroid side chains). The imprinted artificial androstenone receptors showed sharp discrimination for the templates: for the 11α-hydroxyprogesterone imprinted polymers, the template was readily distinguished from the 11β-isomer and the 17α-isomer in chromatography. This polymer also separated 11α-hydroxyprogesterone from corticosterone and cortisone, which were more strongly retained by control polymers of the same composition but prepared in the absence of template. Additionally, the




**Scheme 2.** Non-covalent imprinting of 11α-hydroxyprogesterone with methacrylic acid cross-linked with EGDMA to leave recognition sites used to screen binding of steroids (see Table 1).

corticosterone-MIP was able to separate its template from cortisone and 11-deoxycortisol, which was not observed for the control polymers. The binding specificities of these polymers were reported to arise from differences in the functional group orientations in the imprinted sites and their interactions with complementary functionality on the templates as the positioning and orientation of the 11-hydroxyl group and the side chain 21-hydroxyl moiety were critical to ligand binding. This work suggests that MIPs might be used as synthetic aids for screening drug libraries by mapping out chemical space in receptor sites, which would be of particular use where natural receptors are either not known or are poorly characterized.

**Table 1**.



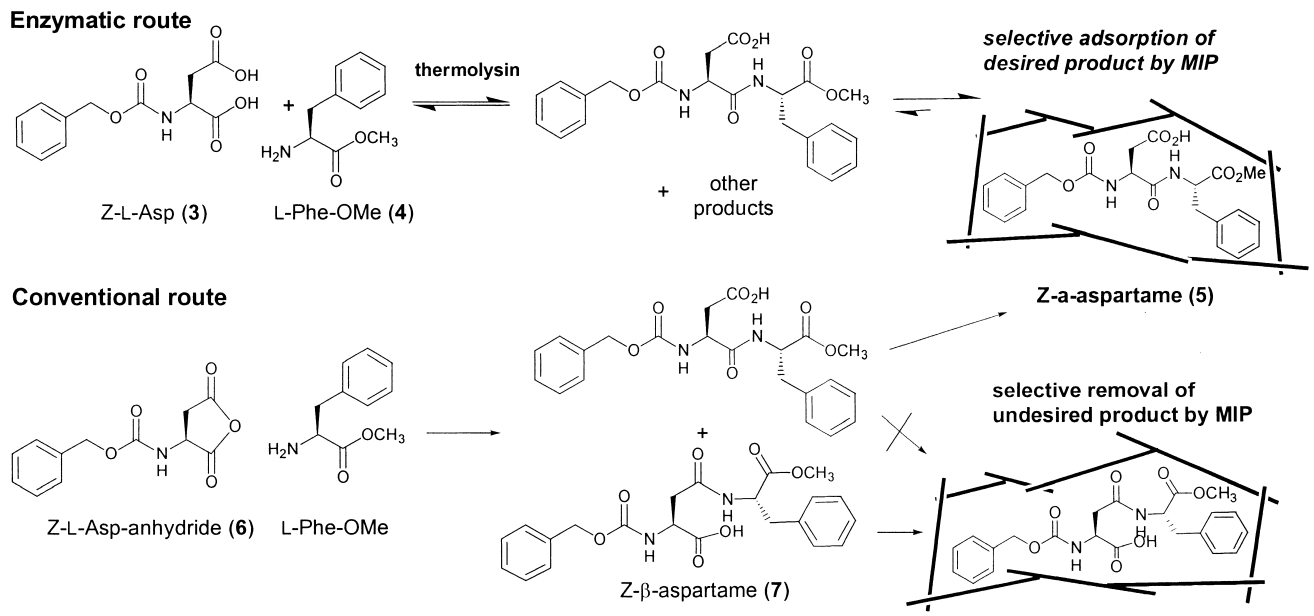

| Steroid | R | Other functionality |
|---|---|---|
| 11α-Hydroxyprogesterone (**1**) | $COCH_3$ | 11α-OH |
| 11β-Hydroxyprogesterone | $COCH_3$ | 11β-OH |
| 17a-Hydroxyprogesterone | $COCH_3$ | 17α -OH |
| Progesterone | $COCH_3$ | |
| 4-Androsten-3,17-dione | =O | |
| 1,4-Androstadiene-3,17-dione | =O | $\Delta^1$ |
| Corticosterone (**2**) | $COCH_2OH$ | 11β-OH |
| Cortexone | $COCH_2OH$ | |
| 11-Deoxycortisol | $COCH_2OH$ | 17α-OH |
| Cortisone | $COCH_2OH$ | 11=O, 17α-OH |
| Cortisone 21-acetate | $COCH_2OAc$ | 11=O, 17α-OH |
| Cortisol 21-acetate | $COCH_2OAc$ | 11β-OH, 17α-OH |

A second example in which MIPs have been used as auxiliaries in a synthetic procedure is in equilibrium shifting[40] and by-product removal[41] during biocatalytic or chemical reactions. The condensation of benzyloxycarbonyl-L-aspartic acid (*Z*-L-Asp (**3**)) with L-phenylalanine-methyl ester (L-Phe-OMe, (**4**)) to generate Z-protected α-aspartame (**5**) was carried out using the enzyme thermolysin, but the product yields were found to be low under standard conditions. The use of a *Z*-L-Asp imprinted polymer to trap the product as it formed enabled yields of up to 63% to be obtained (Scheme 3).

A related polymer was able to extract selectively the *Z*-β-isomer from the crude product mixtures produced during the conventional chemical synthesis of aspartame, which was carried out by ring-opening *N*-protected L-aspartic anhydride (**6**) with L-phenylalanine methyl ester. Whilst the enzymatic route forms only the α-isomer, the chemical route generates both α- and β-isomers of which the by-product *N*-(benzyloxycarbonyl)-β-L-aspartyl-L-phenyl-alanine methyl ester (β-L,L-ZAPM) (**7**) has to be removed. Polymers imprinted against *Z*-β-aspartame were employed as solid-phase extraction media for product purification, enabling an increase in product purity from 59 to 96% and, in comparison, a non-imprinted polymer afforded a final purity of only 86%.[42] It is anticipated that both these applications of imprinted polymers will be utilised in industrial syntheses where product yield and purity are of key importance in establishing commercial viability.

### 2.1. 'Microreactors' for chiral synthesis

Polymers imprinted with chiral or prochiral templates should afford recognition sites that are intrinsically asymmetric, and these cavities can be considered to be similar to the binding domains of enzymes, although more



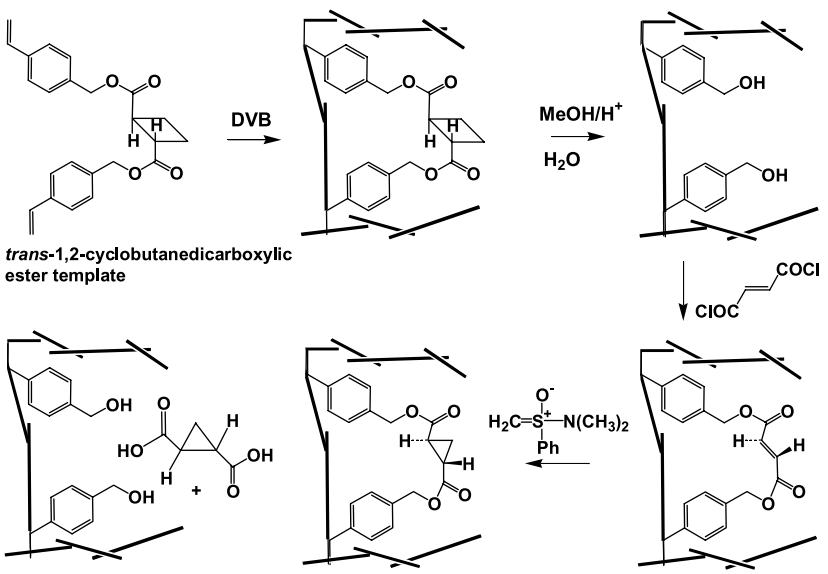

**Scheme 3.** Imprinting of aspartame derivatives: MIP prepared from Z-L-Asp template (**5**) used to trap selectively the desired product from thermolysin-catalysed condensation of the reagents (**3**) and (**4**). A related polymer was used to trap the β-isomer from conventional chemical synthesis to effect purification.

conformationally restrained. The analogy with enzymatic binding sites is most striking where these cavities have been used for catalysis (vide infra), but the exact location and orientation of functional groups within the sites has also been explored for more 'conventional' syntheses. In particular, the spatial definition and directionality of chemical groupings offer the potential for selective and asymmetric synthesis within the imprinted cavities. The first examples in this area were described by Damen and Neckers[43] and, subsequently, by Shea and co-workers.[44] The latter study involved an ingenious investigation of the chemistry of imprinted polymers via esterification with fumaryl chloride of the hydroxyl groups formed in the sites following template hydrolysis and subsequent reaction of the C=C bond with dimethylaminophenyloxosulfonium methylide as a nucleophilic methylene transfer reagent (Scheme 4).

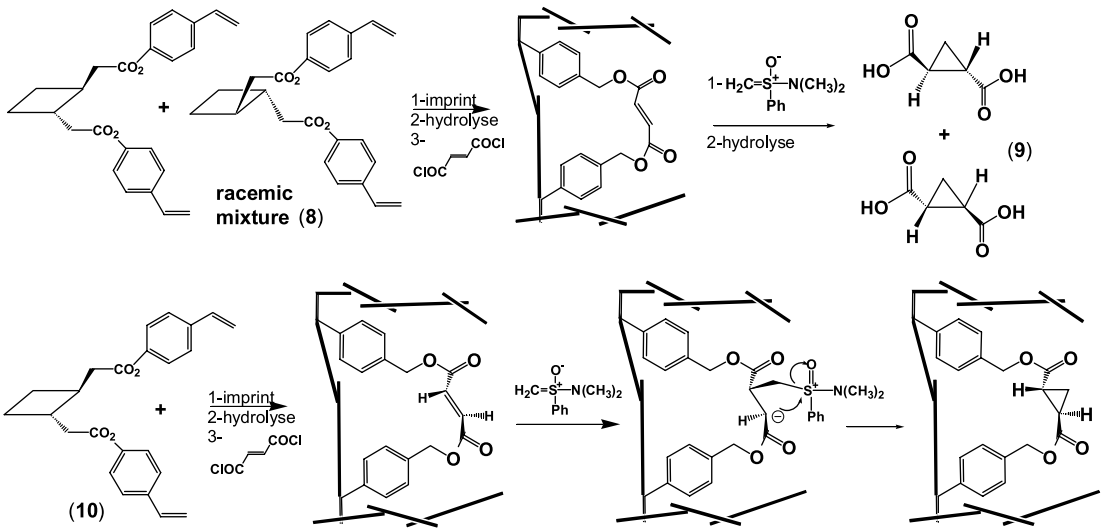
The use of a racemic *trans*-1,2-cyclobutanedicarboxylic ester (**8**) during the imprinting step furnished racemic cyclopropanedicarboxylic acid (**9**), whereas imprinting with the (−)-*trans*-isomer (**10**) gave an increase in enantiomeric purity of the recovered cyclopropane derivative (Scheme 5).

Although the enantiomeric excess induced by the imprinted site was low (0.05%), the results demonstrated that stereochemically defined products could be prepared by this methodology and that the polymer retained a 'chiral memory' for the template.

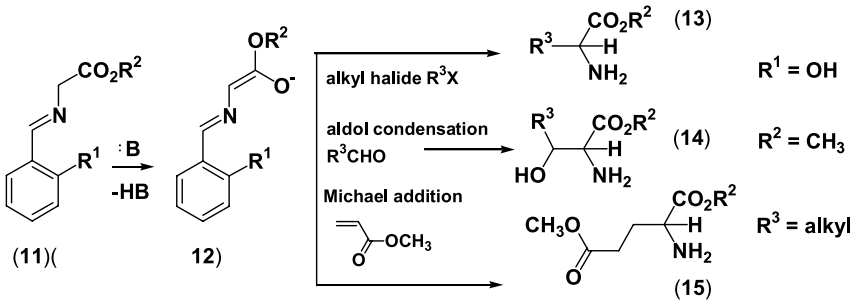
Subsequent to these ground-breaking studies, synthetic transformations including enantioselective protonation–deprotonation[45] and selective hydrolysis[46] were also conducted in imprinted polymer binding sites. A particularly elegant study of the potential of these cavities in syntheses



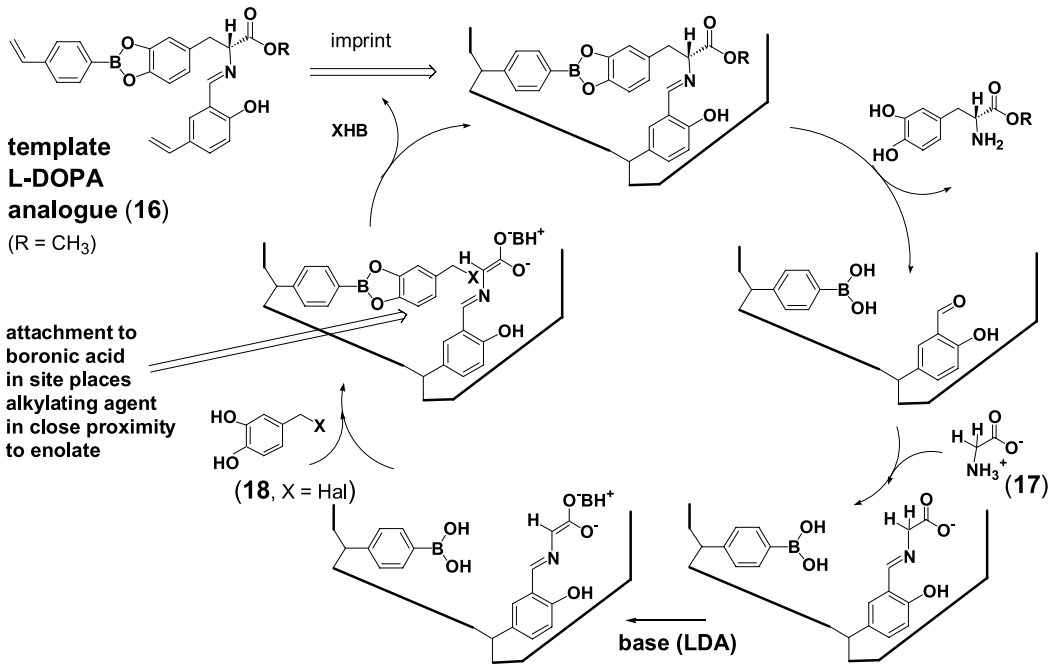

**Scheme 4.** Imprinted polymer microreactors for the synthesis of cyclopropane dicarboxylic acids.




**Scheme 5.** Stereochemical consequences of imprinting racemic cyclobutanedicarboxylic esters (**8**), followed by template removal and reaction of fumaryl chloride in the imprinted sites. Alkylation with dimethylaminophenyloxosulfonium methylide generates the cyclopropane derivatives (**9**). The relative yields of the diastereomers is altered by imprinting with the enantiopure template (**10**).




**Scheme 6.** Scheme set out by Wulff[48] for deprotonation of the Schiff base derivative (**11**) to (**12**) for alkylation to form the amino acid derivatives (**13**–**15**).




**Scheme 7.** Alkylation in an imprinted microreactor. The template (**16**) set up a chiral cavity to enable the alkylation of a deprotonated glycine (**17**) by benzyl halide (**18**) in a stereodirected manner.

was reported by Wulff and co-workers, who evaluated C–C bond formation in a biomimetic synthesis of α-amino acids from glycine.[47,48] The key to this strategy was a glycine-Schiff base (**11**), which, after deprotonation to the enolate (**12**) was accessible to alkylation, yielding the substituted amino acids (**13**), (**14**) and (**15**) in which the stereochemistry of the final products would be determined by the approach of the alkylating agents (Scheme 6).

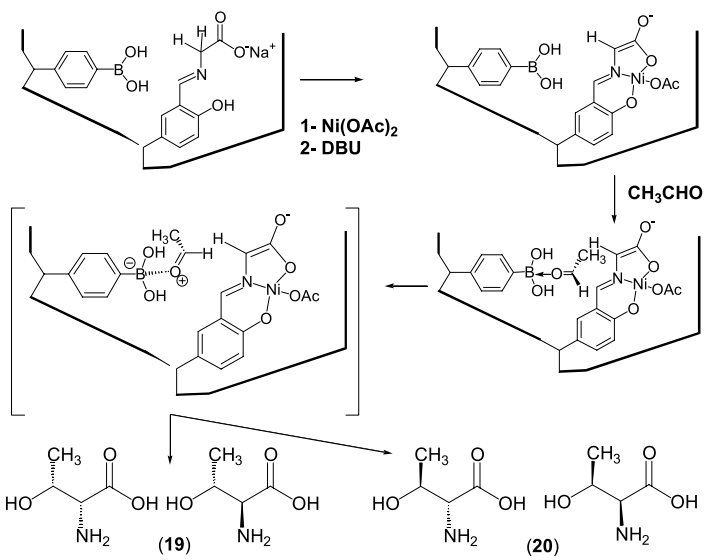
The substituted glycine fragment (**11**) was introduced into the imprinted binding cavity as a polymerisable L-DOPA derivative (**16**), wherein the amino acid part was anchored via an azomethine linkage whilst the 1,2-dihydroxyaromatic side chain was fixed as a boronate ester. In this way, the alkylation site of the substituted glycine derivative was oriented within the chiral recognition site by two points of attachment. Co-polymerisation of (**16**) with divinylbenzene (DVB) or EGDMA, followed by template removal from the resultant polymers, generated binding sites with the corresponding two points of attachment for amino acids, their derivatives or precursors. Subsequent alkylation on the template molecule was proposed to follow Scheme 7.

Glycine (**17**) was reacted with the polymer-bound aldehyde in the imprinted sites and lithium diisopropylamide (LDA) was added to deprotonate the Schiff base to yield an ester enolate in which the enantiotopic methylene protons were rendered diastereotopic in the cavities. The polymer-bound boronic acid was esterified with a dihydroxybenzylic halide (**18**) placing an alkylating agent positioned and oriented to react with the glycine ester enolate in a stereospecific manner. In effect, the imprinting process established a chiral environment that directed the alkylation in an asymmetric fashion. Whilst the stability of 3,4-dihydroxybenzyl halides as alkylating agents proved too low to establish the practical efficacy of this particular synthesis, the same imprinted polymer was used to carry out an aldol condensation with a measured enantiomeric excess of 36% (Scheme 8). Coordination of the glycine ester enolate at a second point in the site required the addition of $Ni^{2+}$, which prevented rotation about the C–N single bond prior to a deprotonation step with DBU. Approach of the electrophile, in this example acetaldehyde, generated the diasteromers, threonine and allo-threonine (**19**, **20**), and the asymmetric induction was aided further by coordination of the approaching acetaldehyde to electron-deficient boron in the polymer-bound boronic acid residues in addition to the orientation of the attached glycine enolate in the cavities.

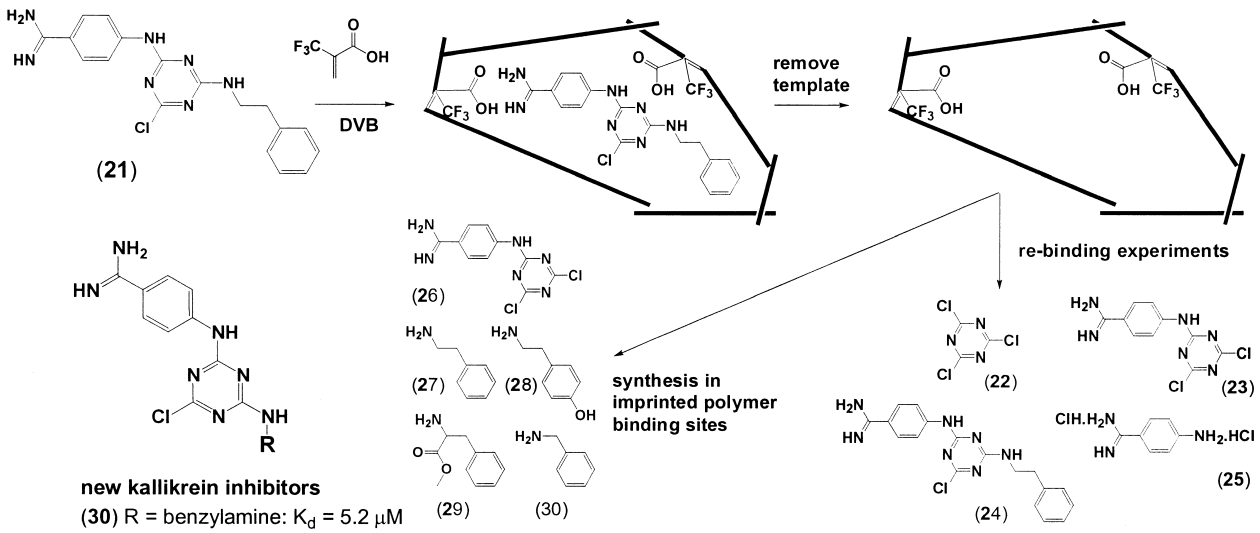
Careful control experiments discounted the possibility that enantioselective binding of the reaction products effected the observed enantiomeric excess, the template, D,L-DOPA, being resolved by the imprinted polymer, whereas no resolution of D,L-threonine was obtained. The observed product ratio suggested that the incoming acetaldehyde was orientated at the *Si*-face of the enolate, with the transition state constrained into a specific conformation by the inherent asymmetry of the imprinted cavity.

The above examples illustrate that the binding site geometry can be used to influence the stereochemical course of a reaction in which one or more reagents and products are covalently bound within the polymer. A number of other methods have been developed whereby the reactive species can be brought together only if there is suitable 'fit' in the site, relying on reagent approach rather than covalent binding interactions in the imprints, thus enhancing the kinetics and facilitating product recovery from the polymer.

Mosbach and co-workers have taken this concept recently into the arena of medicinal chemistry, using imprinted polymer binding sites to mimic those of the enzyme kallikrein and through this functional and spatial similarity to prepare new inhibitors for the enzyme.[49] Kallikrein is a medicinally important proteinase and is inhibited by compounds that mimic the Phe-Arg peptides known to bind to the S2–S1 pocket of the enzyme active site. The inhibitor (**21**) containing a guanidine headgroup and a hydrophobic benzylic tail was used as a template to establish MIP binding sites with structural similarity to

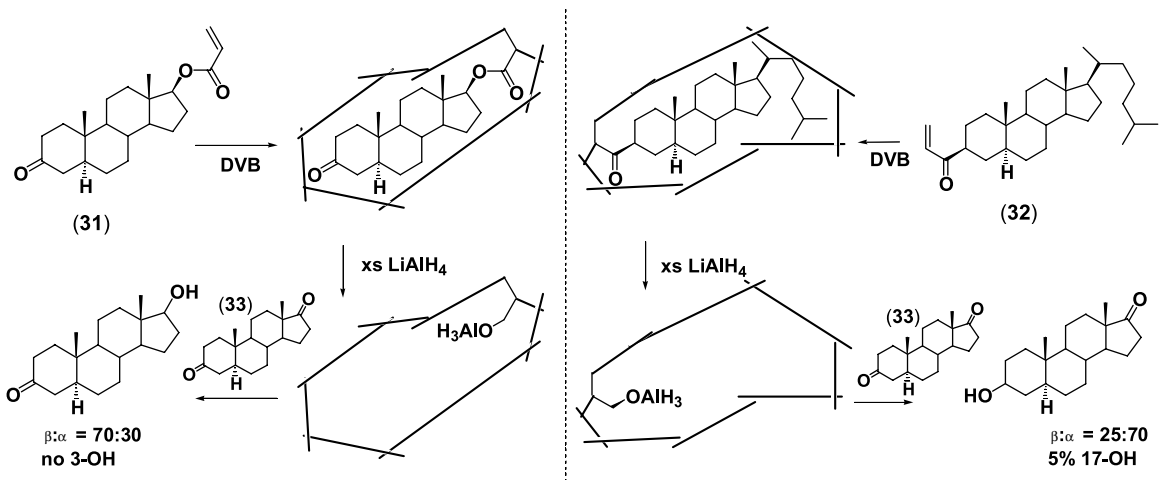



**Scheme 8.** Proposed mechanism for asymmetric induction during $Ni^{2+}$-assisted aldol condensation in the imprinted sites. The approach of acetaldehyde is facilitated by coordination to the electron-deficient boron, whilst the chelation of $Ni^{2+}$ by glycine enolate prevented rotation about the N–C bond.




**Scheme 9.** The anti-idiotypic approach to new enzyme inhibitors.[49] Imprinting of the known kallikrein inhibitor (**21**) resulted in a MIP binding site which was used to direct the synthesis from precursors based on the triazine derivative (**26**) and the alkyl amines (**27**–**30**). The new inhibitor (**30**) was found to bind to kallikrein with a similar $K_d$ as the original template.

kallikrein S2–S1 pockets (Scheme 9). The functional monomer trifluoromethylacrylic acid and the cross-linker DVB were chosen in order to establish acid–base and hydrophobic π–π interactions in the cavities and the resultant polymers were evaluated for binding of the template and of structural homologues with specific molecular 'deletions' corresponding to binding functionality. The affinity for homologues (**22**–**25**) lacking either or both the guanidine or hydrophobic domains was reduced relative to the original template, indicating that cooperative interactions were required for strong binding and for establishing the veracity of imprinted cavity functionality and geometry. The next stage involved the assembly of individual molecular building blocks within the site, such that the directional binding of components would place functional groups in close proximity to react, resulting in the generation of molecules with a close resemblance to the original template. This 'double imprinting' methodology is analogous to the generation of secondary anti-idiotypic antibodies wherein the combining site of the secondary antibody bears a strong resemblance to the antigen to the primary antibody. Kallikrein MIP anti-idiotypes were thus prepared by binding a terminal reactive headgroup, 2-(4-amidinophenylamino)-4,6-dichloro-*s*-triazine, to imprinted and control polymers, and then adding the arylalkylamines (**26**–**30**) of varying structure and hydrophobicity. Once the reactions had attained complete conversion, acetic acid was added to liberate the products from the polymers. The formation of the original template from the components in the sites using phenylethylamine as the alkylarylamine was more efficient in the MIP than in the control polymer (by a factor of 4), whereas the same reaction in solution did not take place under the same conditions of temperature and reagent concentration. Significantly, however, all of the products released from the imprinted sites following templated assembly demonstrated inhibition of kallikrein and compound (**30**) (R=benzylamine), which had not previously been reported as a kallikrein inhibitor, bound the enzyme with an affinity extremely close to that of the original template. This 'anti-idiotypic' approach therefore represents an attractive route to the generation of new pharmaceutical candidates, as a known inhibitor can be used




**Scheme 10.** Steroid acrylates (**31**, **32**) imprinted to generate cavities which, on reductive template removal with $LiAlH_4$, were used for the selective reduction of androstan-3,17-dione (**33**). The reaction products, α,β-hydroxyketones were produced with regio- and stereoselectivity defined by the templates.

to generate a MIP which can then be utilised as both reactor and screen for steric and functional mimics of the inhibitor template. Such an approach is especially attractive where the native enzyme is poorly characterized and/or difficult to express, purify and isolate for biomedical studies.

### 2.2. Imprinted polymer supported reagents

Chiral and polymer supported reagents are well-established in organic chemistry[50,51] primarily for ease of product separation and purification, and the use of 'reactive MIPs' is a logical extension of this concept. Imprinted polymer reagents offer the further advantage of selective substrate or reagent access to or within a site, giving kinetic or stereochemical control over processes that might be difficult to accomplish by other means. In some respects, reactive MIPs are analogous to the well-established supported reagents and chiral catalysts such as binaphthylphosphine metal complexes and alkylborohydrides, but have the additional ability to be produced, in theory at least, for almost any reaction of interest, and the resulting imprinted sites can have 'bespoke' geometries, rather than depending on the 'chiral pool' of accessible reagents.

One of the first examples of imprinted polymer reagents was published by Byström and co-workers[52] Androstane- and cholestane-based sterols were imprinted via their 3-, or 17-acrylates (**31**, **32**) into polymer beads and the templates removed by reductive cleavage with $LiAlH_4$ (Scheme 10). Excess hydride converted the hydroxyl groups in the sites to an aluminohydride functionality, positioned where the sterols had been linked to the backbone. The resultant polymer-supported reagents were then used to reduce androstan-3,17-dione (**33**) and cholestan-3-one.

Polymer beads imprinted with the androstane-17-acrylate (**31**) reduced only the 17- position of the diketone, whereas in the absence of polymer there was almost complete preference for reduction at C-3, as this is the least hindered to the approach of hydride. The reaction was 'switched' using the cholestane-3-acrylate (**32**) imprinted polymers, which reduced androstan-3,17-dione (**33**) at C-3 in preference to C17 (85:15 ratio). In each case, the use of the sterically defined imprinted site allowed access only of the correctly fitting template such that the steroid could come into close enough proximity to the reducing agent to react. Stereochemical control was also exhibited by the cholestane-3-acrylate MIP; the addition of cholestan-3-one to this polymer yielding predominantly the 3α-isomer (72:28 ratio), whereas via solution chemistry a 9-fold excess of 3β- to 3α-OH was obtained. Once again, the approach of the steroid to the polymer-bound alumino-hydride governed the outcome of the reaction: reduction took place from the 'β-face' via a β-template imprinted site i.e above the ring to generate an α-hydroxyl function on the introduced steroid.

It should also be noted that the polymer matrix was an important factor in the relative product yields. In this study, polymer beads were prepared via suspension methods in the absence of a porogen. The resultant beads demonstrated poor swelling characteristics in contrast to standard MIPs, and proved subsequently to be the optimal polymeric reagent in the reduction of steroidal ketones with regio- and stereoselectivity. Conventional 'bulk' polymers were reported to be inferior, presumably as a result of solvent-induced swelling and loss of conformational and stereo-chemical information in the imprinted sites. These results suggest that the properties of the polymer backbone must also be considered when designing and evaluating reactive or catalytic MIPs, especially where well-defined 3-D orientation of functionality is required.

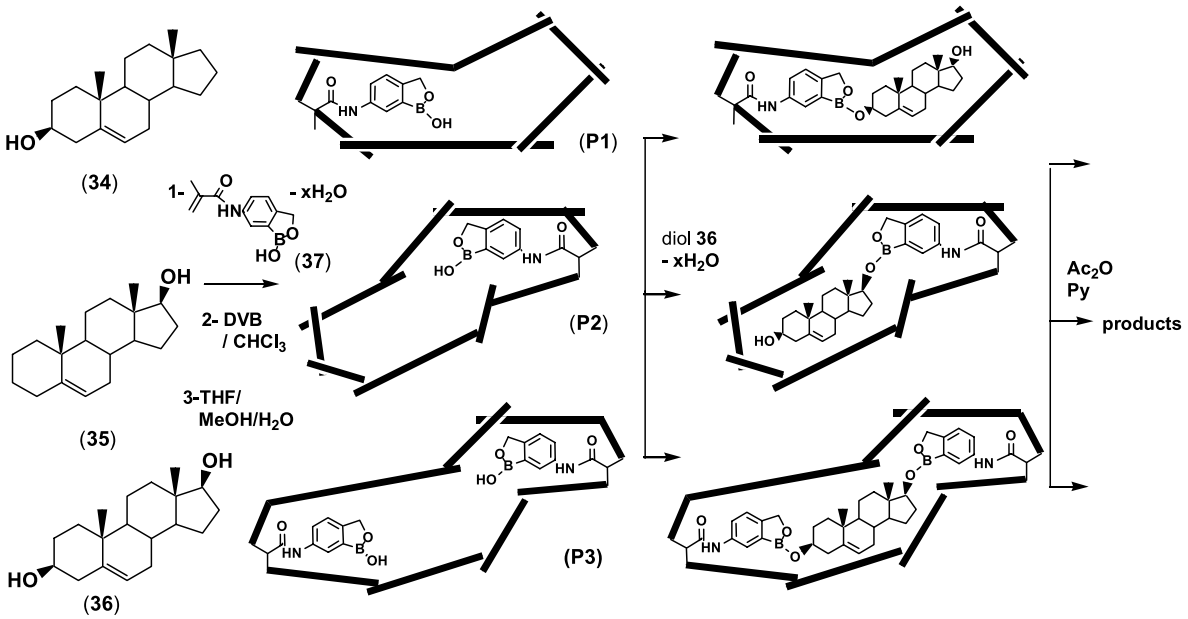
### 2.3. Imprinted polymers and protecting group strategies

Polymers have been used as protecting groups in both a soluble and solid phase format for a large number of synthetic transformations in organic chemistry,[37,53–57] and the stereochemically-defined binding sites of imprinted polymers are of increasing interest as enhancements to conventional protection strategies. Hamase et al.[58] employed polymers imprinted with L-phenylaniline anilide as protecting groups during derivatisation of D- or L-phenylalanine anilide with a fluorescent label, dansyl chloride. This reaction generated considerably less (46%) of the L-isomer than the D-analogue, suggesting that the L-form was bound in preference and was therefore less available to react with the label in solution.

Another approach to stereo- and/or regioselective protection is to react ligands bound by particular residues in a site whilst other functional groups are free as a consequence of their positioning in the molecule relative to those used to attach to the imprint site. Steroidal hydroxyl groups have been investigated for regioselective acylation employing this concept, as these compounds are of medicinal importance and also because conventional steroid chemistry in solution can involve multiple orthogonal protection strategies and is thus frequently complex and time-consuming. The acylation of di- and trihydroxysteroids bound to polymers imprinted with structurally related diols was investigated by Alexander et al.[59] Hydroxysteroids based upon androst-5-ene (**34**–**36**) were esterified with a polymerisable boronophthalide (**37**) employing DVB as the cross-linker and chloroform as an aprotic porogen (Scheme 11). Template removal generated the polymers (**P1**–**P3**) with either one or two boronophthalide groups to which hydroxyl functionality in the steroids could subsequently bind in the imprinted sites.

Androst-5-ene-3β,17β-diol (**36**) was then reacted with each of the polymers (**P1**–**P3**) under dehydrating conditions and the non-covalently bound steroid was removed by an aprotic solvent wash. Reflux of the polymer-bound steroid with acetic anhydride and pyridine under conditions previously shown to acylate both the 3- and 17-hydroxyl groups in solution was carried out, the polymers were washed again with aprotic solvents to remove the unbound steroids, and the reaction products were liberated from the polymers by hydrolysis with aqueous methanol.

TLC analysis of the products indicated that the esterification product of androst-5-ene-3β,17β-diol bound to polymer (**P1**) (androst-5-ene-3β-ol imprinted) was androst-5-ene-3β-ol,17β-acetate, that of the steroid diol bound to polymer



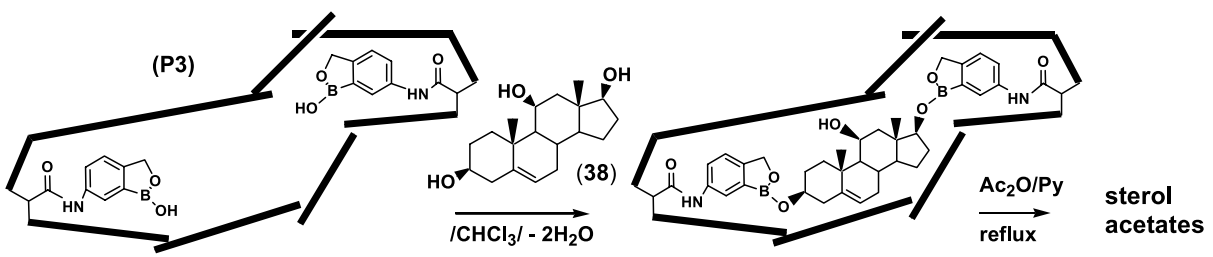

**Scheme 11.** Imprinted polymer 'protecting groups' 1. Androst-5-ene derivatives (**34**–**36**) were polymerised as esters of boronophthalide (**37**) to leave hydroxyl-binding groups in the imprinted cavities of the polymers (**P1**–**P3**). The reaction of these polymers with androst-5-ene-3β,17β-diol resulted in acylation of the diol on polymers (**P1**, **P2**) but 'protection' of the diol on its own polymer (**P3**).

(**P2**) (androst-5-ene-17β-ol imprinted) was androst-5-ene-3β-acetate,17β-ol, whilst the diol bound to its own polymer (**P3**) was protected from acylation to the extent that <3% of the recovered material was esterified. Quantitation of the monoester products was not possible owing to the lack of baseline resolution in GCMS, but it was nevertheless apparent that the diol-imprinted polymer was able to protect both hydroxyl groups against esterification by virtue of their fit in the polymeric recognition site. In addition, the capacity of this polymer was greater, with twice as much sterol recovered from the diol imprints compared to the mono-hydroxysterol imprinted polymers, indicating a better match of the diol to its own imprints.

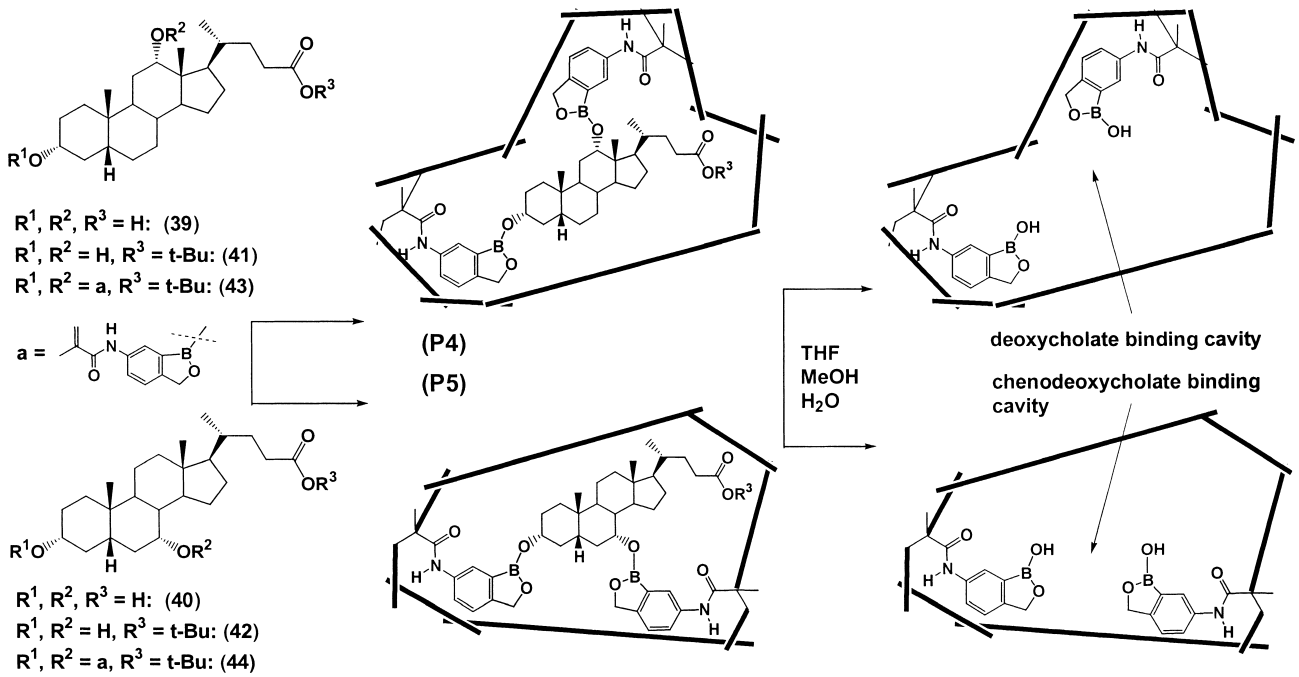
The synthetic utility of this strategy was probed by refluxing the polymer (**P3**) with the trihydroxysteroid androst-5-ene-3β,11β,17β-triol (**38**), and carrying out esterification with acetic anhydride as shown in Scheme 12.

Although the aqueous methanol wash in this example was predominantly the unmodified triol (90%), the $^1$H NMR spectra indicated the formation of a small amount (5–10% by integration) of the 11β-acetoxy product. This low extent of acylation at the 11β-hydroxyl group was most probably as a result of severe steric hindrance of the angular methyl groups at C10 and C13 and access of the *N*-acylpyridinium intermediate would have to take place from 'below' the steroid, which would have been further restricted by the cross-linked polymer backbone. No evidence for modification at the 17β-position was obtained and the ratio of 11β- to 3β-acetoxy products was at least 1:1 by integration, whereas acetylation of the same steroid in the absence of any polymer rapidly gave the 3β,17β-diacetate and more severe conditions were required to achieve complete acylation of all three hydroxyl groups.

Further studies of this protection/acylation strategy were carried out on steroids derived from deoxycholic acid and chenodeoxycholic acid (**39**, **40**), *t*-butyldeoxycholate and *t*-butylchenodeoxycholate (**41**, **42**). These polymers were imprinted as boronophthalide esters at the 3α- and 7α-, or 3α- and 12α- positions, respectively (**43**, **44**). The t-butyl ester group protected the acid functionality on the templates, thus preventing the formation of mixed boronic acid anhydrides at the imprinting stage, and provided steric bulk around which the polymer scaffolds (**P4**, **P5**) were built (Scheme 13). This was designed to create an extra pocket within the imprint site to facilitate access of the reagents to template derivatives bound within the cavities. In this way, two 'isomeric' polymers were prepared, differing only in the position and orientation of the boronophthalide groups in the imprint sites.

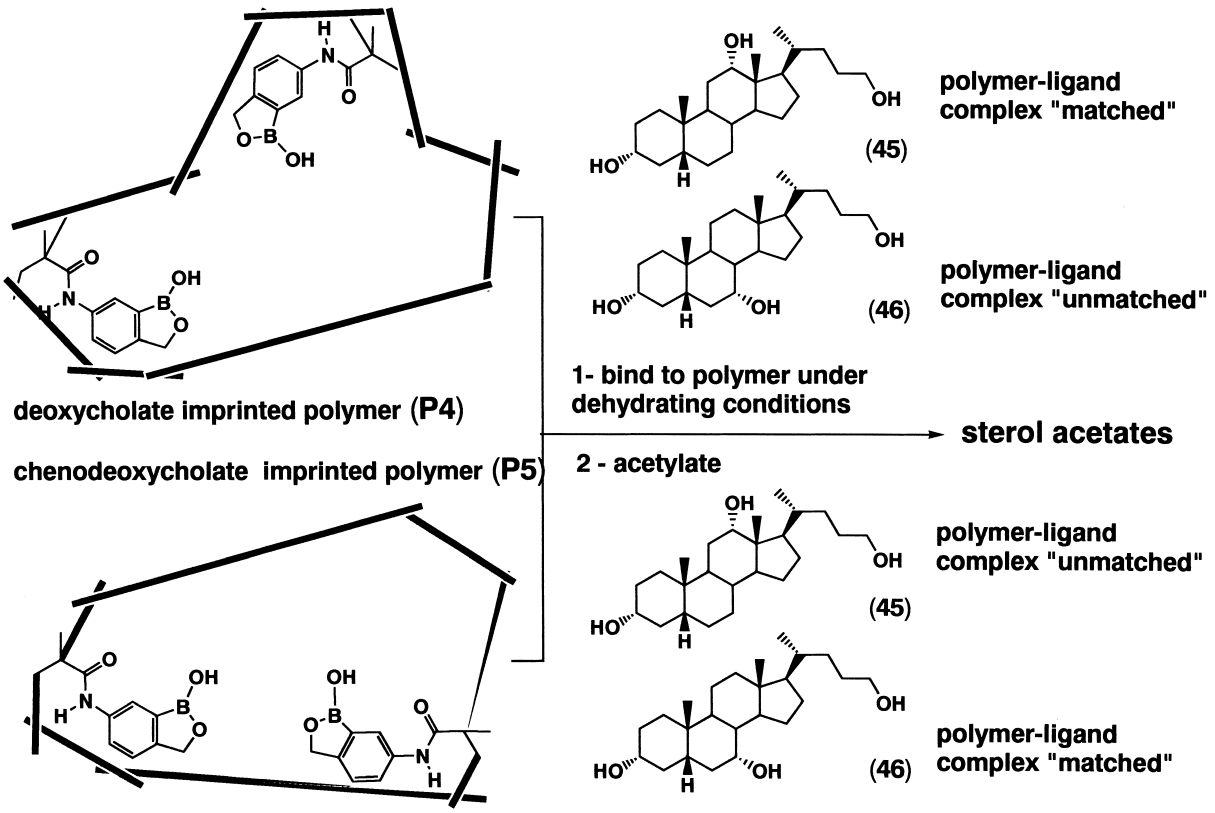



**Scheme 12.** Acylation of androst-5-ene-3β,11β,17β-triol (**38**) on androst-5-ene-3β,17β-diol polymer (**P3**): reaction at the hindered 11β-position took place on the polymer-bound steroid more readily than at C3 and C17 in contrast to solution acylation.




**Scheme 13.** Imprinted polymers prepared from cholic acid derivatives (**43**, **44**). 'Isomeric' polymers (**P4**) and (**P5**) contained cavities predisposed to bind deoxycholate and chenodeoxycholate sterols.

These polymers were employed subsequently as protective cavities for regioselective modification of hydroxysteroids. The isomeric trihydroxysterols (**45**) and (**46**) were prepared by reduction of deoxycholic acid and chenodeoxycholic acid, respectively, with lithium aluminium hydride, and rebound to each of the isomeric polymers (**P4**) and (**P5**) (Scheme 14). In this way, template derivatives were bound to the polymers either in a 'matched' fashion, i.e. by the 3α and 12α position of deoxycholan-24-ol to (**P4**) and the 3α and 7α hydroxyls of chendeoxycholan-24-ol to (**P5**), or in an 'unmatched' manner for deoxycholan-24-ol bound to (**P5**) and chendeoxycholan-24-ol bound to (**P4**). For the




**Scheme 14.** Imprinted polymer protecting groups 2. Isomeric trihydroxysterols (**45**, **46**) bound to polymers (**P4**, **P5**) in a matched or unmatched arrangement. Acylation patterns for hydroxyl groups not involved in binding interactions were dependent on the ligand-polymer 'fit'.

matched polymer–ligand species, the residual hydroxyl group at C24 was predicted to be accessible to acetylation, whereas, for the unmatched systems, a different pattern of acylated products was expected.

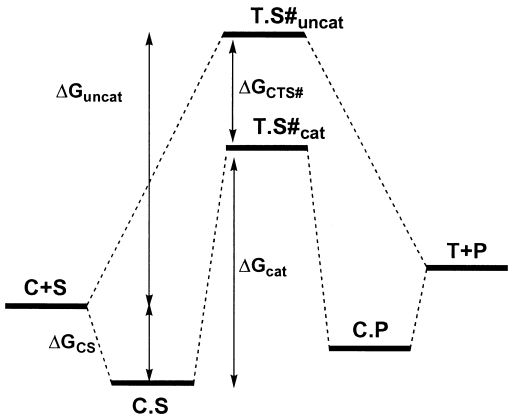
The results of these experiments were in agreement with the predictions, in that when the matched sterols were bound to their corresponding polymers, acetylation at the 24-position was favoured by up to 23.1:1, whereas for the unmatched ligand binding to the same polymers, this ratio fell to 5.4:1. Although complete regioselectivity was not achieved, the product distribution nevertheless indicated a good fit of the matched sterols in the sites, with attachment of the ligands to the polymers via their 3α-position favoured, even though the first rebinding event might be expected to be by the more reactive 24-hydroxyl (see Scheme 14). Indeed, even for the unmatched sterol–polymer systems, the acylation pattern suggested a close overall shape-match of the cavities to the sterol skeleton, in which a proportion of ligand bound via the 3α-hydroxyl, leaving C24 accessible to the acetylating species. In contrast, when the same trihydroxysterol was modified on a polymer imprinted with ethylene glycol, acetylation was directed almost exclusively to the 3α-OH, indicating that the sterol could attach only via its most accessible and reactive primary alcohol group at C-24. The formation of boronophthalide esters in the sites involved the loss of water and it is therefore possible that, in the restricted cavities, multiple binding and hydrolysis cycles took place until the sterols reached their optimal fit, i.e. matched or in the case of the much smaller ethylene glycol imprint, by the side chain primary hydroxyl. The presence of a 'sterol-shaped' cavity in the polymer determined the optimal fit and was therefore responsible both for directing the regioselectivity of binding and the positioning and efficiency of the subsequent reaction chemistry.

It is worth noting in this example, and in an earlier study by Sasaki and Shea,[60] that long reaction times and rather forcing conditions were required to effect covalent rebinding of ligands at two points within the cavities. This phenomenon was most likely to have been the result of slow relaxation and movement of chains within the highly cross-linked network combined with high local viscosities deep within the polymer matrix. Once bound, however, the ligands proved stable to severe acylation chemistries, whilst remaining amenable to cleavage by facile hydrolysis and recovery from the polymer backbone in high (>90%) yields. The above strategy might therefore be very effective for solid phase parallel organic synthesis and combinatorial chemistry, as multiple modifications could be carried out on ligands with a variety of functionalities but bound via a common structural core to a specific cavity. The rapid generation of libraries for biochemical and medicinal screening is an obvious and attractive application of this methodology and has yet to be fully exploited.

## 3. Catalysis

The similarity between the defined geometries and functionalities of imprinted polymer binding sites has long been considered analogous to that of enzymes and, not surprisingly, the catalytic activity of these natural polymers has inspired synthetic chemists to design MIPs that can lower the activation energy barriers to chemical transformation. A number of detailed reviews on MIP catalysis have now appeared that consider the mechanistic aspects, the challenges that face researchers in this area and the potential applications of these materials.[61,62]

In general, the approach taken to effect catalysis in artificial enzyme research, including MIP catalysts, relies upon transition state theories and, specifically, the generation of materials that can stabilise particular species along a reaction pathway. Expressions based on the Michaelis–Menten equation are often used to describe the kinetics of these reactions, and values for $k_{cat}$ and $K_m$ can be derived which denote the efficacy of the catalytic process. Tightly-bound substrates, (where $K_m$ is low) are thus desirable, but the catalytic rate constant $k_{cat}$ relies upon a number of other factors. In order for catalysis to occur, the MIP binding site must stabilise the transition state of a reaction more than the ground state, i.e. in Figure 2, $\Delta G_{CTS\#}$ must be $>\Delta G_{CS}$, a consequence of which is the necessity for MIP catalysts to be designed to interact more favourably with a postulated intermediate than with the reagents. It is important to note that a transition state is not a discrete entity and analogues of postulated species are therefore, by definition, only




$$C + S \rightleftharpoons C.S \rightleftharpoons C.P \rightleftharpoons C + P$$

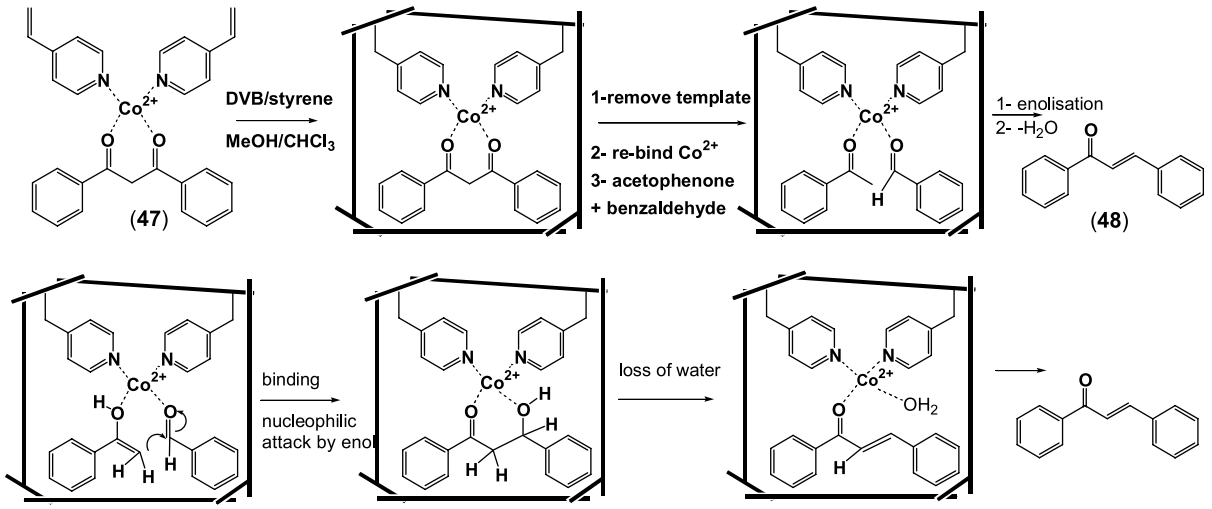
$$\frac{d[P]}{dt} = -\frac{dS]}{dt} = k_{cat}[CS] = \frac{k_{cat}([C] + [C.S])}{1 + K_m/[S]} \quad \textit{Equation 1}$$

when $[S] >> ([C] + [C.S])$ and $[S] << K_m$

$$\frac{d[S]}{dt} = \frac{k_{cat}}{K_m}[S]([C] + [C.S]) \quad \textit{Equation 2}$$

**S** = substrate, **C** = catalyst, **P** = product
**C.S** = catalyst substrate complex
**TS#** = transition state
**C.P** = catalyst product complex
$\mathbf{k_{cat}}$ = rate constant of catalysed reaction
$\mathbf{K_m}$ = Michaelis constant

**Figure 2.** Schematic of pathways involved in enzyme-analogous catalysis. A substrate S associates with catalyst C leading to the products P. Stabilisation of the transition state T.S. by the catalyst lowers the activation energy of the C–P reaction. The rate of conversion of the substrate d[S]/d*t* can be related to the rate constant of the catalysed reaction and the concentrations of substrate and catalyst by the Michaelis–Menten equation (Equation 2).




**Scheme 15.** Class II aldolase:[65] dibenzoylmethane imprinted as the cobalt-bis(4-vinylpyridine) complex (**47**) to leave a metal coordination site. Subsequent rebinding of acetophenone and benzaldehyde in the site, followed by catalysed C–C bond formation and loss of water generated the α,β-unsaturated ketone (**48**).

approximations of the many dynamic intermediates in a chemical reaction.

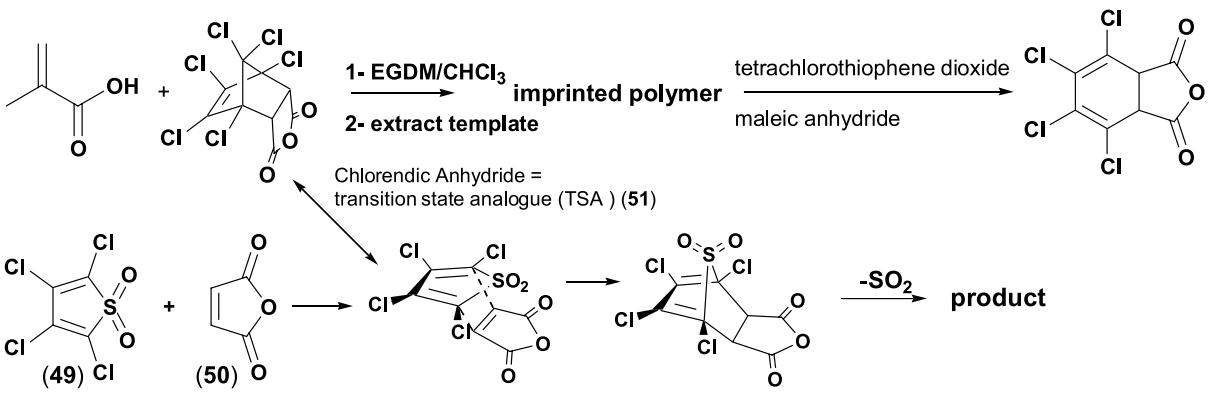
The MIP catalyst must also bind the products of the reaction with a lower affinity than the intermediates to ensure turnover. Indeed, product inhibition is a severe problem where the substrate, intermediate and products bear a strong chemical resemblance. Enzyme catalysts are often able to adopt conformational changes to favour product release, whereas the rigidity inherent in MIP binding sites can hinder the egress of reaction products. In view of these fundamental difficulties, it is not surprising therefore that MIP catalysts have yet to achieve turnover rates as good as those of natural polymers.

Many of the early efforts in this field concentrated on catalytic hydrolysis,[63,64] and this class of reactions is still a significant area of MIP catalysis research (vide infra). In more recent years, however, a wider range of reactions has been addressed, including those regarded as amongst the most difficult in organic chemistry, such as carbon–carbon bond formation and cycloadditions.

### 3.1. Carbon–carbon bond formation

A key paper describing the preparation of an artificial class II aldolase by Matsui et al.,[65] involved the use of polymer-bound cobalt complexes to effect an aldol condensation between acetophenone and benzaldehyde (Scheme 15). The template employed to generate the catalytic site was dibenzoylmethane (**47**), coordinated to a $Co^{2+}$ bis(4-vinylpyridine) complex such that removal of the imprinted species left a binding cavity with functionality positioned appropriately to interact with carbonyl oxygens.

Enolisation in the site, followed by loss of water, took place to yield chalcone (**48**) as the product and the polymer complex between dibenzoylmethane (DBM) and $Co^{2+}$, which utilised two coordinative bonds (**47**) was replaced by a product which could at best bind by only a single interaction, thus favouring product release. The imprinted polymer demonstrated substrate selectivity, turnover, and rate enhancement and although the reaction conditions were still rather severe (100°C, reaction times >24 h), can therefore be considered as a true catalyst. A concentration-dependent inhibition of chalcone production by the template dibenzoylmethane ($K_i$=60±10 μM) was also found, suggesting that catalysis took place at a specific center in the MIP. Replacement of acetophenone in the reaction scheme with more bulky analogues (adamantly methyl ketone, anthracyl methyl ketone) led to the generation of substituted α,β-unsaturated products with




**Scheme 16.** Diels–Alder reaction of tetratchlorothiophene dioxide (**49**) with maleic anhydride (**50**) catalysed via MIP imprinted with TSA (**51**).

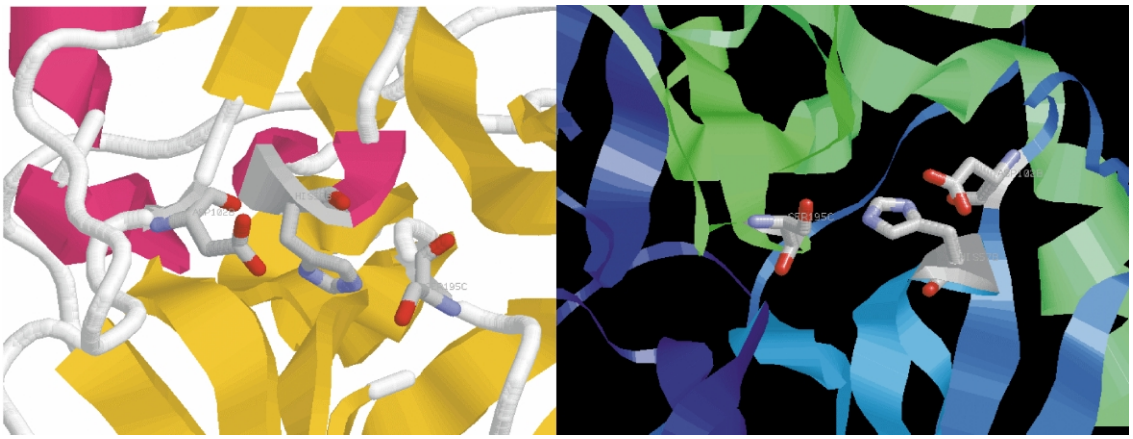


**Figure 3.** Two views of the active site of bovine chymotrypsin, showing the relative positions of amino acids serine 195, histidine 57 and aspartic acid 102—the 'catalytic triad'.

lower reactivity ratios, as expected based on the fit of reagents in the sites. The authors noted that the catalytic turnover and rate enhancements were low using this polymer ($V_{max}$=0.61±0.06 μmol $h^{-1}$; Michaelis constant $K_m$=1.23±0.04 μM) and suggested that these low values may have occurred as a result of the imprinting of an analogue which was closer to the reaction product than the transition state. Nevertheless, in contrast to many other catalytic processes, the carbon–carbon bond formation reported in this study is disfavoured entropically, implying that MIP catalysis may be of considerable potential for difficult reactions, especially where high temperatures and pressures would normally be employed.

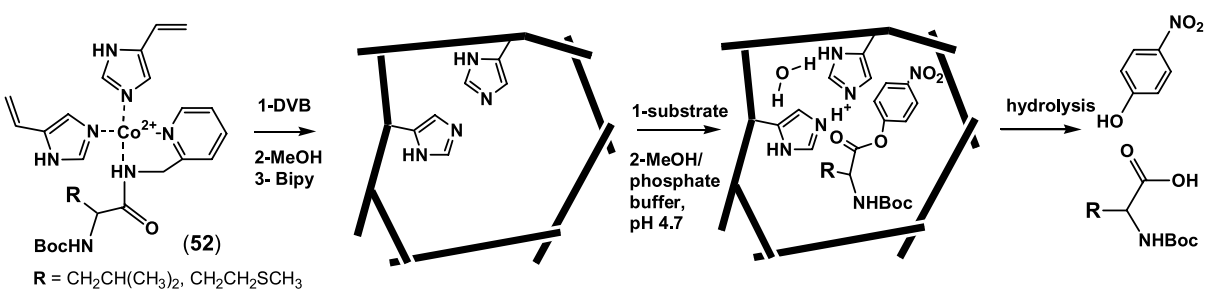
A second example of carbon–carbon bond formation was reported by the group of Mosbach.[66] Diels–Alder cycloadditions have been of interest to researchers in synthetic and catalytic chemistry for many years as 'unnatural reactions', and a number of approaches have been adopted to catalyse these transformations, including artificial receptors and catalytic antibodies.[67–70] The bimolecular Diels–Alder cyclisation of tetrachlorothiophene dioxide (**49**) and maleic anhydride (**50**) was catalysed (Scheme 16) by a polymer imprinted with chlorendic anhydride (**51**), a transition state analogue (TSA) for this coupling process. Kinetic studies utilising the resultant MIP revealed a 270-fold rate enhancement of the reaction in comparison to the non-mediated system.

Further studies carried out by the addition of chlorendic anhydride as an inhibitor demonstrated only a decrease of 41% in the rate of the MIP-catalysed reaction when compared to the uninhibited process. This result indirectly indicated that the reaction most likely proceeded within the cavities of the polymer imprinted against the transition state analogue for the reaction.

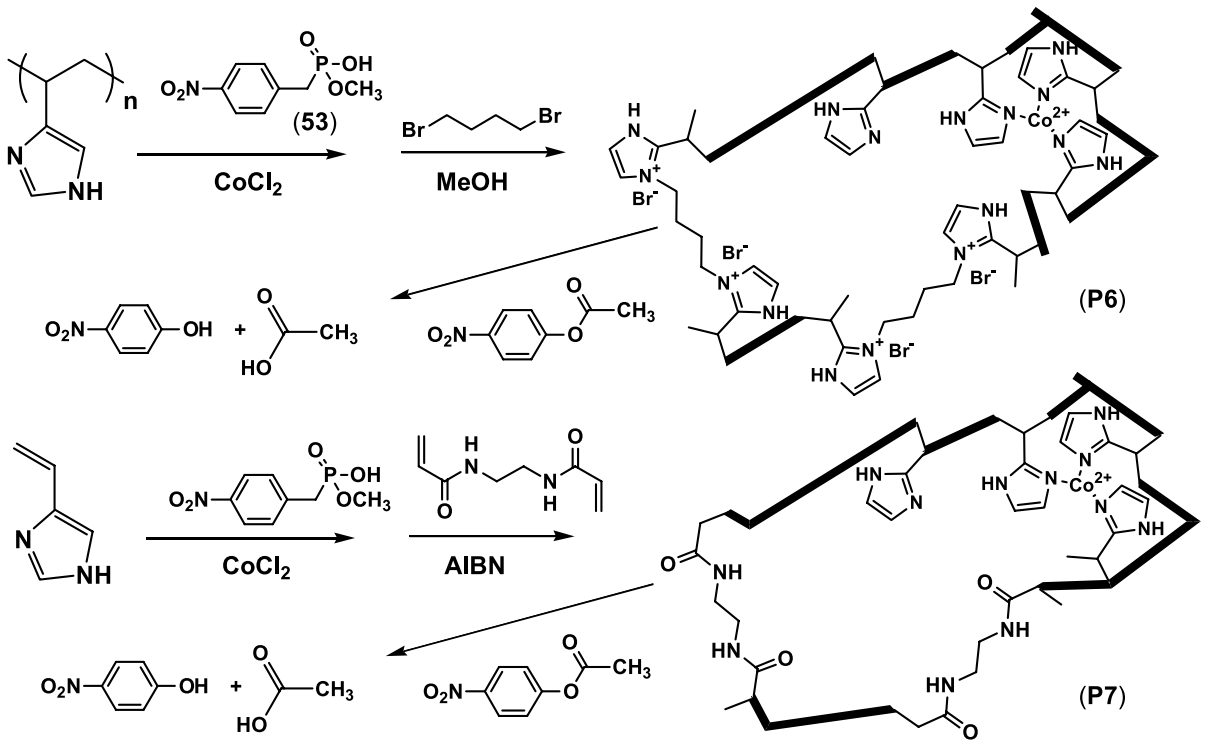
### 3.2. Imprinted polymer catalysts as mimics of natural enzymes

The developing understanding of the mechanisms by which enzymic catalysts function[71,72] has been a major source of inspiration for researchers in MIP catalysis and a number of active imprinted polymer catalysts have now been prepared. In particular, the 'catalytic triad' motif of serine, histidine and aspartic acid (as the carboxylate anion) present in the enzyme family of serine proteases has served as a model around which MIP catalysts have been derived. Bovine chymotrypsin, for example, displays these amino acids at positions 195, 57 and 102, respectively, in order to set up proton relay systems able to cleave peptides adjacent to a hydrophobic residue, which sits in a pocket in the active site (Fig. 3).

By analogy with proteolytic biopolymers, imprinted polymers have been prepared with functionality consisting of some or all of the hydroxyl, imidazole and carboxylate groups present in the enzyme pocket in the MIP binding sites. In an early example, Leonhardt and Mosbach[46] employed imidazole residues to hydrolyse amino acid nitrophenyl esters, as shown in Scheme 17. In this case, cobalt ions were used to coordinate vinylimidazole monomers with a template based on a picolinyl-*N*-Boc protected amino acid (**52**). Removal of the template and reincubation of the polymer with the corresponding 4-nitrophenyl ester of the same amino acid resulted in catalytic activity.




**Scheme 17.** MIP chymotrypsin mimic prepared by Leonhardt and Mosbach[46] for the hydrolysis of activated ester substrates.



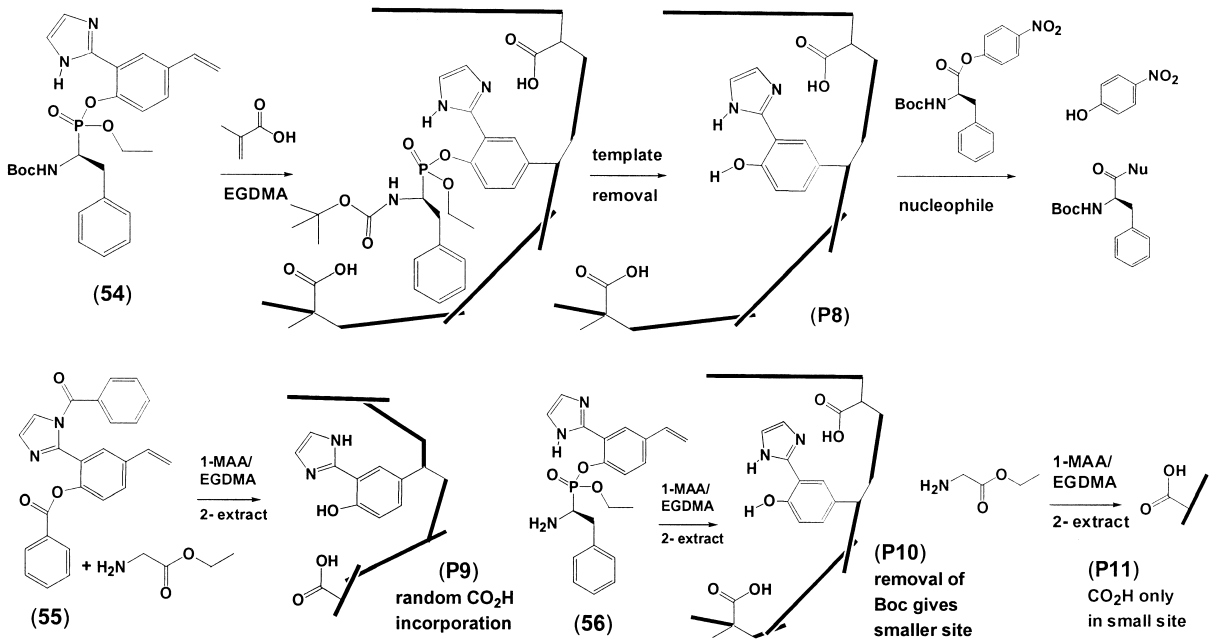

**Scheme 18.** Schematic of chymotrypsin mimic MIPs reported by Ohkubo[74] employing phosphonate ester (**53**) as TSA.

The selectivity in catalysis was demonstrated to be dependent on the amino acid side chain (methionine, leucine), whereas control polymers exhibited lower hydrolytic rates. The authors further noted that the addition of cobalt ions during the hydrolysis step did not affect selectivity or reaction rate, but was, of course, essential in predisposing specific geometries of the imprint species. The relatively low activity of the imprinted polymers compared with the controls (2 to 3-fold enhancement of the reaction rate) can partly be attributed to multiple coordinative states of the template and the use of a substrate rather than a transition state analogue as the template species. In order to address these issues, Robinson and Mosbach[73] imprinted 4-nitrophenylmethylphosphonate (**53**) as a TSA for 4-nitrophenyl acetate using cobalt chloride, a poly-(vinylimidazole) and 1,4-dibromobutane as the cross-linker. The resulting MIP was observed to bind the transition state analogue selectively and an increase in the rate of hydrolysis in comparison to the control polymer (prepared in the absence of template) was reported.

The transition state analogue approach was also investigated by Ohkubo et al.,[74] who prepared esterolytic MIPs using the procedure of Robinson and Mosbach[73] and also by a conventional imprinting strategy employing 5-vinylimidazole, ethylenebisacrylamide and cobalt chloride with the same phosphonate ester (**53**) as the template (Scheme 18). The MIP cross-linked with ethylenebisacrylamide (**P7**) was found to be a more active catalyst under certain conditions than the dibromobutane cross-linked polymer (**P6**), but a strong pH dependence was noted. The $k_{obs}/k_{uncat}$ for **P6** values were 1.3 and 2.5 at pH 7.0 and 8.0, respectively, whereas the $k_{obs}/k_{uncat}$ for **P7** were 6.7 and 1.9 at the same pH values. This was attributed to the extra protonation of imidazole residues, which were present in higher relative amounts in **P6**, and a consequent concentration of the esterolytic hydroxyl ions in the sites at the higher pH. No control polymers, i. e. polymers prepared in the absence of phosphonate ester TSA were, however, reported, and it is thus difficult to gauge accurately the true catalytic efficacy of these systems.

More recently, Ohkubo et al.[75,76] have extended their investigations to amino acid ester hydrolysis catalysed by phosphonate TSA MIPs, and have considered the effects of co-monomer incorporation and cross-linker variation. The hydrolysis of *Z*-L-Leu-4-nitrophenyl ester on a polymer imprinted with a racemic TSA coordinated to an L-His-derived monomer was found to occur at a higher rate than that of the D-isomer ($k_L/k_D$=1.10–2.54). This hydrolytic reaction took place even though the polymers were of rather low cross-link density (11–20%) compared to conventional MIPs.

Lele and co-workers[77] have prepared polymers with functionality analogous to chymotrypsin by a novel surface-grafting procedure, utilising preformed poly(glycidyl methacrylate-co-ethyleneglycol dimethacrylate) beads as a support. Functional *N*-methacrylamido amino acid monomers based on serine, aspartic acid and histidine were complexed with cobalt chloride and template species *N*-nicotinylbenzoyl ester (*N*-Nic-Bz) and adsorbed on to the preformed beads prior to co-polymerisation with EGDMA. This template was chosen for its close structural similarity to tyrosine esters, and the 2-pyridyl functionality of the nicotinyl moiety provided an anchoring point for ligating with cobalt ions. Template species, unreacted monomers and residual cobalt species were removed by a methanol wash, and the beads were then incubated with *N*-benzyloxycarbonyltyrosine-4-nitrophenyl ester (*N*-CBz-Tyr-PNP) and the hydrolysis kinetics investigated. Control

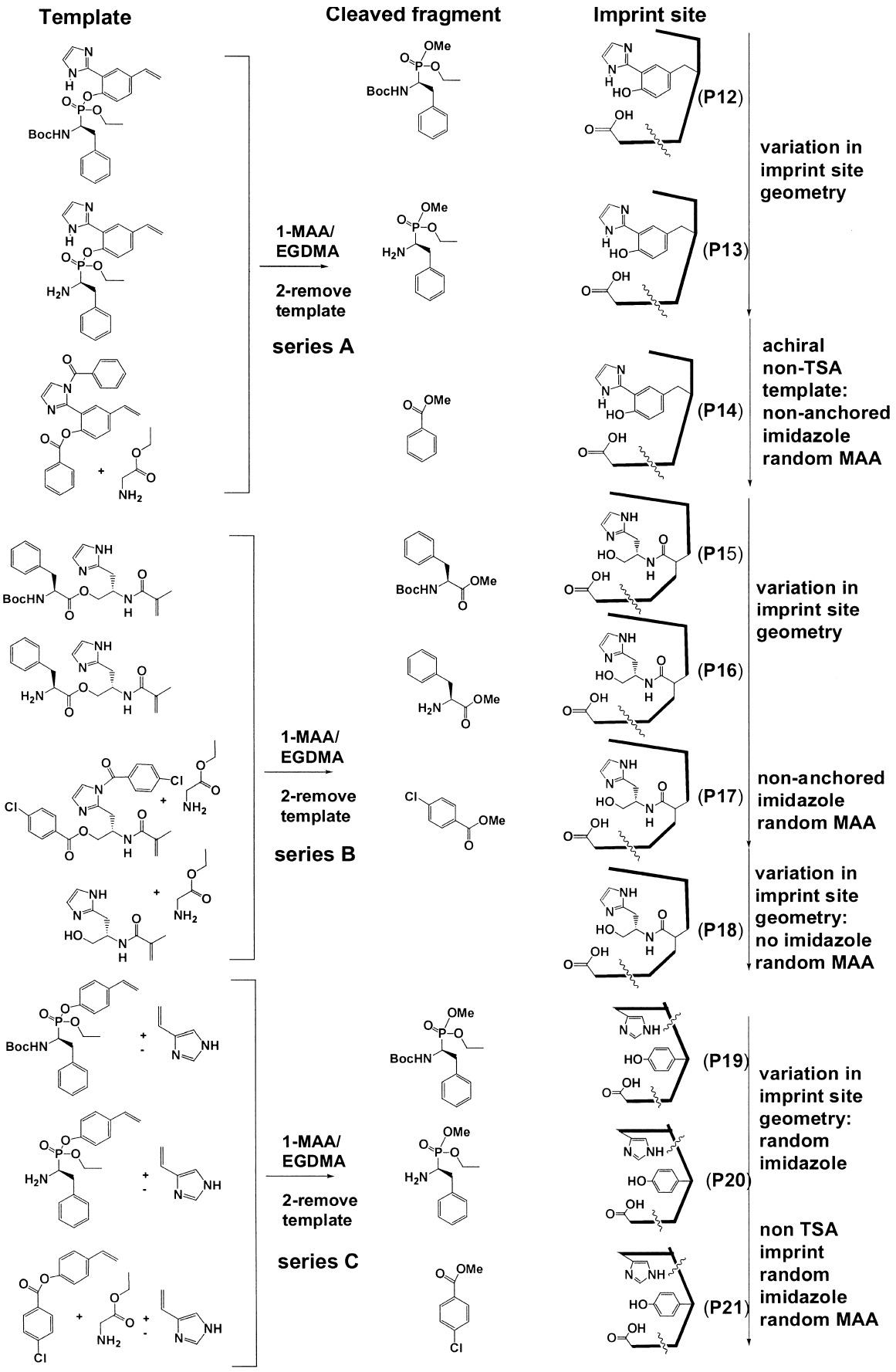



**Scheme 19.** Imprinted polymer chymotrypsin mimics and 'deletion mutants' devised by Sellergren and Shea.[79] The phosphonate ester (**54**) was used as TSA to generate a polymer (**P8**) with oriented and positioned hydroxyl, imidazole and carboxyl groups for ester hydrolysis. Templates (**55**, **56**) and glycine ethyl ester were used to probe the shape and size requirements in the sites for catalytic activity.

polymers were prepared with a further range of functional monomers, including hydroxyethyl methacrylate (HEMA) to mimic serine, methacrylic acid as a replacement for aspartic acid and with or without the template. In all cases the highest catalytic activity was observed for the polymers synthesised with the multivalent cobalt complex containing chymotrypsin amino acid-derived monomers, and the substrate specificity for *N*-CBz-Tyr-PNP was 3-fold higher ($V_{max}/K_m$=6.0×10$^{-4}$ s$^{-1}$ m$^{-1}$) for the nicotinyl template imprinted polymer than for that of an analogous polymer prepared with all three functional monomers, but in the absence of template ($V_{max}/K_m$=1.8×10$^{-4}$ s$^{-1}$ m$^{-1}$). This result suggested that the chymotrypsin mimic synthesised by the surface grafting procedure was able to recognise its substrate specifically to enable catalysis to take place and its catalytic efficiency ($k_{cat}/K_m$) was almost 2-fold that of the non-imprinted analogue. The observed $k_{cat}$ for the non-imprinted polymer was, however, actually marginally higher than that of the nicotinyl template imprinted material. The catalytic efficiency differences between the imprinted and non-imprinted materials thus arose principally as a consequence of the higher substrate binding affinity of the former against the latter, and not as a result of any inherently favourable interactions along the reaction pathway from functional group positioning set up by the imprinting process. In a following article,[78] the effect of increasing nucleophilicity in the serine mimics imprinted, and of the flexibility/chain length of the aspartate substitute for hydrolysis of the substrate *N*-CBz-Tyr-PNP was evaluated. A higher catalytic activity was found for the polymers containing SH nucleophiles compared to the hydroxyl analogues for a given set of histidine and aspartate mimics in the binding sites, whilst a polymer imprinted with a β-alanine-derived monomer was a better catalyst for ester hydrolysis than a similar polymer imprinted with methacrylic acid. In addition, the catalytic action was decreased in polymers with low surface areas, which was attributed by the authors to a reduced access of the substrate to the binding sites. This would have affected the $K_m$ values for these materials: hindered access or blocked sites would reduce the measured affinity constant of the MIP-substrate binding interaction, and thus $k_{cat}/K_m$ would have decreased even though there is no connection between $k_{cat}$ and $K_m$. Immobilized enzymes also show reduced catalytic activity on account of difficulties in substrate access and so in this respect the MIP system has no further disadvantages. Overall, the studies by this group indicated that rational choices of functional monomers and imprinting conditions were able to influence strongly the activity of MIPs in esterolysis, but a number of mechanistic questions arising from this work await further clarification.

The most detailed investigation to date of a designed chymotrypsin mimic was reported by Sellergren and Shea[79] and further elaborated by Sellergren et al.[80] Template molecules (**54**–**56**) were prepared (Scheme 19) such that following polymerization into a cross-linked network, functional mimics of serine, histidine and aspartate remained in the sites, which were subsequently used to catalyse ester hydrolysis. These studies were especially elegant in that the nature of the recognition and catalytic species was systematically evaluated by imprinting of several individual and combined fragments expected to contribute to the activity of the MIP and compared with polymers imprinted with a template that should contain all the necessary components. In this way, a series of MIPs (**P8**–**P11**) were produced that were analogous to enzymic deletion mutants and, significantly, which assessed the role of a transition state analogue imprint compared to a ground state template.

In the first study, the rates of hydrolysis of D- and L-(*N*-*t*-butoxycarbonyl)phenylalanine nitrophenyl ester (Boc-PheONP) were assessed and the D-isomer was found to




**Scheme 20.** Templates and polymers used to map out the chemistry of MIP chymotrypsin mimics by Sellergren et al.[80]

hydrolyse most rapidly on polymer (**P8**), i.e. the MIP prepared from the D-phosphonate ester template. The rate of hydrolysis using this polymer was 10-fold higher than the reaction in solution using a soluble phenol-imidazole derivative as the nucleophile. Significantly, this level of rate enhancement occurred only when complete template cleavage had been effected, such that the phenolic hydroxyl and imidazole groups were present in the sites. The 'deletion mutants' were less active (by >2-fold) and a notable difference was obtained between polymer (**P8**) imprinted with the TSA and (**P9**) that had been imprinted with an achiral 'ground state' substrate. The polymers additionally exhibited a degree of enantioselectivity, albeit low, with the D-imprinted polymer (**P8**) hydrolysing the D-substrate more rapidly than the L-enantiomer. This situation was reversed when a polymer was imprinted with the L-substrate, demonstrating that, not only was a true active site established in the MIP, but also that designed chiral induction was effected by this process. The use of more forcing conditions for template removal (aqueous base at elevated temperatures) caused a loss in enantioselective esterolytic activity, indicating that whilst the sites were still active for hydrolysis, the stereochemical configuration established during imprinting was lost as a result of this treatment. A marked pH effect was also observed, with a high catalytic activity only at low pH, presumably on account of the proton transfer requirements during esterolysis: under basic conditions these polymers, which were rich in carboxylic acid residues, would most likely inhibit ingress of hydroxide ion into the sites.

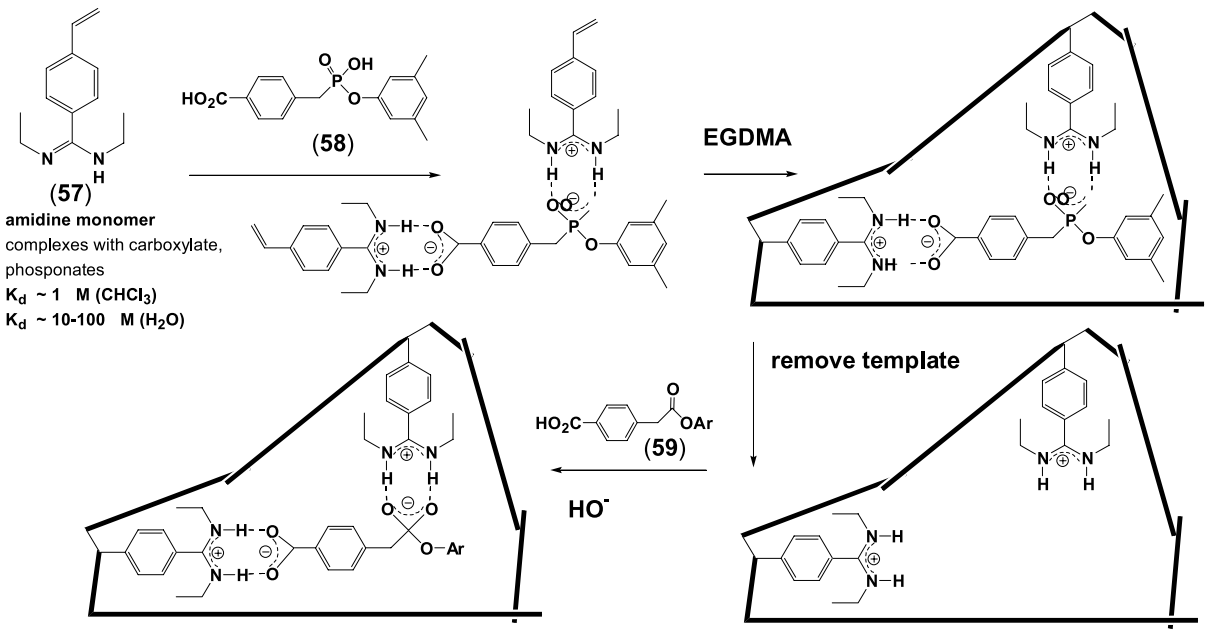
The second report extended the data set for templates and transition state analogues, as shown in Scheme 20. In the series A polymers (**P12**–**P14**), the effect of incorporation of a TSA template, an achiral template, the steric demands of the site and the imprinting effect of stereospecificity vs random incorporation of carboxylate groups from methacrylic acid was assessed. For the series B polymers (**P15**–**P18**), the effect of a chirally-positioned histidinyl moiety was probed, as well as the spatial requirements of differentially-substituted templates and specifically-positioned carboxylates, whilst for series C polymers (**P19**–**P21**), the positioning and role of the imidazole group were investigated. A number of different hydrolysis conditions were also evaluated to ensure that the desired functionality remained in the imprinted cavities and the integrity of the dimethacrylate backbone, which was susceptible to degradation under strongly basic hydrolysis. The polymers were further characterised in terms of their degrees of swelling after template removal; aqueous base treatment yielding more swellable polymers, owing, most probably, to some hydrolysis of the EGDMA backbone. Chromatographic studies were performed to demonstrate selective binding of template species and stereochemical analogues and, as expected, only the complementary polymers were able to resolve their templates.

The catalytic activity of the three series of MIPs was assessed against D- and L-BOC-PheONP and D-/L-PheOEt and, again, a marked pH effect was obtained. At low pH, the carboxylate and imidazole groups in the site would be expected to be protonated and the binding of the substrate by carboxylic acid residues would therefore effect placement of the ester in the sites in the desired position, enabling the imidazole groups to promote nucleophilic attack by water according to a general acid catalysis mechanism. These mechanistic predictions were confirmed in that the placement of carboxyl and imidazole groups, which varied systematically across the series of polymers evaluated, was found to be crucial in determining the binding and catalytic activity, respectively. Perhaps surprisingly, the highest rate enhancements were observed for the polymers that exhibited the greatest degree of swelling, suggesting that some flexibility in the backbone may have been beneficial to catalysis, even though some longer range order may have been lost during basic cleavage of the templates from the polymer. The enantioselectivity of esterolysis was also surprisingly high for base-hydrolysed MIPs, with $k_D/k_L$ factors of up to 1.85 being obtained for the activated NP ester, whilst even for the non-activated D- and L-PheOEt substrates, $k_D/k_L$ values of 1.2–1.4 were found. The polymers were further shown to retain their enantioselectivity after storage for an extremely long period (~10 years), in stark contrast to the conventional enzyme-based catalysts.

These studies are significant in the field of MIP catalysis, for, although the rate enhancements are relatively modest compared to enzymes, the work clearly establishes structure-function relationships that should enable future researchers to design and prepare more active and also selective imprinted polymer catalysts.

The most active imprinted polymer catalysts for esterolysis to date have been reported by Wulff and co-workers using a strategy they have described as stoichiometric non-covalent imprinting.[81,82] Careful consideration of the mechanistic aspects of substrate binding and enzyme catalysis led to the design of a new functional monomer to imprint anionic functionality such as carboxyl groups and phosphonate esters. The methodology was based on reasoning that, since a complementary shape to a transition state analogue is not in itself sufficient to produce strong esterolytic activity, new functionality must be introduced into the polymer in order to improve the efficiency of catalysis. Since arginine residues play an important role in the catalytic mechanism of some antibodies raised against phosphonate ester haptens, Wulff et al. devised monomers with a carbon analogue of the guanidine side group of arginine, namely an amidine functionality. The association constants of this amidine monomer (**57**) with carboxyl groups were found to be $\geq 10^6\ M^{-1}$ in $CHCl_3$ and the formation of template-monomer complexes in this solvent was therefore essentially complete. The consequence of this investigation is shown in Scheme 21, where the monoaryl phosphonate (**58**) was imprinted with stoichiometric amounts of the amidine monomer (**57**), which has high association constants for both carboxylic and phosphonic acid groups.

The strong ionic, double-bridged interaction between the amidine and the phosphonate TSA was able to set up a site into which the ester substrate should fit in a position for carbonyl group activation and also which should provide an 'oxyanion hole' for transition state stabilisation in the subsequent hydrolysis reaction. Although the phosphonate ester imprinted was not the true transition state (vide supra),

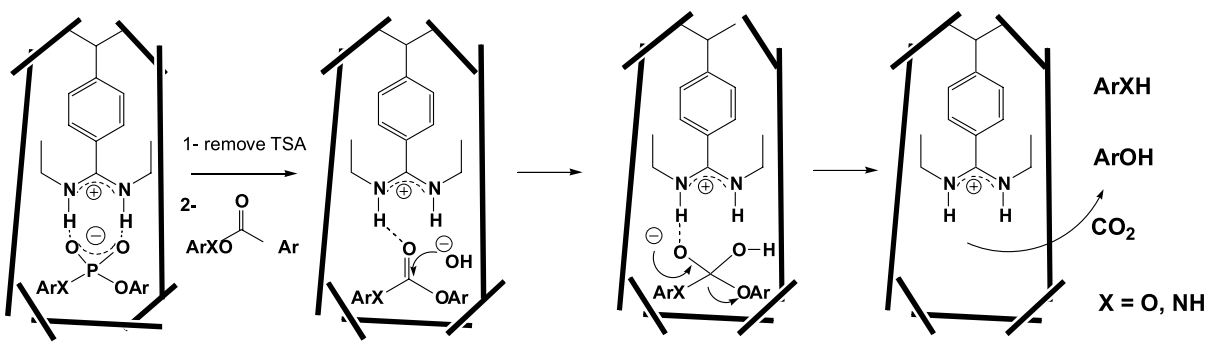



**Scheme 21.** Stoichiometric non-covalent approachto MIP catalysts employing the amidine monomer (**57**) and bifunctional TSA phosphonate ester. Polymers imprinted with (**58**) showed activity in hydrolysis of non-activated esters (**59**) and were inhibited by carboxyl group-containing products.

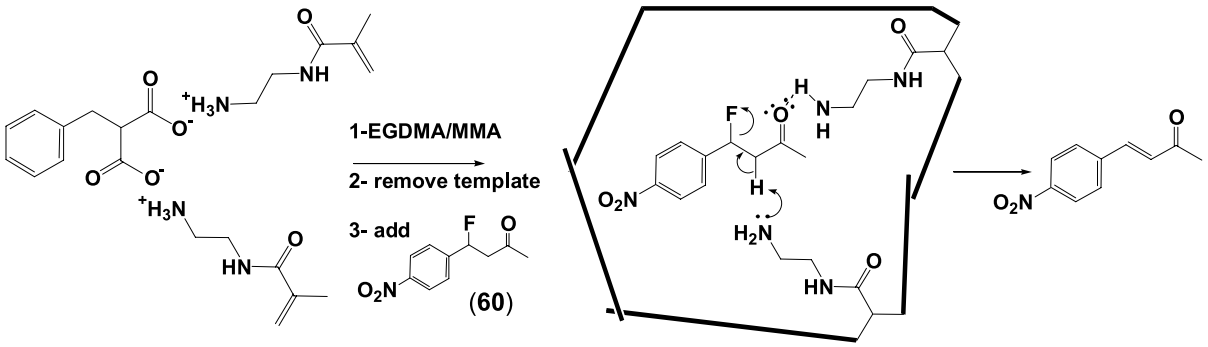
it nevertheless represented an intermediate along the reaction pathway, and would be expected, based on the Hammond postulate, to be of a similar energy to the actual TS and stabilisation of this intermediate by the MIP should indeed therefore lower the activation energy for esterolysis. The resulting polymer catalysed the alkaline hydrolysis in water/acetonitrile (1:1) of the carboxylic ester substrate (**59**) with Michaelis–Menton kinetics and a rate acceleration of >100-fold. Both the template (**58**) and the product of hydrolysis, homoterephthalic acid, are competitive inhibitors of catalysis and a relatively low level of turnover was achieved. Some substrate preference was also observed for polymers imprinted with two different aryl esters, and direct comparison between cross-linkers DVB and EGDMA used to form the bulk matrix revealed that the DVB-based polymers afforded considerably higher rate enhancements in comparison to imprinted polymers featuring EDMA. This enhancement in catalytic activity was attributed to hydrophobic interactions between the phenyl rings of the substrate and those of the polymer backbone.[83]

The realisation that the product of the reaction during esterolysis, i.e. a carboxylic acid, could bind to the amidine groups (product inhibition) suggested that a higher turnover might be achieved for substrates where the product liberated was less able to bind to the imprint site. Accordingly, the same polymers were tested for catalytic activity against carbonates and carbamates, which do not yield carboxylic acids on hydrolysis. Significant rate enhancements were observed in these systems, with the rate constant for the imprinted polymer catalyst being up to 1400–3860-fold higher than that of the same reaction in solution. It is interesting to note that non-imprinted polymers (those with the same functional groups but prepared without a template) also enhanced the hydrolysis rates, whereas the amidine monomer alone did not. A $B_{Ac2}$ type mechanism was proposed in the imprinted sites (Scheme 22) on the basis of analogous studies with catalytic antibodies raised against phosphonate ester haptens.

The use of the stoichiometric non-covalent imprinting procedure additionally enabled the preparation of imprinted beads via suspension polymerisation, and although the rate improvements for these polymers compared to the solution




**Scheme 22.** Mechanism proposed by Wulff[84] for catalysis in amidine-phosphonate ester TSA-imprinted polymers. Binding of carbonate or carbamate carbonyl in the site and nucleophilic attack of hydroxyl proceeded to form a stabilised anionic tetrahedral intermediate. Loss of $CO_2$ gave products which were not able to bind strongly to the site, thus enhancing the catalytic turnover.




**Scheme 23.** MIP-Catalysed dehydrofluorination of (**60**) as a result of imprinting benzylmalonic acid and the consequent placement of amine functionality in the cavities. Michaelis–Menten kinetics were observed, with similar constants in benzene to those obtained using catalytic antibodies in water.

reaction were lower than those with the bulk imprinted materials, the selectivity compared to non-imprinted polymers was much higher.

The stoichiometric non-covalent approach has also been used to prepare MIPs with cholesteryl esterase activity and Michaelis–Menten kinetics were again observed ($k_{cat}$=2.2×10$^{-4}$ min$^{-1}$, $K_M$=3.7 mM).[84] The MIP system catalysed cholesteryl carbonate hydrolysis at a rate ~27-fold higher than that in solution. Although these catalysts were less active than those prepared against aryl aryl carbonates and carbamates, the robustness of the methodology suggests that this approach is extremely promising: rate enhancements of >10$^3$-fold have been achieved and are therefore close to those obtained with catalytic antibodies for the same reactions. In addition, a high-throughput MIP synthesis and screening system has now been developed by Wulff and co-workers,[85] enabling large numbers of MIP catalysts to be prepared and tested robotically, further enhancing the potential of this methodology.

### 3.3. MIP-Based coenzyme analogues

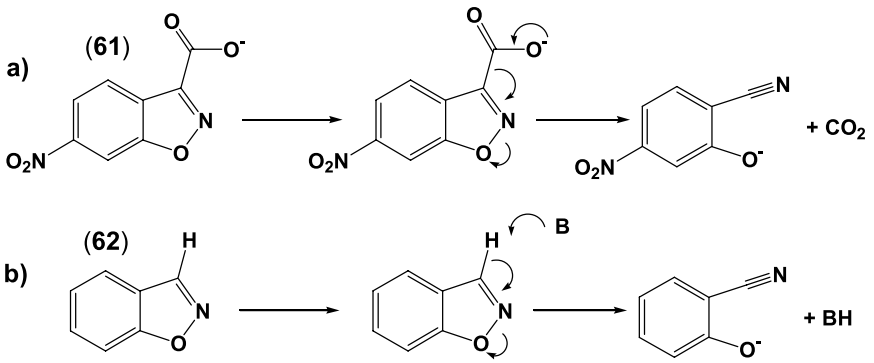
In related enzyme mimic studies, synthetic analogues of naturally occurring coenzymes may provide a useful tool for the study of catalytic mechanisms.[86] An early example of the study of enzymatic reactions was described by Andersson and Mosbach.[87] The template chosen was the novel compound *N*-pyridoxyl-L-phenylalaninanilide, a coenzyme analogue of the naturally occurring pyridoxal-5′-phosphate, a coenzyme involved in many enzymatic transformations of amino acids in nature. This was thought to be the first example of the imprinting of a synthetic coenzyme analogue. In addition to the efficient separation of the enantiomers of the template (separation factor of 2.5), an enhancement in the rate of the condensation of phenylalanine anilide and free pyridoxal was observed (the first step of many pyridoxal-catalysed reactions), thereby demonstrating the potentially high catalytic ability of these molecular imprinted polymers.

### 3.4. MIP-Catalysed elimination reactions

In general, for many catalytic reactions—especially bond formation processes—large product molecules often exhibit a strong affinity for the catalyst in comparison to the component starting materials. Such greater product affinity for the catalytically-active site results in a potentially detrimental inhibition and this of course is not confined to imprinted polymer catalysts.[88] Product inhibition of this type was suspected by Brüggemann[89] in the dehydrofluorination of 4-fluoro-4-nitrophenylbutan-2-one (see **60** in Scheme 23). In this study, it was observed that the catalytic MIP prepared was only capable of catalysing 50% of the starting material—implying catalyst inhibition by the product. For simple hydrolysis reactions, however, sufficient catalytic turnover is relatively easy to achieve, since the product molecules (being smaller than the starting materials) do not bind strongly to the catalyst and are released freely into solution. Efficient product decomplexation thereby renders the catalytic binding sites free for further reaction with the starting material. Despite the observation of catalyst inhibition, catalytic polymers of this type have been tested in batch- and continuous-flow reactors.

Amongst the earliest examples of imprinted polymer catalysts for elimination reactions were those reported by Beach and Shea,[90] who demonstrated catalysis of the dehydrofluorination of 4-fluoro-4-nitrophenylbutan-2-one (**60**). The polymer was imprinted with benzylmalonic acid to orient *N*-(2-aminoethyl)-methacrylamide functional monomers in the matrix with methyl methacrylate and ethyleneglycol dimethacrylate. Removal of the template left pendant amine groups in the polymer orientated to facilitate the β-elimination of HF and rate enhancements of >10-fold were achieved compared to the solution reaction. The postulated mechanism involved a hydrogen-bonded substrate–polymer complex and therefore substrate binding and subsequent elimination would be expected to be favoured in non-polar solvents, but decreased in more polar media. This mechanism was indeed supported by observation, with relative reaction rates dropping by ≥2-fold in ethanol, whereas for conventional E2 eliminations in solution, more polar solvents increase the rate of dehydrofluorination.[91] In further studies analogous with enzyme site-directed mutagenesis, the use of templates with 'misplaced' functionality altered the activity of the imprinted polymer catalysts; an isomeric dicarboxylic acid ((4-carboxyphenyl)-3-propanoic acid) generating a polymer with the amino functionality placed away from the site of elimination and no catalytic rate enhancement therefore being observed.

The most active polymer, i.e. that imprinted with benzenemalonic acid, exhibited Michaelis–Menten kinetics in




**Scheme 24.** Decarboxylation of benzisoxazoles (**61**, **62**) as performed by the groups of Shea[93] and Mosbach.[66]

benzene ($K_m$=27 mM, $k_{cat}$=1.1×10$^{-2}$ min$^{-1}$) which compares well with an antibody designed to catalyse the same reaction in water ($K_m$=0.182 mM, $k_{cat}$=0.193 min$^{-1}$) and the expected template inhibition of the polymer by benzenemalonic acid was also observed.

The same β-elimination was also carried out by Müller et al.,[92] via the non-covalent imprinting method employing *N*-isopropylbenzylamine as the template with methacrylic acid as the functional monomer. In this case, the dehydrofluorination was catalysed by an orientated carboxyl group, with rate enhancements by 2.4-fold compared to the same reaction carried out in the presence of a polymer with randomly-oriented carboxylates. The above examples can be considered as 'bait-and-switch' strategies, as interaction of the template with the appropriate functional monomers results in the specific and precise arrangement of these catalytic groups within the MIP binding site. In this respect, the template molecule acts as a 'bait' for the required catalytic functionality during the imprinting process. When catalysis takes place the template is replaced by a substrate (the β-fluoroketone) and the positioned functional groups promote the required reaction. The efficient use of bait-and-switch strategies may aid in the correct orientation of catalytic functional groups within synthetic MIPs, such that substrates that are otherwise difficult to imprint can be chemically transformed.

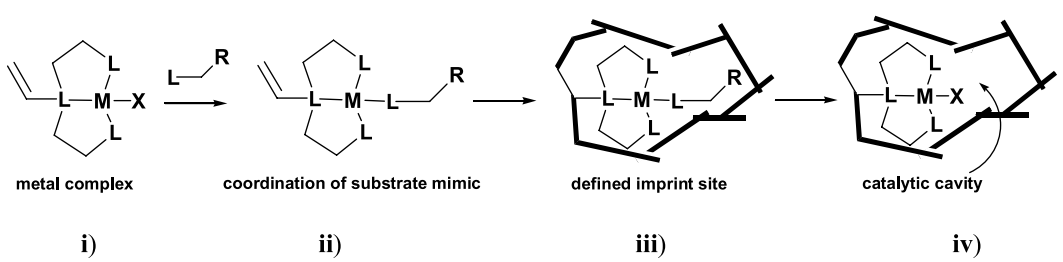
The research groups of Shea[93] and Mosbach[66] have also investigated other imprinted polymer-catalysed eliminations, with a particular target being the decarboxylation of benzisoxazoles.

The use of a covalent imprinting strategy to place amino groups via azomethine chemistry generated MIP catalysts which decarboxylated 5-nitro-3-carboxybenzisoxazole (**61**, Scheme 24a) with an 11-fold rate enhancement compared to a control polymer, whereas the non-covalent route catalysed the decarboxylation of benzisoxazole (**62**, Scheme 24(b)) by 7.2-fold.

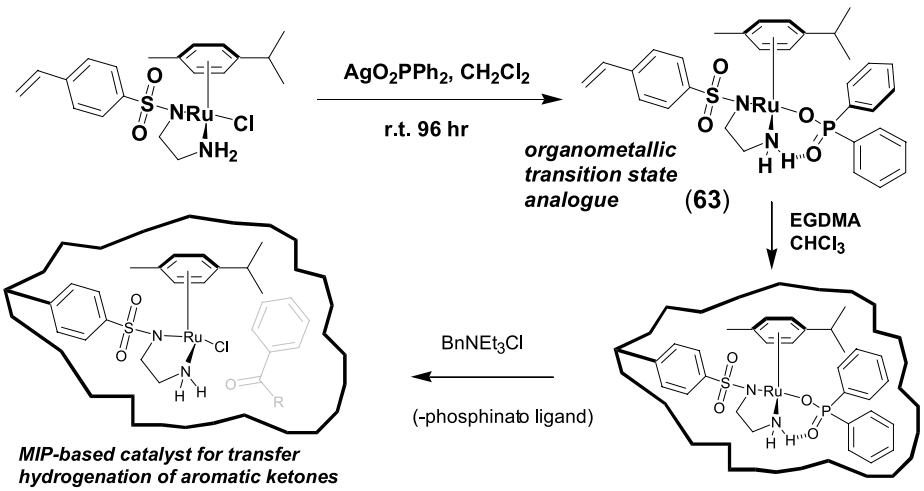
### 3.5. Transition metal-mediated catalysis

In nature, reactive metal centres are located within enzymes in well-defined sites, attenuating their otherwise unselective chemistry. Highly cross-linked MIPs should provide ideal matrices for transition metal catalysts as the access of substrates is constrained by the polymer backbone and site geometry. A combination of immobilised transition metal catalysts within molecular imprinted polymers should therefore, in theory at least, afford catalytic systems that closely resemble metalloenzymes, since the catalytic process is controlled by a well-defined second co-ordination sphere.[94]

Amongst the first examples in this area were pursued by the group of Lemaire[95] who investigated the enantioselective hydride transfer reaction at a catalytic rhodium complex. An intrinsically chiral complex was used as the catalytic species which catalysed the enantioselective reduction of acetophenone in the presence of isopropanol and the chirality of the 1-phenylethanol product was determined by the configuration of the chiral diamine ligand used to form the catalyst. Polymerisation of this chiral complex into a cross-linked network to produce polymer-supported chiral sites generated a heterogeneous catalyst which yielded enantiomeric excesses (ee) of the product alcohols of only 25–33% compared to that achievable with an equivalent homogeneous catalyst (up to 67%). In contrast, cross-linking of the same catalyst into the polymer matrix in the presence of a template ligand, chiral sodium 1-phenylethoxide, resulted in a heterogeneous MIP catalyst with enhanced enantioselectivity, approaching that of the homogeneous reaction.




**Scheme 25.** Schematic of polymer binding sites obtained by imprinting of transition metal catalysts. In (i) the approach of a ligand to a transition metal with coordinated polymerisable group leads to (ii) displacement of X and coordination of template R–L. Polymerisation (iii) followed by template removal results in (iv) imprinted metal catalysts with a designed second coordination sphere.




**Scheme 26.** Metal TSA (**63**) developed for the imprinted polymer-mediated transfer hydrogenation of ketones.

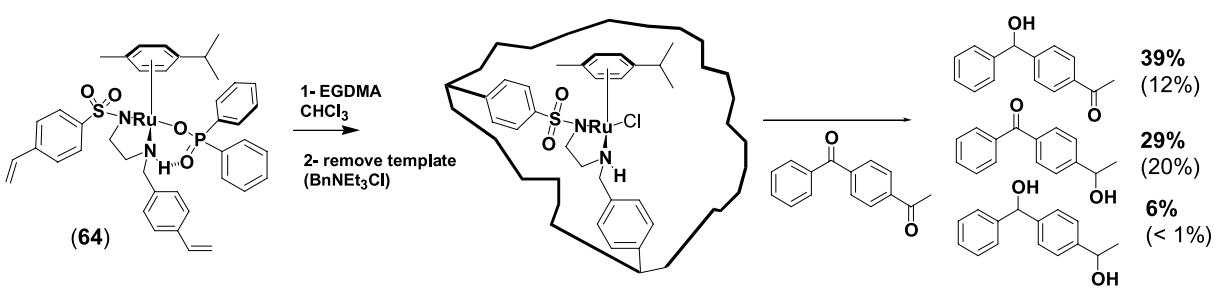
Careful control of the cross-linking ratio (mixture of tri- and di-isocyanates) gave polymers which catalysed the production of (*R*)-1-phenylethanol with an ee of 70%, exceeding the value for the homogeneous catalysis. Further extension of this work substantiated the templating effect and catalysts that revealed ligand size discrimination as well as enantioselectivity were prepared.[96]

A second approach to artificial metalloenzymes involves the co-ordination of a transition state analogue to a metal complex featuring a suitable polymerisable unit (Scheme 25). This stage can be regarded as analogous to the formation of template-functional monomer complexes in non-covalent molecular imprinting protocols. Polymerisation of the co-ordinated species and subsequent cleavage of the transition state analogue should yield a macroporous polymer possessing specific cavities in close proximity to the active sites in the form of the immobilised ruthenium catalyst. In order for this process to take place, it is important that the coordinative linkage between the transition state analogue and the polymerisable metal catalyst is sufficiently strong to ensure that high concentrations of the conjugate are maintained throughout the polymerisation process. Coordinative bonds of this type are in general thermodynamically stable yet kinetically labile, allowing for efficient ligand exchange processes, whilst many transition metal complexes show wide functional group tolerance, enhancing their suitablity for imprinting chemistry. Complex stability characteristics of these species are especially favourable when combined with the highly chemoselective, directional nature of such bonds.

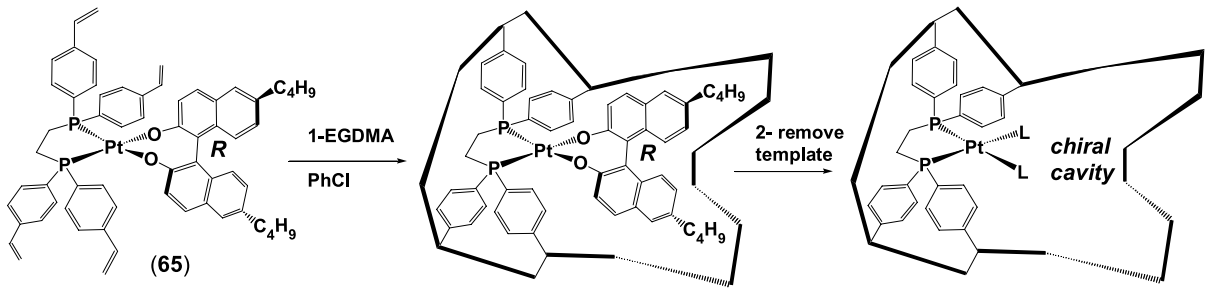
Polborn and Severin[97] developed this technique to produce a MIP catalyst for the reduction of benzophenone, employing an immobilised ruthenium catalyst ligated to a transition state analogue (**63**, Scheme 26) as the catalytic precursor.

The combination of recognition specificity of an imprinted polymer binding site with the high catalytic efficiency of transition metal catalysts was predicted to yield catalytic materials capable of demonstrating both rate enhancements and highly-selective substrate binding. When the ability to catalyse the reduction of benzophenone was assessed, the MIP systems displayed high activities with rate enhancements >3-fold in comparison to the uncatalysed process. These findings indicated efficient incorporation of the ruthenium complexes within the polymeric matrix and also their accessibility within the imprinted sites. The MIP catalyst exhibited a high selectivity for benzophenone in comparison to other ketone substrates. The work was extended to include ruthenium species locked in the matrix by two ligands to avoid conformational 'flipping' during and after polymerisation.[98] The dual anchored MIP metal catalyst (**64**) displayed selectivity for benzophenone against other ketonic substrates and also regioselectivity (Scheme 27).

A similar approach was adopted by Cammidge et al.[99] who used polymerisable triphenylphosphine ligands to fix otherwise labile palladium species in a cis geometry for catalysis of Suzuki and Stille coupling reactions. A particular desire in this work was to combine the advantages of selectivity displayed by homogeneous chiral catalysts with the convenience of heterogeneous reagents. Whilst




**Scheme 27.** 'Dual-anchor' metal TSA (**64**) for catalytic hydrogenation with regiospecificity.



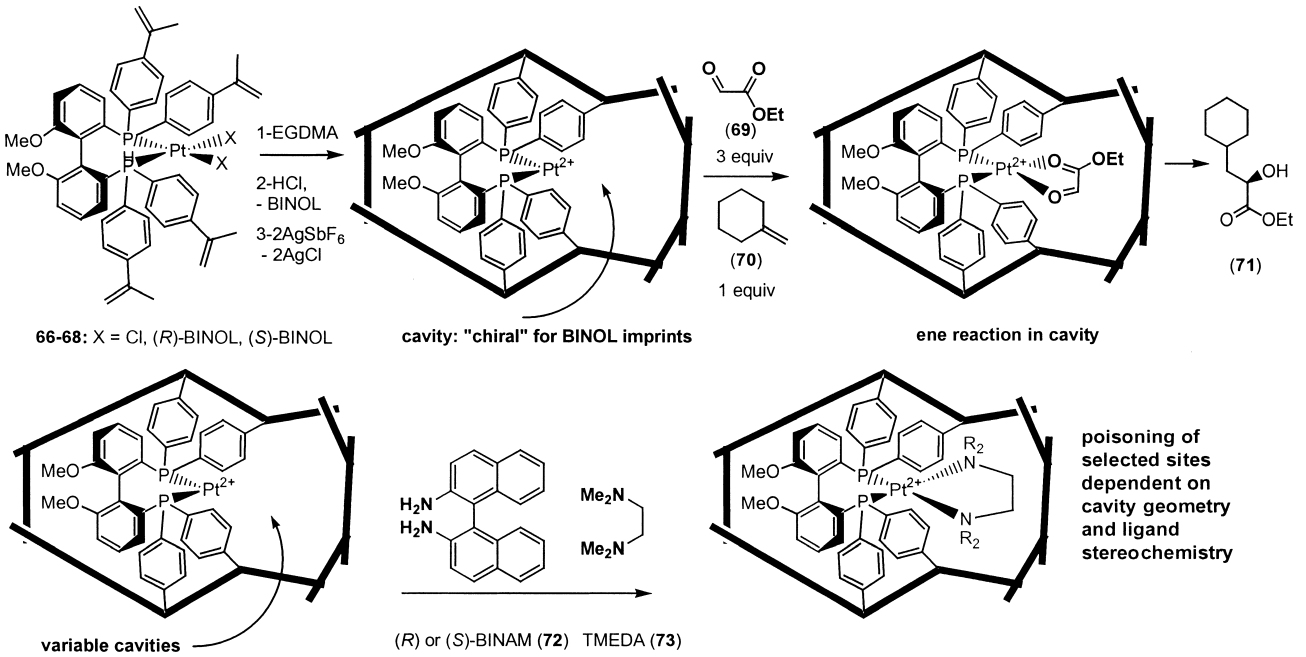

**Scheme 28.** Platinum BINOL-based catalysts (**65**) devised by Gagné for the preparation of chiral cavity MIPs.[101] A defined coordination to the metal-based template was designed to set up geometry and stereochemistry in the site that would define subsequent ligand binding and transformation processes at the metal centre.

commercial polymer-supported metal catalysts are, for example, superior in terms of reuse and recovery, many suffer from the drawback of non-uniform spatial distribution of ligands and their activities are therefore unpredictable compared to solution phase systems. Imprinted polymer palladium catalysts were found to be as active and selective, or more so, than homogeneous systems, which was attributed to their semi-rigid ligand geometry retained by the imprinting process. The incorporation of palladium(II) as its (bis(4-ethenylphenyl)diphenylphosphine)-1,2-dihydroxybenzene complex in a *cis* square planar geometry was postulated to be responsible for the improved performance, as the catalytically-active species, Pd(0), during the coupling reactions would have been slightly strained (whereas a tetrahedral geometry is preferred). As a consequence, the rate of oxidative addition of the aryl species in the resulting cross-coupling was probably faster, and the reductive elimination of the coupled biaryl occurred more quickly. In addition, as there were no competing *cis*/*trans* geometries, rearrangements to give side products could not occur.

The potential for induced chirality at transition metal catalysts within MIP binding sites has been elegantly explored by Gagné et al.[100,101] in some very detailed and rigorous investigations employing metals as diverse as rhodium, titanium and platinum. Central to this strategy was the realisation that the MIP binding environment could be designed to generate a transition metal catalyst with a highly-defined outer coordination sphere. This was accomplished by attaching a non-polymerisable imprinting ligand to the metal centre as a template, whilst incorporating the metal into the cross-linked backbone by one or more polymerisable ligands. In this way, an achiral catalyst system could be rendered asymmetric by ligation of the chiral template species such that on template removal after polymerisation, a tightly-defined metal-ligand coordinating environment remained. This strategy is shown for platinum catalysts (**65**) in Scheme 28, with R-BINOL as the chiral templating ligand.

The properties of these polymer systems were investigated by $^{31}P\{^{1}H\}$CP/MAS HP-NMR and the site geometries were probed by ligand exchange experiments. Whilst the NMR signals were, in general, too broad to allow definitive assignment (a common feature of imprinted polymers owing to the high cross-link densities and heterogeneity of chemical environments), ligand displacement studies indicated that as the displacing species increased in size, the amount of bis(7-butyl-2-hydroxy)binaphthol ((*R*)-$Bu_2$-BINOL) removed decreased. This showed that there was a distribution of binding site geometries and sizes in the final polymers, with some allowing access of bulky ligands to displace (*R*)-$Bu_2$BINOL, whilst others were too small for the incoming ligands or were completely inaccessible to solution. The ligand size was not the only factor in these exchange reactions, as the processes taking place on the metal coordination sphere involve H-bonding and subsequent proton transfer; more acidic ligands binding more strongly to the Pt centres. As a consequence, subsequent catalytic events were predicted to reflect the site heterogeneity, and this was indeed observed. Imprinted sites that were very tightly defined were of low accessibility and hence low reactivity, whereas those more loosely defined were more reactive. This reactivity/selectivity balance is, of course, often observed in homogeneous catalysis with mixed species, but is rather different from many other MIP systems where the reactive sites are more selective, primarily because there is no reactivity in sites where the reagents are not able to gain access. The results are of some significance for transition metal MIP catalyst systems, as, unless the site heterogeneity problem can be overcome, it is inevitable that unselective active sites will dominate over metal centres constrained in specific cavities. These sites are most likely concentrated at the surfaces/interfaces of MIPs and thus the observed products of catalysis may be those due to open ill-defined metal second coordination spheres. In order to overcome this limitation, Gagné and co-workers adopted a 'poisoning' strategy, using diamine derivatives of binaphthol intended to block the most easily accessible sites in a MIP containing chiral phosphine platinum complexes (**66**–**68**).[102] The catalytic process investigated was the ene reaction of a diketone (**69**) with an alkene (**70**) catalysed by a dicationic metal-diketone intermediate to give an α-hydroxyester (**71**, Scheme 29).

In the poisoning experiments, when the MIP-$Pt^{2+}$ catalyst contained an (*S*)-BINOL shaped-cavity, it was selectively poisoned by the similarly-shaped (*S*)-BINAM (**72**), most probably because the (*S*)-BINAM ligand bound more strongly and thus inhibited catalysis more efficiently. Similarly, the (*R*)-BINOL-derived MIP was poisoned more effectively by (*R*)-BINAM; in both cases the changes in catalytic efficiency being different to those obtained by poisoning the analogous chiral phosphine Pt complexes in solution. These results indicated that the cavity shape was the controlling factor in subsequent ligand binding to Pt and not the chiral dimethoxydiaryl ligand in the Pt first




**Scheme 29.** Imprintng of Pt-BINOL catalysts for the ene reaction of ketoesters, e.g. (**69**) with an alkene (**70**) to produce α-hydroxyester (**71**). Cavities of differing sizes and coordination spheres were prepared for binding of the ketoester, and selective 'poisoning' experiments were carried out using the amine-containing ligands (**72**) and (**73**) of varying size and stereochemistry.

coordination sphere. Whilst the poisoning experiments demonstrated that chiral cavities were indeed templated, the product enantioselectivities were unaffected by the amount of BINAM added, suggesting that open or surface cavities were not selectively blocked whilst leaving the better-defined sites still active. Unselective catalyst poisoning by TMEDA (**73**) showed that nearly all the sites in the (*S*)-BINOL MIP were catalytically competent, and the observed enantiomeric excesses were therefore indicative of cavities which, despite imprinting, were not sufficiently defined to alter the ene reaction transition states as desired. Nevertheless, enantioselective deactivation of specific cavities in each 'chiral' MIP was accomplished and the reaction selectivities were enhanced depending on the original chiral diphosphine platinum species imprinted. Taken as a whole, these data suggest that structural and chemical information present in a transition metal complex during a templated polymerisation process can be retained in the final material and that immobilised catalysts produced via this methodology can be made more selective through chemical modification with suitable 'poisons', a result which parallels other studies[103] into post-imprinting derivatisation of imprinted polymer binding sites.

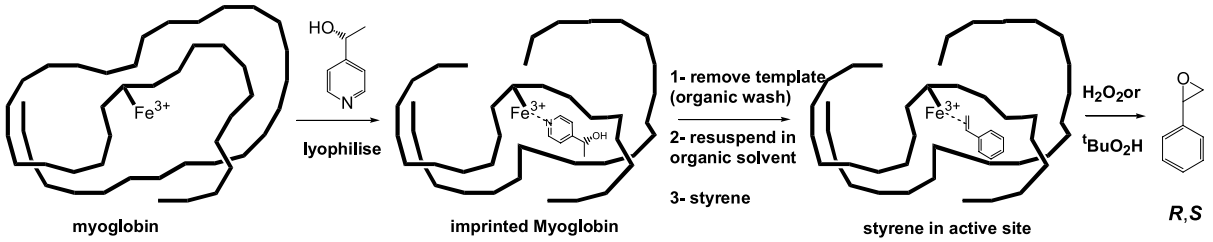
For less mechanistically complex chemistries, MIP-bound metal catalysts have proved to be effective and have been applied to problems of very practical importance. A recent example was reported by Yamazaki et al.,[104] who described the preparation of a synthetic enzyme mimic for the hydrolysis of phosphotriesters—in particular the organophosphate pesticide paraoxon. Catalytic groups designed to mimic the enzyme phosphotriesterase, which contains two bivalent metal ions and four histidine residues within its active site, were introduced into a polymer via imprinting of a cobalt(II)/4(5)-vinylimidazole complex. The rate of hydrolysis of paraoxon by the MIP enzyme mimic was considerably lower (catalytic turnover of $5.6\times10^{-5}\ s^{-1}$) than that of the biological enzyme ($\sim2200\ s^{-1}$), but was noticeably higher (by ≥20-fold) than the corresponding control polymer, thereby indicating that the molecular imprinting approach had increased the organophosphate hydrolysis rate. Whilst not yet optimised, the MIP phosphotriester catalysts offer the possibility for use in demanding environments or in areas wherein the poor long-term storage or application of phosphotriesterase enzymes is problematic.

### 3.6. Catalytic imprinted biopolymers

The wealth of functionality in naturally occurring polymers such as proteins and polysaccharides provides an obvious starting point for the development of catalytic systems, even for biopolymers that do not exhibit catalytic functions in nature. Conceptually, the process of converting a biopolymer into an imprinted material ('bioimprinting') is not significantly different to that of conventional molecular imprinting except that the 'monomers' are already linked together in a linear fashion. The 'locking in' of conformation around a template can be accomplished by a number of methods, such as chemical cross-linking, or freeze-drying in the presence of a substrate.

Amongst the first efforts in this area were those of Keyes et al.,[105] who used partially denatured proteins that were cross-linked around a template using dialdehydes. The catalytic activity was altered in a designed manner; the cross-linking of a trypsin enzyme around an inhibitor (indole), for example, generating a semi-synthetic polymer with decreased trypsin activity, but enhanced chymotrypsin activity.

The group of Klibanov applied a lyophilisation strategy for




**Scheme 30.** Schematic of the 'bioimprinting' of myoglobin with alkylpyridines.

the imprinting of proteins, utilising the unusual stability of certain biopolymers in organic solvents in subsequent catalysis events.[106–109] This methodology involved dissolving a protein in a concentrated aqueous solution of a template species, freeze-drying the enzyme-template complex to lock in conformational order, washing out the template with organic solvents and re-suspending the imprinted enzyme in an appropriate organic solvent for subsequent rebinding experiments or catalysis. A recent example of this technique was the bioimprinting of myoglobin, using ligands designed to bind to the heme iron in the protein, to activate this enzyme for catalytic epoxidation of styrene (Scheme 30).[110]

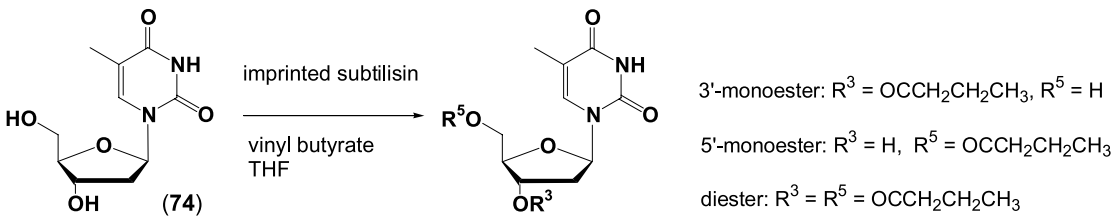
The bioimprinted myoglobin showed rate enhancements of ≤20 fold (160 $\mu M\ h^{-1}$) compared to the native enzyme (8 $\mu M\ h^{-1}$), although enantioselectivity was not observed.

The lyophilisation method has been used by Rich and Dordick to generate imprinted subtilisin capable of catalysing the acylation of nucleosides (Scheme 31) with substrate specificity.[111]

Native subtilisin Carlsberg preferentially acylates thymidine (**74**) with vinyl butyrate at the 5′-hydroxyl in THF, although the regioselectivity is not high (5′:3′ ratio=2.15:1).

Imprinting of this enzyme with thymidine resulted in 50-fold rate enhancements of acylation, whereas imprinting with thymidine fragments, thymine and ribose, resulted in rather lower rate enhancements for thymidine acylation (20–30-fold). In substrate specificity experiments, subtilisin was imprinted with sucrose or thymidine, and the enzymes tested for acylation activity for both substrates. The sucrose-imprinted enzyme catalysed sucrose acylation 40-fold faster than the native enzyme, whilst thymidine acylation was accelerated by 4-fold. For the thymidine imprints, the situation was reversed, with rate enhancements of 9- and 52-fold for sucrose and thymidine, respectively. The substrate specificity of the same enzyme therefore correlated directly with the templating species. Similar results were obtained by imprinting subtilisin with the structurally-related nucleosides, dG, dA and dC.

More recently, the group of Dordick have extended this strategy for the regioselective acylation of paclitaxel and 17β-estradiol derivatives (**75**–**79**, Fig. 4), using two different approaches.[112] In the first study, hydrophilic derivatives of these otherwise water-insoluble compounds were used to imprint thermolysin, subtilisin or lipase TL. Increases in acylation rates as a result of imprinting were ≤20-fold in comparison with the native enzymes. The second method involved lyophilisation of the same enzymes




**Scheme 31.** The acylation reaction of thymidine (**74**) with vinyl butyrate catalysed by subtilisin Carlsberg studied by Rich and Dordick.[111] Imprinting of the enzyme with thymidine enhanced the rate of acylation.

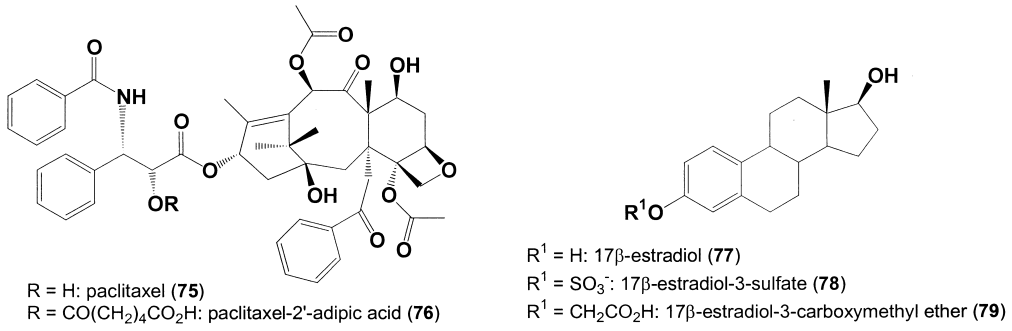



**Figure 4.** Compounds (**75**–**79**) used as templates for imprinting of proteins prior to enzyme-catalysed acylation. Rate enhancements of 110-fold were observed for the acylation of paclitaxel at the 2′-position with an imprinted thermolysin compared to the native protein.

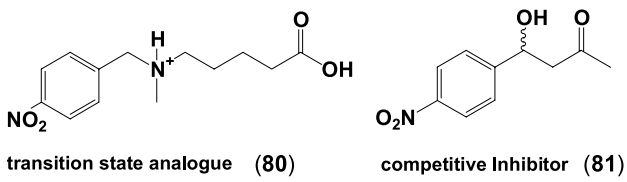



**Figure 5.** TSA Approach to enzymatic dehydrofluorination. BSA imprinted with template (**80**) was 4.4-fold more active than a non-imprinted protein, and was competitively inhibited by (**81**).

with palitaxel or 17β-estradiol in aqueous organic solvent mixtures (*t*-butanol or 1,4-dioxane). The rate enhancements using this approach were of a similar order to those obtained by imprinting of the hydrophilic template derivatives. In addition, the imprinting of thermolysin with the water-soluble paclitaxel derivative (**76**) in aqueous media containing a high salt (KCl) concentration, or of paclitaxel (**75**) in *t*-amyl alcohol, resulted in rate enhancements by ≤110-fold compared to that of the non-imprinted enzyme.

An interesting hybrid approach to bioimprinting was developed by Piessker and Fischer,[113] who adapted a protocol first reported by Ståhl et al.[114] for the imprinting of chymotrypsin with *N*-acetyl-D-tryptophan. Whereas in Ståhl's work the imprinted protein was used for enzymatic synthesis in organic solvents without further modification, Piessker and Fischer derivatised chymotrypsin with itaconic anhydride prior to precipitation from aqueous solution with a template (*N*-acetyl-D-tryptophan) in 1-propanol. The enzyme was then cross-linked with EGDMA, the template was removed and the cross-linked imprinted protease (CLIP) was used to catalyse the hydrolysis of *N*-acetyl-D-tryptophan ethyl ester in phosphate buffer, and also to esterify ethanol and *N*-acetyl-D-tryptophan in cyclohexane. The reaction rate enhancements compared to the non-catalysed processes were $10^4-10^5$ $mM^{-1}$, which is the same order of increase as obtained with catalytic antibodies even though the cross-linked enzymes were of a lower surface area and were subject to mass transfer limitations. The CLIP systems displayed repeated activity for both ester hydrolysis and ester formation in the respective solvents, clearly demonstrating the versatility of these imprinted modified enzymes.

The transition state analogue approach to imprinted catalysts has also been applied to bioimprinting. The same dehydrofluorination reaction catalysed using wholly synthetic MIPs by the groups of Shea and Mosbach (vide supra) was investigated by Ohya et al.,[115] who imprinted bovine serum albumin (BSA) with *N*-methyl-*N*-(4-nitrobenzyl)-δ-aminovaleric acid (**80**) (Fig. 5) as the TSA.

The resulting imprinted BSA catalysed the dehydrofluorination of racemic 4-fluoro-4-(4-nitrophenyl)butan-2-one at a rate 4.4-fold higher than the non-imprinted polymer and Michaelis–Menten kinetics were demonstrated. The imprinted polymer was also competitively inhibited by racemic 4-hydroxy-4-(4-nitrophenyl)butan-2-one (**81**), further indicating that a specific active site was generated by the imprinting process.

It should be noted that whilst the ready availability of many biopolymers suggests that bio-imprinting should be a facile route to catalysts with enhanced activity, the complexity of these materials in terms of variety of functionality and difficulty in characterisation has somewhat hindered their development. The use of adequate controls, for example, has been problematic and, in some cases, non-imprinted lyophilised proteins have shown considerable catalytic activity,[116] suggesting that the placement of functional groups in appropriate orientations and geometries for catalysis can occur unintentionally. If these problems can be overcome, however, it is likely that imprinted enzymes will form a useful addition to the organic chemist's 'toolkit', as these altered natural polymers may offer new routes to complex bioactive molecules, whereas conventional chemistries, e.g. nucleoside derivatisation, often require extensive protection/deprotection strategies. Whilst native enzymes are undoubtedly effective in accomplishing otherwise difficult chemical transformations, their lack of stability in organic solvents can preclude the use of reagents that are not soluble in water, and alteration of their substrate selectivity is generally only possible by site-directed mutagenesis or protein engineering. The ability then to tailor substrate specificity of enzymes by imprinting is of considerable potential benefit.

### 3.7. Inorganic polymer MIP catalysts

The above examples of imprinted materials have all been based upon organic polymer backbones or biopolymers, but there is, in principle, no reason why other building blocks cannot be used to form the matrix, or to act as the support to which functional groups are attached. Indeed, the earliest examples of what is now termed molecular imprinting were based on silica supports, and there is a very considerable literature on imprinted silicas, including some comprehensive reviews.[117–150] In general, very similar strategies to those developed for organic MIPs have been used for imprinted silica materials, but some recent examples are of special interest.

The TSA approach was investigated by Markowitz et al[151,152] who prepared silica particles with enantioselective amide

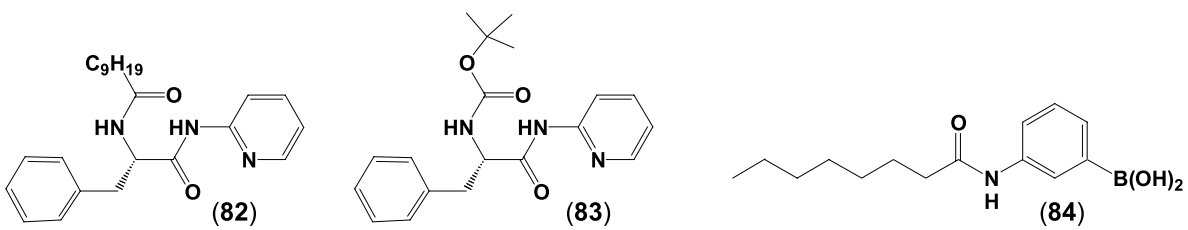



**Figure 6.** Amphiphilic templates (**82**, **83**) and surfactants imprinted into silica surfaces via a sol-gel route. The resultant catalysts displayed a remarkably stereospecific trypsin-like activity when imprinted with chymotrypsin-mimic functionality.

hydrolysis activity based on chymotrypsin functionality. Nonionic surfactants were mixed with surfactant TSA groups or a designed inhibitor (**82**–**84**, Figure 6) and cyclohexane, ammonia in ethanol and water. Silica sol-gel precursors (tetraethoxysilane) and functional silanes with end-groups based on the active site of chymotrypsin (amine, imidazole and carboxylate) were then added and the reactions thus generated silica particles. As a consequence of their surfactant properties, the template TSA species assembled at the interface between the growing particles and the bulk solution.

Removal of non-ionic and template surfactants yielded imprints at the surfaces of the silica. These silica particle 'negatives' of the chymotrypsin transition state analogues displayed almost 5-fold increases in the amide hydrolysis rates compared to the non-imprinted controls. Remarkably, the most active catalysts exhibited trypsin- rather than chymotrypsin-like behaviour; silica particles imprinted with *N*-α-decyl-L-phenylalanine-2-aminopyridine catalysing the hydrolysis of benzoyl-D-arginine-4-nitroanilide at a rate 10-fold higher and 39-fold more efficiently than that of the L-isomer. Whilst the differential isomer hydrolysis was to be expected in terms of chiral selectivity set up by the negative imprint process, the results are perhaps indicative of a better packing of the substrate compared to the template within the site. The observed enantioselectivities are the highest reported to date for imprinted catalysts.

A novel generic approach to the imprinting of inorganic surfaces with imprinted supported metal catalysts has recently been reported by Suzuki et al.[153] These studies addressed niobium and rhodium species on silica supports with a view to producing catalytic domains with sub-nanometer precision and also the generation of imprinted cavities on alumina surfaces with intrinsic esterolytic action. For the latter approach, benzyldiethylphosphonate was chosen as a TSA template, and adsorbed onto the surface of precleaned $Al_2O_3$ surfaces to give a total of 0.5 wt% template:catalyst ratio. Tetramethoxysilane (TMOS) was added via consecutive chemical vapour deposition (CVD) and hydrolysis steps until the alumina surface and template were completely covered with a silica layer. Extraction of the materials with ethanol removed 86% of the template, leaving imprinted pores at the silica surface (19 μmol $g^{-1}$ catalyst) that were of ~0.52 $nm^{-2}$ in density. A similar procedure was followed for rhodium hydrogenation catalysts, employing $Rh_2Cl_2(CO)_4$ supported on $TiO_2$ with $P(OCH_3)_3$ as the TSA template for half-hydrogenated intermediates and a surface layer built around the rhodium centres by CVD of TMOS.

The phosphonate ester (**85**)-imprinted materials showed activity in the hydrolysis of a number of alkyl esters (**86**–**92**, Fig. 7). The highest rate enhancements compared to non-imprinted silica-alumina hybrids occurred for ethyl isobutyrate (**88**), which corresponded in cross-sectional area (0.53 $nm^2$) most closely to that of the template pores (0.5 $nm^2$), even though the putative cross-sectional area of the imprinted TSA was ~0.7 $nm^2$.

The reason for the difference between the cavity area and the template is not clear, but some annealing of the surface after template removal and the role of the solvent during the imprinting process are likely to have been contributing factors. The activation energy of the phosphonate TSA imprinted catalyst was found to be 12 kJ $mol^{-1}$, considerably less than that of the non-imprinted analogue (79 kJ $mol^{-1}$), whilst the activation entropy for the imprinted material was more highly negative (−312 J $mol^{-1}$ $K^{-1}$) compared to the control (−50 J $mol^{-1}$ $K^{-1}$). The activation energy of the non-imprinted catalyst in the esterolysis reaction was very similar to that of conventional acidic catalysts: the much lower value for the imprinted material suggests that the templated pores contained acidic sites in a defined geometric arrangement, perhaps enhancing their acidity whilst the cavity was able to hold the substrate in a stabilised arrangement.

The imprinted rhodium catalysts also displayed enhanced activity; ≤2-fold higher for alkene hydrogenation than for conventional supported catalysts. Defined shape specificity was not observed in these experiments, although longer chain alkenes were more rapidly hydrogenated, presumably on account of the template $P(OCH_3)_3$ used.

These results, whilst undoubtedly impressive in terms of reaction rate enhancement, show that templating of inorganic materials during synthesis can lead to highly active catalysts with improved performance over conventional supported metal catalysts. More work is required on these systems to demonstrate a specific imprinting effect, i.e. designed shape and regio- or stereoselectivity.

## 4. Conclusions

The growing number of reports in which imprinted polymers have been used as reagents, supports or aids for synthetic chemistry suggests that there is much potential for exciting future applications of these materials. In particular, as preparative organic chemistry increasingly moves to

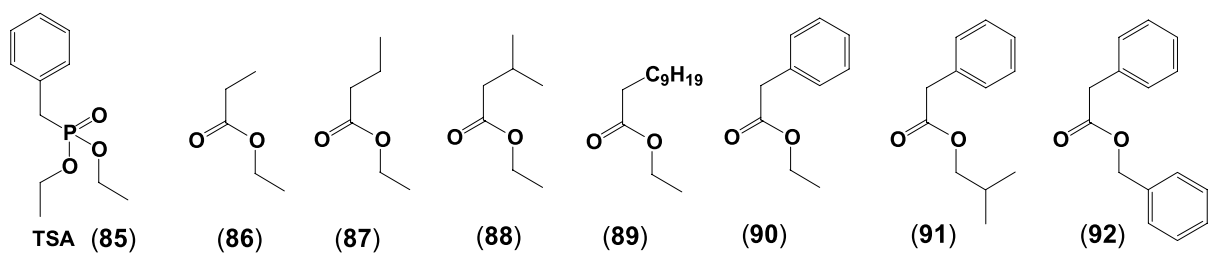
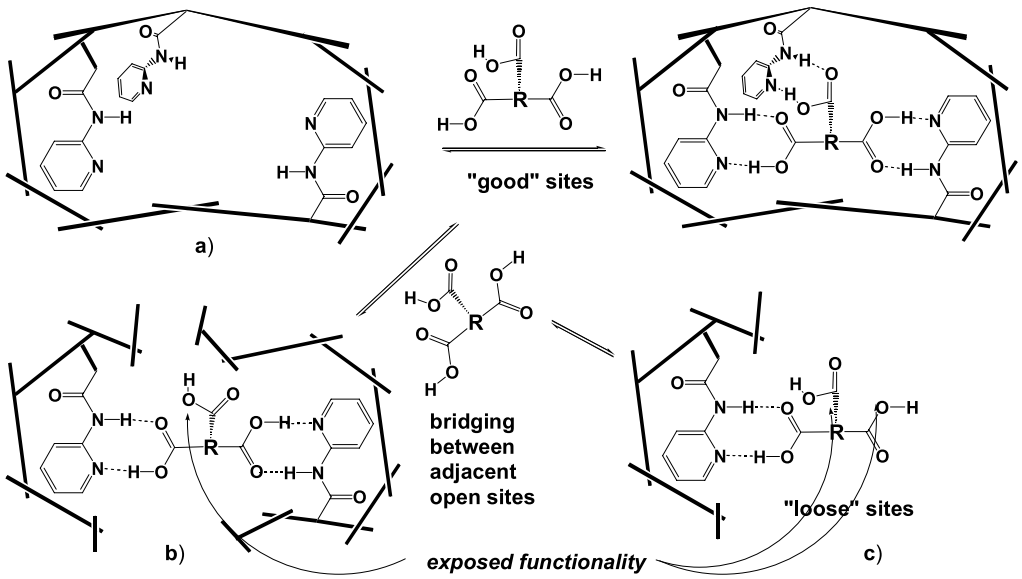



**Figure 7.** Phosphonate TSA imprinted by Suzuki et al.[153] using the controlled CVD hydrolysis method, and esters (**86**–**92**) hydrolysed by the supported silica MIPs produced. The highest rate enhancement was found for the ester (**88**) which was of similar calculated cross-sectional area to the observed imprinted pores.



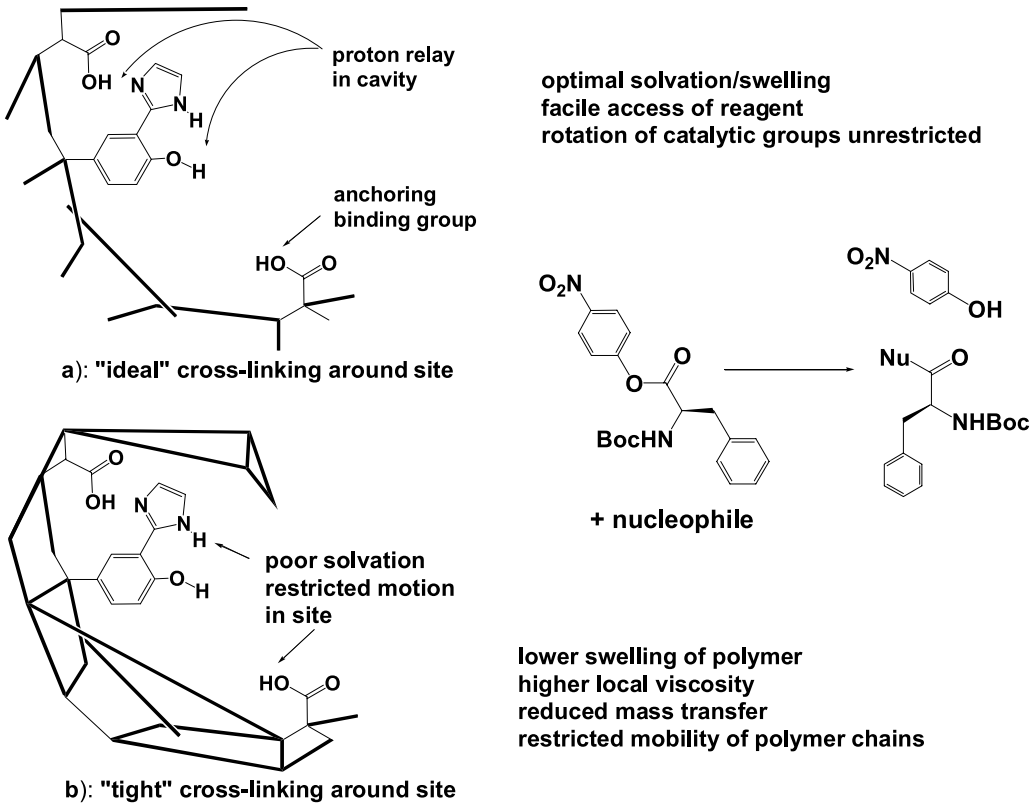

**Scheme 32.** Schematic of productive and unproductive binding in MIP cavities. In (a), binding takes place in an isolated cavity via all desired interactions, leaving no attachment points or reactive functionality. By contrast, in (b) binding sites are open and closely positioned together in space and the template can bridge adjacent sites. For (c), exposed surface sites cannot satisfy all the template functionality and the site environments (b) and (c) do not therefore enable specific further chemistry to be carried out.

automated solid phase and supported solution phase methods, the need for materials able to hold a reagent in a particular stereochemical environment or conformation during functional group interconversion, or to scavenge specific by-products or effect rapid and selective clean-up becomes ever more apparent: imprinted polymers may soon become the materials of choice for these applications. Admittedly, there are still problems to be solved concerning the accessibility of imprinted sites in bulk MIPs and the retention of stereochemical memory under extreme conditions, but, if these can be solved, imprinted polymers as protecting groups and 'microreactors' are likely to be increasingly used as synthetic tools. The development of 'surface-imprinting' techniques, new and more active or specific functional monomers and more refined imprinting matrices and formats will undoubtedly help to address some of the current disadvantages of these materials.

There is also an increasing awareness of the need to understand the processes taking place in imprinted polymers at a more mechanistic or molecular level of detail, and to characterise these materials rather better if they are to be adopted by synthetic organic and medicinal chemists, or by industrial scientists. A number of important papers have been published in which exceptionally rigorous analyses of highly complex chemical transformations on imprinted polymers have been carried out.[21,47,48,60,101,102,148,149,154–162] and the lessons from these need to be considered carefully. The heterogeneity of conventional MIP binding sites is well known, and the practical consequences have been addressed from both a thermodynamic (batch re-binding) and kinetic (chromatographic separations) standpoint[163–169] but it is highly significant where applications in synthesis and especially catalysis are considered. It is generally accepted that in order to define a binding site, at least two points of attachment are needed and, for true stereochemical recognition, a minimum of three independent interactions are required—ideally these will be from the same cavity. For the high site densities which may be required if MIPs are to be used as supports for synthesis, however, it is important that these imprints are sufficiently isolated to ensure that ligands do not 'bridge' adjacent cavities and thus bind in an unintended (and likely unproductive) fashion. In the hypothetical example shown in Scheme 32, heterogeneous site distribution leads to undesired binding events: if chemical transformations were carried out on these ligands in the sites, the wrong functionality may be modified either due to their being exposed rather than bound, or as a result of reaction in solution following dissociation from low-affinity cavities.

A second important aspect of MIP structure is that, ideally, the imprinted sites should all be rapidly and equally accessible, such that mass transfer or kinetic factors do not control binding or chemical transformation events. A clear target for MIP research is therefore to ensure homogeneity ('monoclonality') of imprinted cavities, not only in terms of their microenvironment (geometry, placement and orientation of functional groups within the sites), but also in terms of their 'presentation', i.e. their positioning in or on a polymer backbone/support such that their macroenvironment is the same. This last point is often overlooked in a discussion of molecular imprinting, partially because it has not yet been possible to achieve absolute site homogeneity in any case, but such a situation might be envisaged where pre-formed binding sites are subsequently assembled into a cross-linked network. In this case, a kinetic preference for recognition events at surface or near-surface sites would not only occur, but would be detectable and perhaps also be useful synthetically for selective binding or modification chemistries.

Binding site heterogeneity is not generally found in individual natural macromolecules (with the exception of certain glycopolymers) and, of course, proteins have very highly-specified and localised binding pockets that are




**Scheme 33.** Schematic to show the origin of heterogeneity in catalytic sites. Cavities of optimal cross-linking allow the access of reagent and the desired degree of rotation and movement of functional groups in the site, whereas more tightly-cross-linked cavities constrain tethered functionality and are less able to swell, rotate or move.

easily and rapidly accessible to substrates. Importantly, biopolymers possess greater or lesser degrees of conformational flexibility, which in the case of enzymes allows maximal favourable binding interactions to take place with substrates (induced fit or allosteric effect). By contrast, conventional imprinted polymers are highly cross-linked and rigid materials, and chain-chain motion, rotation and relaxation processes are very slow. When this is combined with solvent effects in the rigid cavities, high local viscosities are likely, and these will vary with differential polymer swelling across the network, further complicating the deconvolution of any chemical events taking place in the sites. Two very interesting approaches to make MIPs more like enzymes have recently been developed, one via the use of reversibly collapsing and expanding polymers in the MIP backbone to produce responsive hydrogels,[170–172] and the second method involving the preparation of microgels that are essentially single (and soluble) cross-linked macromolecules with 10–100 binding sites per polymer.[173] Whilst neither of these methods has yet been adopted for applications in synthesis or catalysis, they are nevertheless important steps on the route to producing synthetic materials that are truly biomimetic in terms of dynamic behaviour or binding site characteristics. Until these new formats of imprinted polymers become more established it is likely that the development of MIPs as synthetic aids will continue to be in the areas of reaction scavenging and directed synthesis where monodisperse binding site structure may not always be required, e.g. in the cleaning-up of reactions with multiple similar by-products.

The most important issues in MIP catalysis also stem from binding site heterogeneity and polymer microstructure, as the most highly active sites may not always be accessible, and the least selective or most open sites may be the most 'visible' to ligands. In the latter case the low selectivity sites will exert a disproportionate influence on the reaction products. Secondly, quite marked effects on the reaction chemistry can be observed simply as a consequence of changing between a homogeneous and a heterogeneous phase, and microstructural factors such as local solvation/swelling will then have an impact on the activity of catalytic sites across the polymer matrix, e.g. as might occur in a chymotrypsin mimic (Scheme 33).

In addition, as outlined very clearly by Wulff,[174] there are a number of quite specific phenomena associated with catalysis that need to be taken into account before MIPs find widespread use in catalytic processes. Enzyme-like Michaelis–Menten kinetics are often reported for MIP catalysts, but the very high substrate selectivities of natural polymers are rarely obtained with imprinted polymers. Does this mean that the catalytic site has arisen from a true molecular imprint? It is possible, for example, that close and catalytically useful 'clustering' of functional groups can occur at random in these heterogeneous polymers and these may be more active because of local ordering, p$K_a$ variation or solvation, rather than definitive ordering around a template. Even where the 'control', that is the non-imprinted polymer, exhibits a lower catalytic activity this does not necessarily mean that true imprinting has occurred,

as the presence of a template may induce changes in the polymer-forming reaction by enhanced solvation of functional monomers and subsequent variations in polymer microstructure as described above. In this respect, a correct control polymer is one wherein the polymer forming reactions are as closely similar as possible, i.e. where the control is built around a second 'template' of like chemistry. An obvious case is the internal control of 'isomeric' MIPs prepared around different template enantiomers. Although by far the most common 'controls' in catalytic studies are the same reactions carried out in solution (and thus, from the discussion above, not directly comparable), there have been notable examples of MIP catalysts and synthetic reactors where these more mechanistically correct control experiments have been carried out[80,175,176] and these have shown an imprinting effect, suggesting that the desired cavities have indeed been generated by templating.

A number of more general issues relating to artificial enzyme mimics have been elegantly expounded in recent reviews[61,177,178] A consideration of the use of transition state analogues, and particularly the fact that these represent points along a reaction pathway, suggests that artificial enzymes, including MIPs, must be able to do more than just stabilise a single species in that reaction path. The need for dynamic response has already been mentioned in the context of biopolymer binding site mimicry, but for catalysis there is the need to introduce an element of encoded dynamic behaviour such that stabilisation of one intermediate leads on to the next in the reaction path—the 'helping hand' referred to by Motherwell et al.[61] Catalysts with functionality and response predisposing sequential stabilisation of the desired intermediate states until products are formed and then leave the site would be truly effective. Methods to address this encoded behaviour are being developed, as are dynamic molecular imprints, but combining the two facets has yet to be achieved. The innovative approach to synthetic receptor and catalyst design by Sanders and co-workers via dynamic combinatorial libraries is certainly a significant advance in this direction.[179–184] Although the word 'dynamic' in this sense relates to how the receptor is assembled rather than to its final properties as a catalyst, this strategy is both conceptually elegant and eminently practicable and can in many ways be considered as a solution state analogue of molecular imprinting.

To date, whilst imprinted polymers are not currently as active or selective as biological systems for catalysis, the favourable properties of synthetic polymers compared to their natural counterparts still render MIPs to be of great potential benefit for many applications, and justify further investigation. Of particular importance are the low tolerances of enzymes to extremes of temperature, pressure and pH in comparison to the high stabilities and long lifetimes of MIPs, and the ability inherent to synthetic chemistry of being able to produce novel materials in a variety of formats for specific end use or application.

Ultimately, for the organic chemist, imprinted polymers offer some fascinating possibilities as well as demanding challenges and, as these intriguing materials are better understood, their application in new and diverse areas of synthetic chemistry is certain to grow.

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**Biographical sketch**

**Cameron Alexander** graduated in Chemistry from the University of Durham, UK in 1987 and stayed on at Durham to undertake post-graduate research under the supervision of Professor W James Feast, FRS, receiving his PhD in Organic and Polymer Chemistry in 1990. He then moved to the Melville Laboratory for Polymer Synthesis, University of Cambridge (1991–1992) to work with Professor Anselm Griffin, before taking up Higher Scientific Officer (1992–1994) and Senior Scientific Officer positions (1994–1999) in the Macromolecular Science Department of the BBSRC Institute of Food Research. In January 2000 he was appointed to a Senior Lectureship at the School of Pharmacy and Biomedical Sciences at the University of Portsmouth and in October 2000 commenced an EPSRC Advanced Research Fellowship to investigate 'The Rational Design of Templated Surfaces'. Research interests range from polymer chemistry to medicine, with particular emphasis on the development of novel polymer systems for directing chemical and biological processes at surfaces and interfaces. This has included extensive work in the area of molecular imprinting and polymer self-assembly. Current projects are focusing on 'responsive' and imprinted polymers for control of bioadhesion, molecular recognition and targeted delivery of drugs and biopolymers.

**Louise Davidson** was born in Enfield, UK in 1976. She obtained a first class honours degree in Chemistry from the University of Surrey in 1999 and then joined the Hayes research group at the University of Reading to undertake a PhD degree developing novel molecular imprinted materials. Following completion of her postgraduate studies in October 2002, she joined Castrol as a development chemist within the Product Design and Technology Group at Pangbourne, Reading.

**Wayne Hayes** was born in Wegburg, Germany in 1968 and spent the majority of his formative years in a small village called Eagle near Lincoln, England. He graduated from the Nottingham Trent University in 1992 with a BSc Honours Degree in Applied Chemistry and subsequently undertook a PhD at the University of Birmingham under the supervision of Professor J. Fraser Stoddart F.R.S. In 1996, he moved to the United States of America to undertake a post-doctoral position in the research group of Professor Jean M. J. Fréchet, initially at Cornell University and then subsequently at the University of California, Berkeley investigating functionalised hyperbranched polymeric architectures, free radical polymerisation processes and the use of MALDI-TOF MS for the characterisation of polymer systems. In 1997, he returned to the UK to take up a Lectureship at the Nottingham Trent University and then moved to the Department of Chemistry at the University of Reading in early 1999. He is currently a Lecturer in Organic Chemistry—his research interests focus upon the development of novel macroporous materials for applications in fields such as separation science and biotechnology, the synthesis of novel hyperbranched polymers and in addition, the development of new MALDI-TOF mass spectrometric analysis techniques.