Pergamon

Tetrahedron 57 (2001) 4663–4686

TETRAHEDRON

Tetrahedron report number 569

Recent progress in the design and synthesis of artificial enzymes

W. B. Motherwell,* M. J. Bingham and Y. Six

Department of Chemistry, University College London, 20 Gordon St, London WC1H 0AJ, UK

Received 23 February 2001

Contents

1. Introduction

Enzymes catalyse reactions with remarkable regio and stereoselectivity under very mild conditions. They are a source of inspiration to chemists, demonstrating what could be achieved with a full understanding of the underlying principles of the subject, and have long provided a stimulus for research into synthetic equivalents. The basic principle behind enzyme catalysis: molecular recognition and stabilisation of the transition state of a reaction, was originally proposed by Pauling over 50 years ago[1]and later expanded upon by Jencks.[2] However, despite some impressive advances in the field of artificial enzymes, chemists have yet to fully put this theory into practice to produce a synthetic equivalent which can rival enzymes in rate acceleration, turnover and specificity.

Enzymes have had billions of years to evolve into the sophisticated three-dimensional structures of today. As chemists, we need to concentrate this period into a feasible timescale for research. In recognition of this fact, recent developments in the field of artificial enzymes have tended to move away from the rational design and multistep synthesis of complex molecules where the smallest flaw in conception can have catastrophic results. Instead, current strategies have tended to focus on selection approaches. Advances in the fields of molecular biology, biochemistry and more recently combinatorial and polymer chemistry have all furnished unique and often co-operative solutions to the synthesis of artificial enzymes, and it is the aim of this overview to discuss some of the more recent and diverse approaches taken by organic chemists towards the creation of effective enzyme mimics.

In general these different approaches can be divided into three categories;

The 'design approach'. A host molecule is designed with

Keywords: artificial enzymes; cyclodextrins; cyclophane; enzyme mimics.
* Corresponding author. Fax: +44-207-679-7524;
e-mail: w.b.motherwell@ucl.ac.uk

PII: S0040-4020(01)00288-5

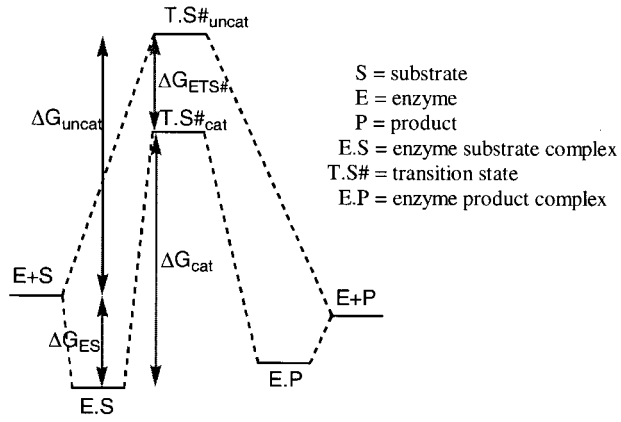


Figure 1.

salient functionality (often also present in the natural enzyme counterpart) which is expected to be involved in catalysis of the chosen reaction. Catalytic cyclodextrins are one such example.

The 'transition state analogue-selection approach'. A library of hosts is generated in the presence of a transition state analogue (TSA) and the best host is then selected from the library. This latter approach has been employed with considerable success in the field of catalytic antibodies and has more recently inspired the process of 'molecular imprinting' (vide infra).

The 'catalytic activity-selection approach'. This takes advantage of the combinatorial chemistry revolution wherein a library of possible catalysts is generated and screened directly for enzyme-like activity.

Although a review on artificial enzymes would not be complete without mention of the more established enzyme mimics such as cyclodextrins and catalytic antibodies, these areas are justifiably deserving of review articles in their own right,[3,4] and only selected examples will be discussed here. Equally, much research into enzyme mimetic systems has been carried out in the field of bio-inorganic and co-ordination chemistry and has been summarised elsewhere.[5] This review, written from the perspective of the organic chemist, will instead concentrate on less developed areas and will conclude with a discussion of some of the more recent developments in 'selection approaches' towards artificial receptors. In particular, dynamic combinatorial libraries (DCLs) and related research will be described with reference to possible applications in the field of enzyme mimics.

2. Transition state theory

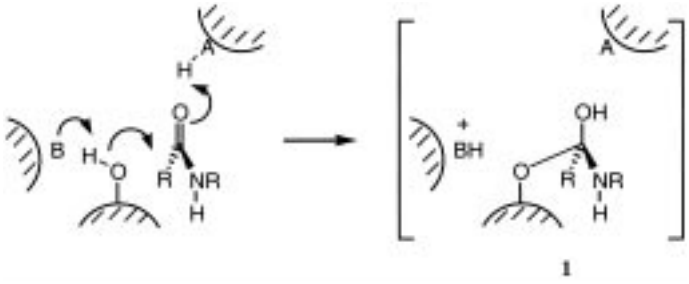
The currently accepted view is that catalysis rests on the enzymes ability to stabilize the transition state of a reaction relative to that of the ground state. This principle is illustrated (Fig. 1) for a unimolecular example where the enzyme–substrate complex is stabilised relative to the free species in solution. The activation barrier to reaction is represented by the difference ΔG_{cat} and ΔG_{uncat} for the enzyme catalysed and the uncatalysed reactions respectively.

It is clear from this picture that, for catalysis to work, the difference $\Delta G_{ETS\#}$ must be larger than ΔG_{ES}. In other words, the enzyme must stabilise the transition state of the reaction more than it stabilises the ground state of the substrate.[6] For true catalysis, a system also needs to exhibit turnover. If the product binds to the enzyme in a significantly stronger way than it binds to the substrate (E.S<E.P), then product inhibition of the reaction can result. In this case substrate binding can be beneficial. The most important consequence of this picture of enzyme action is that the design of an enzyme mimic must not only consider transition state binding relative to substrate binding, but also ensure that the active site is designed such that product release is a thermodynamically favourable process.

The discussion above is a simplification of the real situation since enzyme catalysis of a transformation often involves an alternative reaction pathway from that taken in the non-catalysed process, usually taking advantage of the enzymes ability to reduce the molecularity of multistep sequences. The situation also becomes more complicated for bimolecular processes and reactions involving covalent enzyme-bound intermediates,[7] such as the intermediate **1**, invoked in the initial step of the mechanism for amide bond cleavage by serine proteases (Scheme 1).[8]

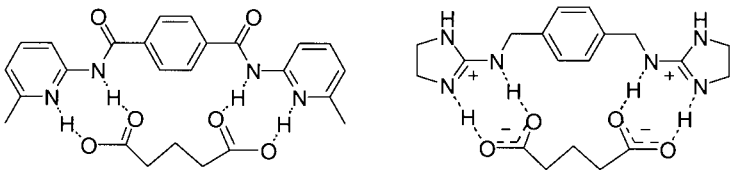
In these more complex systems, application of the above model of transition state stabilisation is less straightforward. However, the foregoing discussion is sufficient to appreciate the basic principle behind many of the various approaches to artificial enzymes.

Perhaps the biggest obstacle to the synthesis of enzyme mimics is that in order to design an artificial enzyme it is necessary to understand *how* enzymes achieve this selective binding of the transition state. Many studies have been carried out in attempts to quantify the contributions of the many weak intermolecular forces involved. In general the



1

Scheme 1.



2 3

Figure 2.

overall binding is a product of electrostatic forces, hydrogen bonding, and cumulative hydrophobic and Van der Waals influences.[7] Since enzymes operate in water, desolvation effects and the resulting entropy changes are also very important factors.[9,10]

Hydrogen bonding and electrostatic interactions contribute significantly to the binding affinity between the substrate or transition state and the enzyme, although, since enzymes operate in water, the contribution of these effects is greatly moderated by solvation. In fact, in some cases, desolvation of both the polar group on the ligand and the complementary group in the enzyme may cost as much in enthalpy as is gained by bringing the two groups together.[7] Many studies into the influence of hydrogen bonding in particular have been carried out, in an attempt to quantify the energy difference gained upon the formation of a ligand–host hydrogen bond in water.[11–13] The value for a neutral–neutral hydrogen bond has been generally found to be in the order of 1.5 kcal mol^{-1}, which represents a perhaps surprisingly modest energy gain.[14] As a result it has been suggested that such interactions 'may play less of a role in enhancing association of the correct ligand than they do in creating a penalty for binding the wrong ligand, i.e., in determining ligand specificity'.[7]

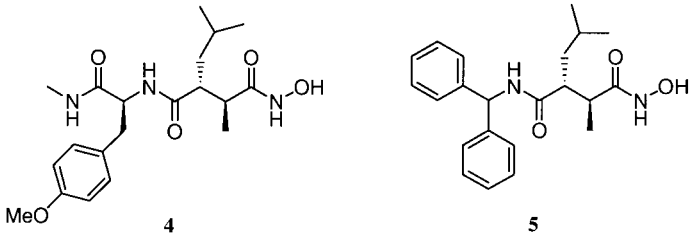
By way of contrast, the contribution of charged hydrogen bonds to binding enthalpy is more significant. This difference between neutral and charged hydrogen bonding is elegantly illustrated by comparison of the two model receptors **2** and **3** for glutaric acid (Fig. 2).[15,16]

Both receptors create the same number of hydrogen bonds with glutaric acid. However, although the neutral diamide **2** binds glutaric acid strongly in choloroform (K_{ass}= 60000 M^{-1}), in DMSO binding is not observed,[15] whilst the receptor **3** which incorporates two charged electrostatic hydrogen bond interactions is almost as good a receptor in DMSO (K_{ass}=50000 M^{-1}) containing 5% THF, moreover, binding is still measurable in the presence of 25% water.[16] In quantitative terms, the presence of a charged hydrogen bond has been estimated to contribute up to 4.7 kcal mol^{-1} to the stability of an enzyme–ligand interaction.[12,14]

More recently the importance of the hydrophobic effect in selective binding has been highlighted.[14] This stabilising influence arises from the transfer of a hydrophobic surface out of water and into contact with a complementary hydrophobic region of a ligand or receptor. The driving force behind this favourable interaction is the beneficial change in free energy as ordered water molecules surrounding the hydrophobic surfaces are released into bulk solvent. The significance of this phenomenon has been beautifully exemplified in a recent review into molecular recognition in drug design.[14] A wide range of examples where hydrophobic interactions play an important role in binding is discussed. Most importantly, the concept of an 'induced fit' in which the receptor undergoes a conformational change in order to optimise hydrophobic interactions with the ligand is emphasised. The fact that the shape of a molecule, rather than it's polar functional groups, is of vital import, has often been overlooked in drug design.

An example can be found in the interactions involved in the complexes of inhibitors **4** and **5** with the matrix metalloproteinase stromelysin.[17] Replacement of the *N*-methyl amide group in **4** by a phenyl ring **5** (Fig. 3) was accompanied by an unpredicted conformational shift of a loop in stromelysin which allowed an unexpected Leu residue to bind to the hydrophobic benzhydryl moiety. This elegantly demonstrates the inherent flexibility of enzymes, which are able to undergo conformational change in order to accomplish binding.

All of the above effects, as well as π-stacking and Van der Waals interactions,[7,18,19] involve binding between discrete ligand and receptor moieties. Perhaps the most difficult binding effects to understand are those interactions at the catalytic centre of the enzyme site between the transition state and the host. This is because the system, and hence the bonding interactions, are dynamic in nature. It is these binding phenomena, termed 'dynamic binding' interactions,



4 5

Figure 3.

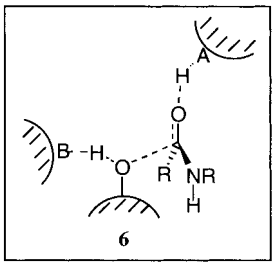


Figure 4.

which distinguish between an active artificial enzyme and a synthetic receptor. To revisit the acyl transfer step in the serine proteases discussed earlier (Scheme 1), Kirby[20] has used this example to illustrate the concept of dynamic binding. The transition state for this step is illustrated in Fig. 4 below, in which at least six bonds are being made and broken.

Although the exact nature of the transition state is unknown the important feature is that the "binding" of this transition state by the enzyme involves more than ordinary molecular recognition. The partially formed covalent bonds at the reaction centre represent "dynamic" binding interactions which have no conveniently modelled ground state counterpart. It can also be expected that these interactions must make a major contribution to transition state binding and stabilisation, not least because they clearly represent a difference between substrate and transition state recognition.

All of the factors described above contribute to the overall binding of the transition state. Despite the many reviews available on the factors which influence molecular recognition and binding in enzyme systems,[19] the practical application of these hypotheses remains the true test of our understanding. It is thus of relevance to investigate the design and synthesis of artificial enzymes since this will hopefully also lead to a better understanding of molecular recognition itself.

3. The 'design approach'

The traditional approach to enzyme mimics has focused on the de novo design of macromolecular receptors with appropriately placed functional groups. These catalytic groups are usually chosen to mimic the amino acid residues known to be involved in the enzyme catalysed reaction. The realisation of ideas in such a process can be an arduous affair and although there are some impressive successes (vide infra), efficient catalysis rivalling enzyme rate accelerations still seems a long way down the line.

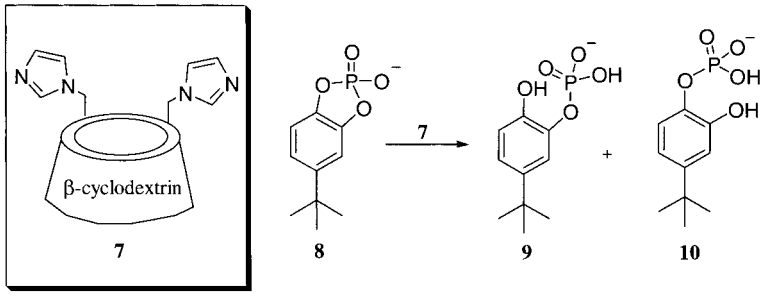
3.1. Cyclodextrins as enzyme mimics

One of the most prodigious aspects of enzyme mechanism is the functional group cooperation often displayed in the active site. The electrostatic environment in the binding site maintains the delicate balance of pK_as required for the various groups to participate in a catalytic fashion. In particular, histidine is often able to function as both an acid and as a base in simultaneous bi or multifunctional catalysis. Intrigued by the challenge of imitating this phenomenon Breslow and co-workers chose to mimic the enzyme ribonuclease A.[21] This enzyme uses His^{12} and His^{119} as its principle catalytic groups in the hydrolysis of RNA.

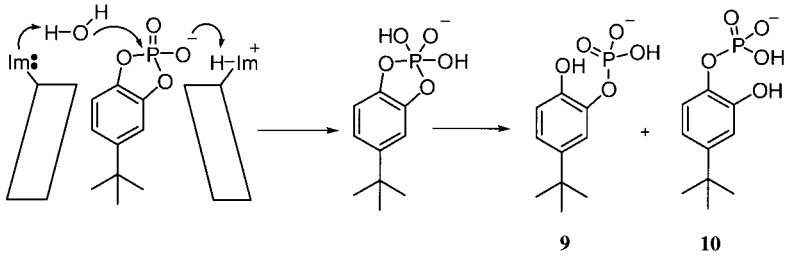
To mimic this enzyme, two imidazole rings were attached to the primary face of β-cyclodextrin as depicted below in Scheme 2. This mimic **7** catalyses the hydrolysis of the cyclic phosphate **8** with a k_{cat} $120\times10^{-5}.s^{-1}$ compared to k_{uncat} $1\times10^{-5}.s^{-1}$ for the uncatalysed reaction and shows greater than 99:1 selectivity for **9**. This is in comparison to the simple solution reaction with NaOH which gives a 1:1 mixture of both products. Isotope effects showed that the two catalytic groups were operating simultaneously and the pH rate profile, which was almost identical to the enzyme itself,[21a] shows that one imidazole functions in its protonated form whilst the other is unprotonated.

The relative positioning of the imidazole groups on the ring of the β-cyclodextrin was found to be crucial. Only when the imidazole groups were attached to adjacent sugars was a single product **9** detected. This regioisomer of β-cyclodextrin was not only more selective but also provided the fastest rate of hydrolysis and displayed the strongest binding to the substrate **8**. Importantly, this result gave information about the mechanism involved since the imidazolium ion in this isomer would be better placed to protonate the phosphate anionic oxygen, which it can access more easily than the other catalyst isomers (Scheme 3).

This observation, when coupled with the evidence that the imidazoles function in a simultaneous co-operative way, allowed the group to postulate that the mechanism was as depicted in Scheme 3 and hence was the same as used by the enzyme, ribonuclease itself.[3a]



Scheme 2.



Scheme 3.

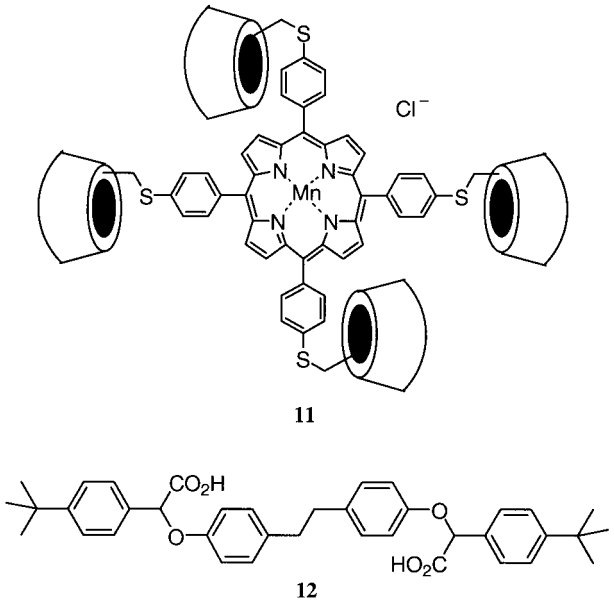
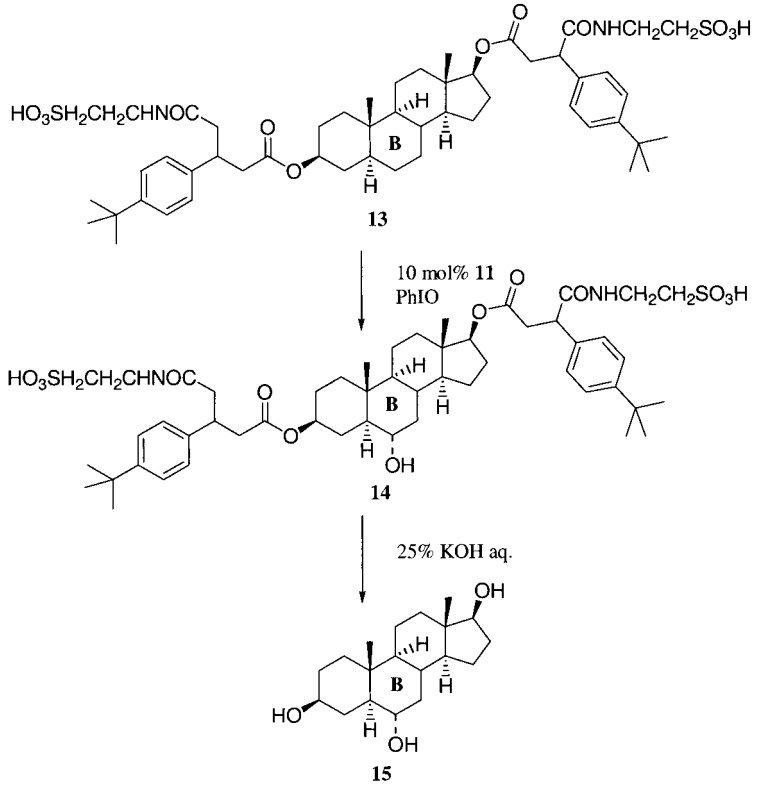


Figure 5.

The use of cyclodextrins as enzyme mimics has been extended to incorporate dimers and trimers of cyclodextrins, as well as a range of transition metal complexes.[3,22] An example demonstrating both these features is the cytochrome P450 mimic **11** (Fig. 5).[23–26] When used with iodosobenzene as co-oxidant the β-cyclodextrin tetramer is capable of the selective epoxidation of stilbene substrates,[24,25] aswell as the hydroxylation of dihydrostilbene **12** at the benzylic position (Fig. 5). [23,26]

However, perhaps most impressively, this mimic is also capable of selectively oxidising the 6-CH_2 position in the B-ring of steroid derivative **13** (Scheme 4).[23,26] Molecular modelling indicated that the two *tert*-butylphenyl groups in the substrate **13** bind into two *trans* β-CD rings of **11** thus placing the steroid B-ring directly above the porphyrin ring. The reaction is carried out with 10 mol% of catalyst **11** and iodosobenzene as co-oxidant. The ester groups are hydrolysed in situ to afford 40% of the oxidised product **15** along with unreacted starting material. This yield corresponds to at least 4 turnovers and although this may be considered



Scheme 4.

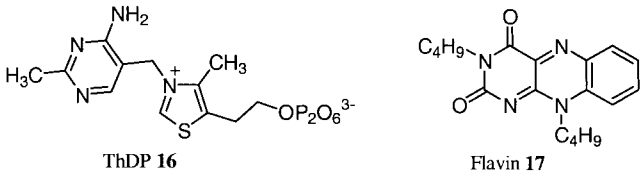
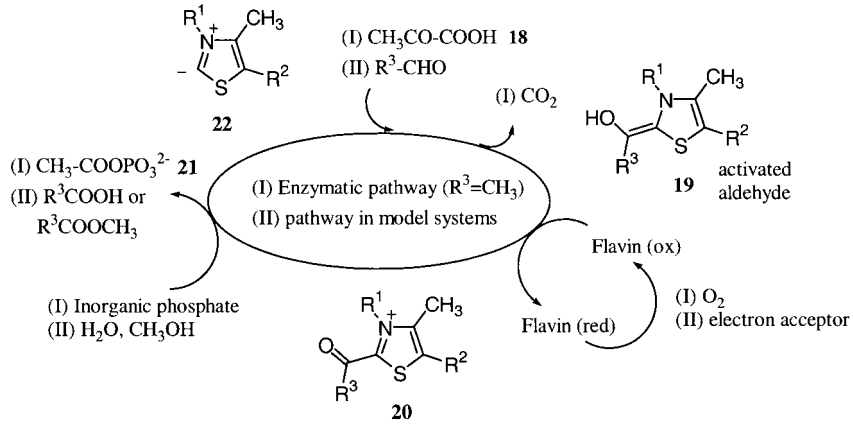


Figure 6.

catalyse the transformation of pyruvate **18** to acetyl phosphate **21** (Scheme 5). The thiazolium group of ThDP forms an activated aldehyde **19** which is oxidised by the flavin to give an electrophilic intermediate **20**. This is then attacked by the inorganic phosphate nucleophile to release the product and regenerate the thiazolium ylide **22** (Scheme 5). In an analogous fashion aldehydes are oxidised by either



Scheme 5.

modest, the example nevertheless demonstrates the power of using designed binding constraints to influence the selectivity of a reaction.

3.2. Cyclophane enzyme mimics

Another impressive application of the design approach is Diederich's pyruvate oxidase mimic.[27] Pyruvate oxidase employs two co-factors ThDP **16** and Flavin **17** (Fig. 6) to water or alcohols to carboxylic acids or esters respectively by simple thiazolium ions in the presence of flavin.

Diederich's pyruvate oxidase mimic **23** combines a well defined binding site with both the flavin and thiazolium groups attached in covalent fashion (Fig. 7).[27] The proximity of the groups to the binding site and the intramolecularity of the oxidation step was therefore expected to improve catalysis relative to previous two component systems.[28] It should also mimic the situation in the enzyme where the cofactors are bound in the enzyme active site thus increasing the effective molarity of the reagents.

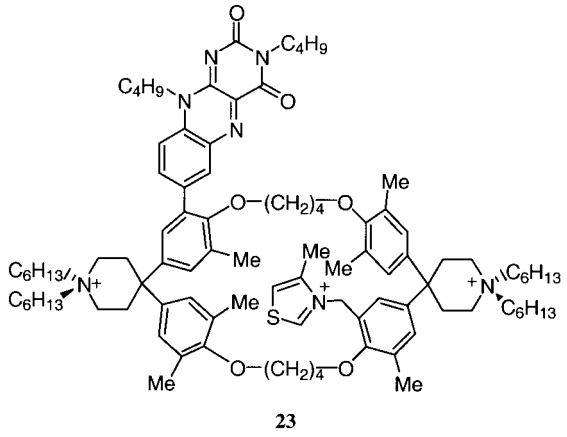
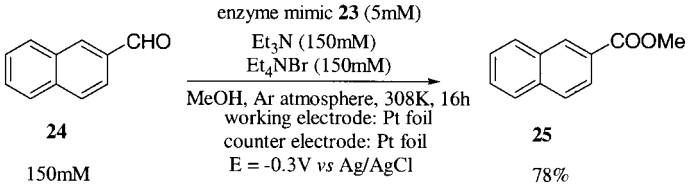


Figure 7.

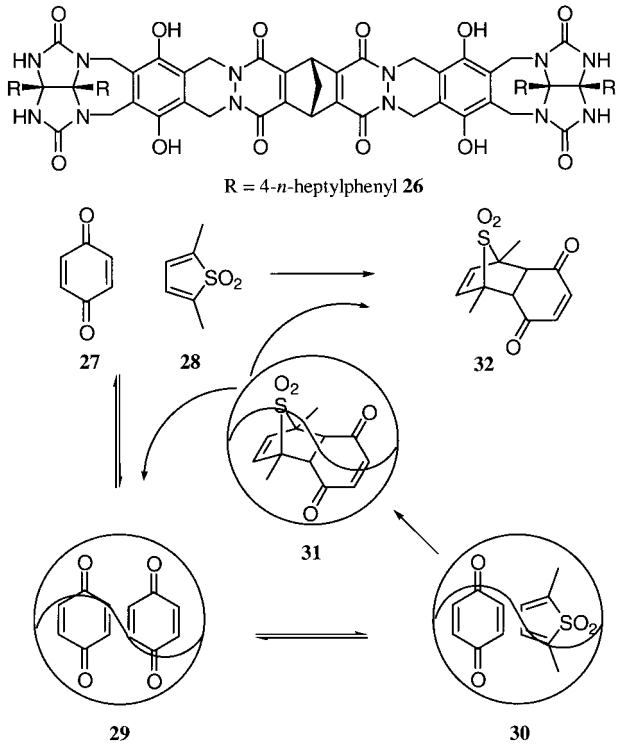
The transformation of naphthalene-2-carbaldehyde **24** to methyl naphthalene-2-carboxylate **25** in basic methanol is indeed catalysed by **23** with a k_{cat} 0.22 s^{-1} (Scheme 6). In order to demonstrate true catalysis the attached flavin derivative needs to be reoxidised in situ. This was achieved by regeneration at a working electrode potential of −0.3 V vs Ag/AgCl. Under these conditions the enzyme mimic **23** is able to act as a catalyst on a truly preparative scale with a catalytic turnover of up to 100 cycles.

3.3. Reversibly self-assembled dimers as enzyme mimics

In a different approach, Rebek et al. have investigated the influence of a designed binding cavity on the rate of the



Scheme 6.



Scheme 7.

Diels–Alder reaction.[29] In this case, no catalytic groups are required. Furthermore, although a Diels–Alderase is known,[30] no natural enzyme catalyst has been isolated or is available for synthetic applications as yet. This is important, since the desirability of designing artificial enzymes for reactions which have no convenient natural enzyme equivalent is self evident.

Rebek and co-workers have carried out much research into the synthesis of reversibly self-assembled dimers.[31] The extended polycyclic system in **26** (Scheme 7) exists as a hydrogen-bonded dimer in organic solvents and adopts a pseudo spherical structure (described as a 'hydroxy-softball') which is able to form and dissipate on a timescale of milliseconds. This dynamic behaviour, coupled with the microenvironment provided by the 'softball' led Rebek et al. to investigate the catalytic potential of **26** towards the Diels–Alder reaction of thiophene dioxide **28** and benzoquinone **27** (Scheme 7).[29]

Earlier attempts at Diels–Alder catalysis for a different reaction were hindered by product binding, necessitating stoichiometric amounts of host.[32] However, in this case, binding studies with product **32** showed that the adduct is an unwelcome guest and is driven out of the cavity by benzoquinone. This strong preference for benzoquinone is however, also a problem since the resting state of the species in solution is **29**. However, true catalysis and turnover were observed in the desired Diels–Alder transformation when compared to a reference reaction in the presence of an isomer of **26** which was unable to form a dimer.

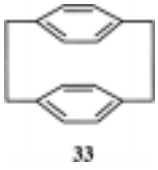


Figure 8.

That the reaction was taking place in the capsule was confirmed by the addition of the competitive inhibitor **33**, known to be an excellent guest for the 'softball' **26** (Fig. 8). Several other examples of both inter and intra-molecular Diels–Alder catalysis have been reported with designed enzyme mimics including β-cyclodextrin catalysis[33] and the porphyrin trimers of Sanders et al.[34] These along with many other highlighted examples of the design approach have been discussed in greater depth in several excellent reviews on the subject.[35]

4. The 'transition state analogue-selection approach'

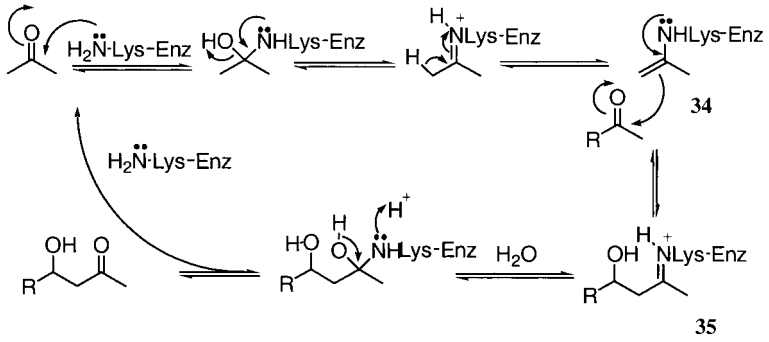
The traditional approach to enzyme mimics is the design approach described above. Whilst this has furnished us with much information on the recognition processes involved in binding and the criteria required for successful catalysis, the realisation of a project from original conception to experimental studies on an enzyme mimic can be a long and laborious process. A case in point is Diederich's pyruvate oxidase mimic (Section 3.2) which required an 18 step synthesis of the host **23**. In an attempt to move away from this linear approach, several techniques have been developed which make use of a selection strategy. This allows for the simultaneous screening of a wide range of possible candidates thus significantly reducing the time required and hopefully allowing for the detection of better hosts.

The earliest examples of a selection approach chose affinity for a transition state analogue (TSA) as the screening criteria. The logic behind this is that any macromolecule which shows strong binding to a molecule resembling the transition state of a reaction, should also bind to and stabilise the real transition state. As this stabilisation of the transition state is the basis behind enzyme catalysis, the hosts selected should behave as enzyme mimics for the chosen transformation.

More recently, it has been recognised that TSA binding alone may not be enough to obtain the rate accelerations needed to rival enzyme catalysis. Nowadays, the incorporation of catalytic groups in the host is often a designed aspect of the selection process and it is this, in combination with the TSA host selection, which has led to some of the most impressive advances described below.

4.1. Catalytic antibodies

The most established applications of the above TSA selection strategy lie in the field of catalytic antibodies, pioneered by Lerner[36] and Schultz[37] in the mid eighties. The immune system generates a natural library of hosts, known as antibodies, in response to the introduction of a foreign molecule into the bloodstream. Advances in molecular biology



Scheme 8.

techniques, notably the process of isolating monoclonal antibodies, allow the selection of a chosen antibody library member on the basis of function.

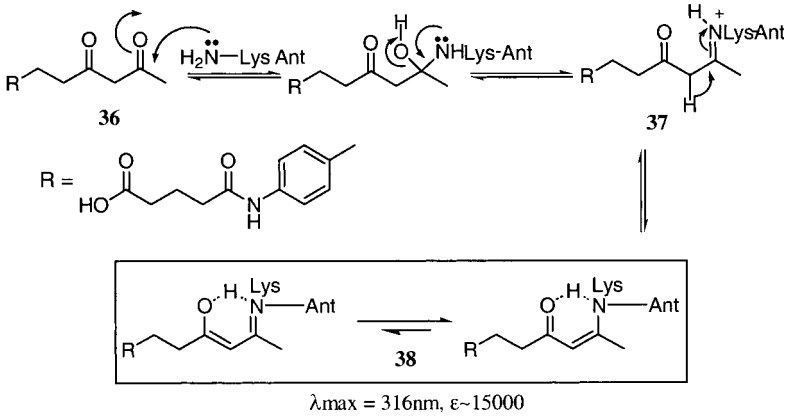
Traditionally, catalytic antibody technology focussed on a purist TSA approach: the TSA was designed, synthesized and then used as a hapten in immunisation (a hapten is a small molecule attached to a carrier protein which is used to stimulate the immune response). The desired monoclonal antibody was then selected from the polyclonal population on the basis of binding affinity to the TSA. Early efforts produced a range of successes in various synthetic transformations, affording artificial antibody catalysts for ester hydrolysis,[36,37] the Diels–Alder reaction,[38] cationic cyclisations,[39–41] cyclopropanation,[42] elimination reactions,[43] the oxy-Cope rearrangement,[44] and an allylic sulfoxide–sulfonate rearrangement,[45] amongst others. However, rate accelerations have always fallen short of their enzyme catalysed equivalents. Furthermore, detailed mechanistic investigations often revealed that a mechanism other than that originally assumed for the design of the TSA was involved.[46] This has important consequences. Since the selection event is based on binding to the TSA and not on the basis of catalytic activity, the antibody selected may not be the best catalyst. The transition state is after all, not a discrete molecular entity and any TSA can only be expected to be an approximation of the true charge distribution required.

Within the last few years, there have been significant advances in the field of catalytic antibodies using the process of 'reactive immunisation'. In this method, the selection criterion is changed from simple binding to chemical reactivity. Illustrative examples are the antibody aldolases 38C2 and 33F12.[47]

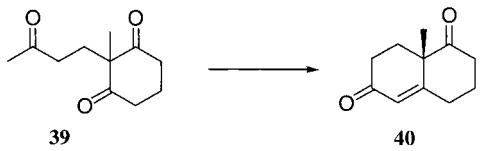
The natural aldolase mechanism was known to operate as shown in Scheme 8.[48] A lysine residue in the enzyme active site forms a covalently bound intermediate with the substrate. Initial attack by the lysine amino group affords a carbinolamine which eliminates water to give a Schiff base imine. This can tautomerise to give an enamine **34** capable of reacting with a further equivalent of the carbonyl substrate to give the Schiff base **35**. Hydrolysis with water furnishes the aldol product and regenerates the active lysine group of the enzyme to complete the catalytic cycle. The Lerner group reasoned that if their selection criteria was the ability to form an enamine intermediate such as **34**, then the antibodies chosen should hopefully act as artificial aldolases with a similar mechanism. The challenge was then experimental; how to observe the covalently bound antibody intermediate.

The enamine intermediate **38** of the 1,3-diketone **36** (Scheme 9) is a vinylogous amide due to the presence of the β-carbonyl group. This vinylogous amide **38** has a strong ultraviolet absorption at (316 nm) which is outside the range of the protein. The rationale was thus to generate a library of antibodies against diketone **36** and to select successful candidates on the basis of their ability to absorb in this region.

Two catalytic antibodies isolated in this manner, 38C2 and



Scheme 9.



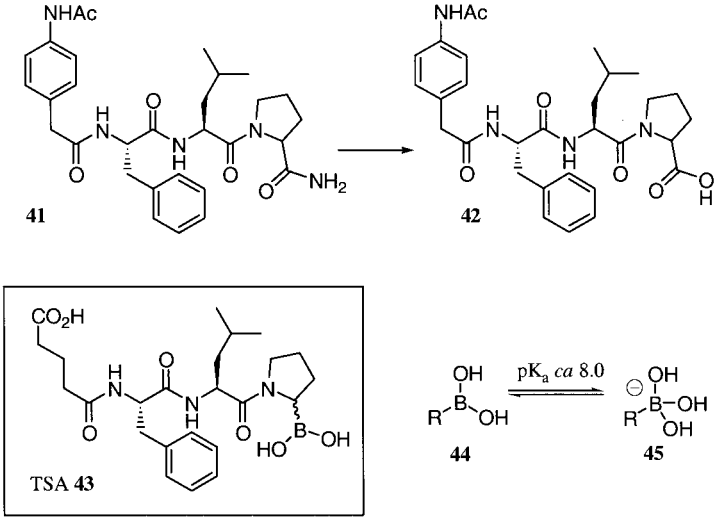
Scheme 10.

33F12 were broad in scope, catalysing over 100 different aldol additions involving aldehyde–aldehyde, ketone–aldehyde and ketone–ketone transformations. The spectacular example below of the formation of the (*S*)-Weiland–Miescher ketone **40** operates with a rate acceleration of k_{cat}/k_{uncat} 3.6×10^6 and affords an enantiomeric excess (ee) of >95% (Scheme 10).[47b]

Not only do these catalytic antibodies accept a wider range of substrates than their natural enzyme counterparts, the catalytic turnover achieved by these antibodies is within 10 times that of the natural enzyme for the optimal substrates of each.

The mechanism of action was shown to be the same as for the natural aldolase. This is significant since the mechanism involves a nucleophilic lysine residue, a group which would usually be protonated at physiological pH. The origin of catalytic activity in the antibody is believed to derive from a greatly perturbed lysine residue in the binding pocket. Cloning and sequencing of the 33F12 genes allowed the group to obtain enough material for an X-ray crystal structure. This indicated that the active Lys residue is contained within a hydrophobic pocket which may explain its enhanced nucleophilicity.[47a]

In a very recent example, which employs a novel selection method involving construction of a Fab (The Fab fragment is the antigen binding domain of an antibody) library, Janda et al. have reported the isolation of a catalytic antibody for primary amide bond hydrolysis[49] as shown for the conversion of **41** to **42** in Scheme 11. The hapten **43** chosen for immunisation contains a boronic acid group **44** which is in equilibrium with the tetrahedral hydrate **45** (Scheme 11). This latter moiety is expected to mimic the transition state for the addition of water to a carbonyl centre. Moreover, the



Scheme 11.

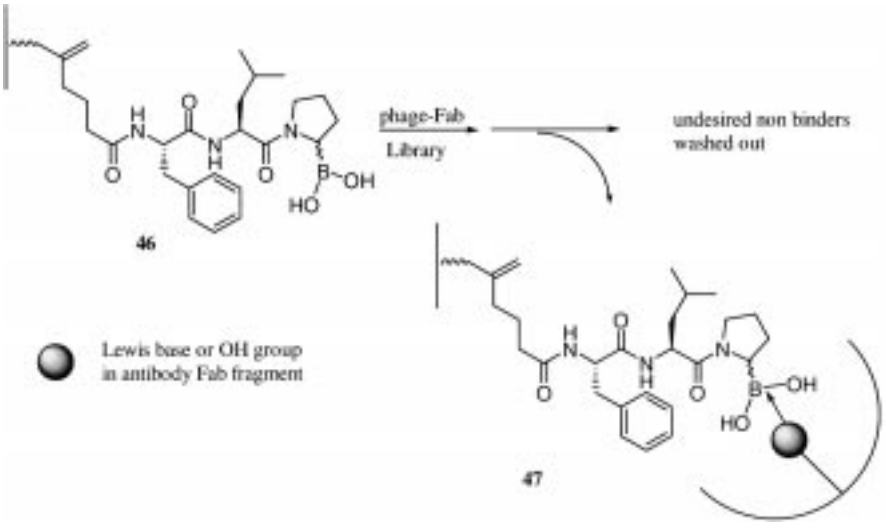


Figure 9.

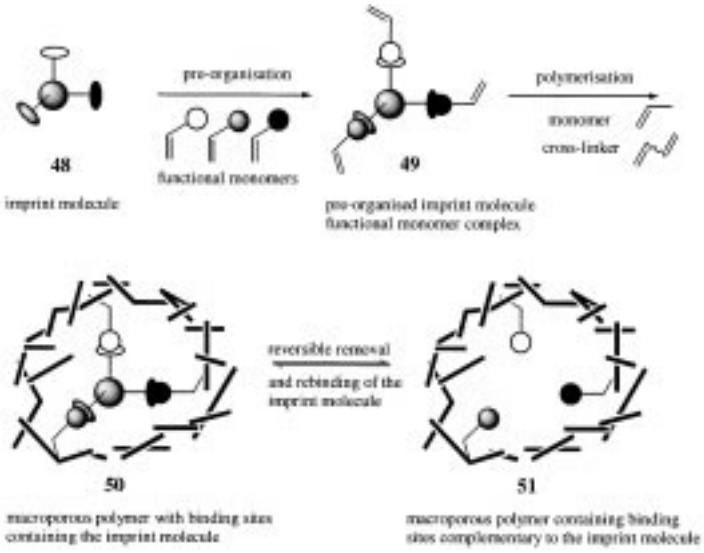


Figure 10.

boronic Lewis acid hapten could form this tetrahedral intermediate by a covalent interaction with a complementary Lewis base or serine hydroxyl in the antibody binding site. This immobilising interaction was used as a basis for the screening protocol.

Immunisation and subsequent manipulation produced a Fab library expressed on phages which was exposed to immobilised hapten **46** and incubated. After washing off the non bound Fab library members the selected candidates could be isolated by washing with acid, since at pH 2.2 the equilibrium of the boronic acid groups should be shifted towards the free acid (Fig. 9). Isolated library members were then amplified and tested for catalysis. The Fab fragment BL-25, isolated in this manner reduces the half life of the primary amide **41** (Scheme 11) from ca 17.5 years to 3.9 h which is greater than two orders of magnitude higher than for a catalytic antibody elicited using a phosphinate TSA. Studies into the mechanism used in the antibody are still in their early stages, although preliminary results suggest that the catalytic power of BL-25 is derived from transition state stabilisation of the anionic tetrahedral intermediate.[49]

Notwithstanding these impressive achievements some problems still remain with the implementation of catalytic antibody technology. The necessity of using mice to generate the antibodies is undesirable, furthermore, the molecular biology techniques involved are highly specialised. Most of all perhaps is that the effort required for the isolation of a monoclonal antibody can be a very time consuming process and, once a truly catalytic antibody is found and separated, as opposed to an antibody which binds the TSA but does not exhibit catalytic action, it may be a process of many months (to years) before the structure of the active site is characterised.

4.2. Molecularly imprinted polymers (MIPs)

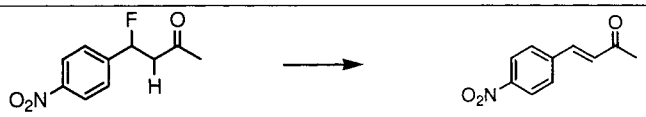
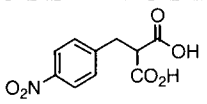
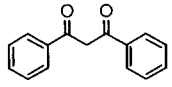
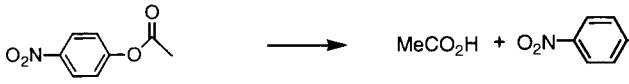
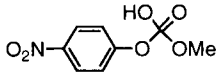
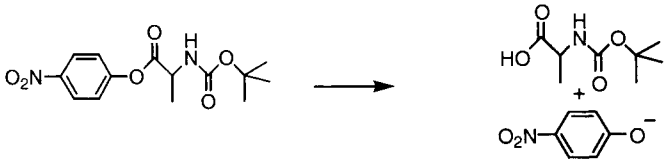
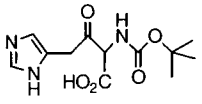
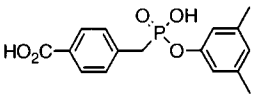
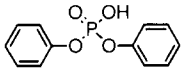
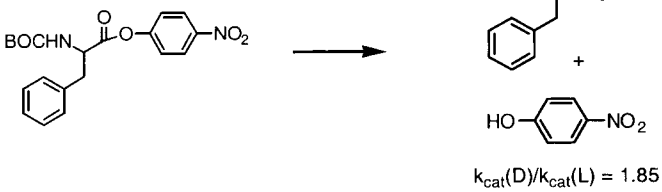
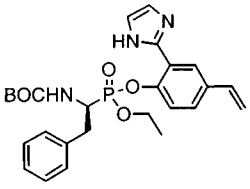
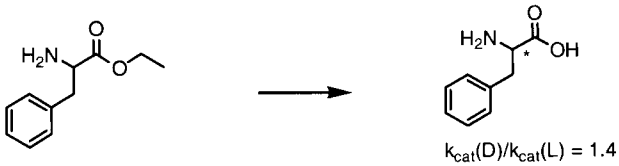
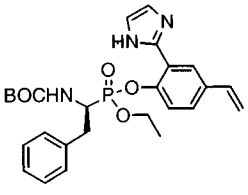
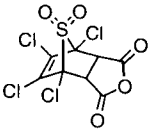
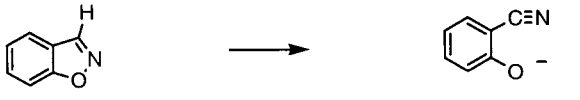
The field of catalytic molecularly imprinted polymers is much younger than its biological counterpart; catalytic antibodies, but the basic concepts underlying both subjects are very similar. Molecular Imprinting[50] is a polymerisation technique which produces macroporous polymers with binding sites which can selectively rebind the molecule with which they were 'imprinted'. If this 'imprint' molecule is a TSA, then the resulting molecularly imprinted polymer's (MIPs), should behave as artificial enzymes for the reaction chosen.

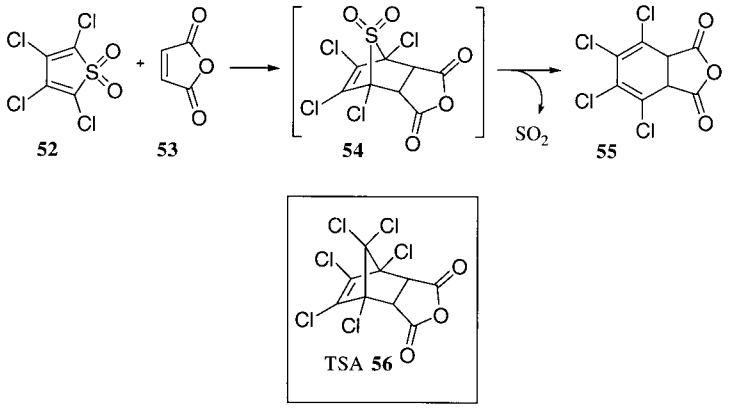
The process of molecular imprinting is outlined in Fig. 10. In the first step monomers containing functional groups which can interact with the imprint molecule, are pre-organised around the imprint molecule **49**. A mixture of standard monomer and cross-linker is then co-polymerised around this imprint molecule monomer complex in a radical polymerisation process, to form a macroporous polymer which contains sites at which the imprint molecule is bound **50**. Finally, the imprint molecule is removed from the polymer to leave well defined, shape specific cavities which are spatially and functionally compatible with the imprint molecule **51**.

The interactions between the imprint molecule and the functional monomers can be either covalent or non-covalent. In the latter case the description pre-organisation can be a slight misnomer, since the combination of weak intermolecular forces involved lead rather to a dynamic associated complex in constant exchange with solution. This, along with other factors, leads to the inherent heterogenearity of the molecular recognition sites produced within the polymer. This has proven to be one of the major sticking points in catalytic applications of MIPs. Despite this drawback, the manifest stability of MIPs when compared to natural enzymes or other artificial analogues, means that the realisation of catalytic MIPs remains a highly desirable goal.

Several successes have been reported exploiting the molecular imprinting technique, however, the scope of catalytic MIPs remains relatively limited. Table 1[51–61] contains a list of organic reactions in which MIPs which have been used as artificial enzymes. In certain cases molecular imprinting has also been used to imprint transition metal catalysts.[62,63]

Table 1.

MIP catalysed reaction	TSA used as imprint molecule	Ref.
		51
		52
		53, 54
		55
		56
 X= NH, O		57
 $k_{cat}(D)/k_{cat}(L) = 1.85$		58
 $k_{cat}(D)/k_{cat}(L) = 1.4$		59
		60
		61



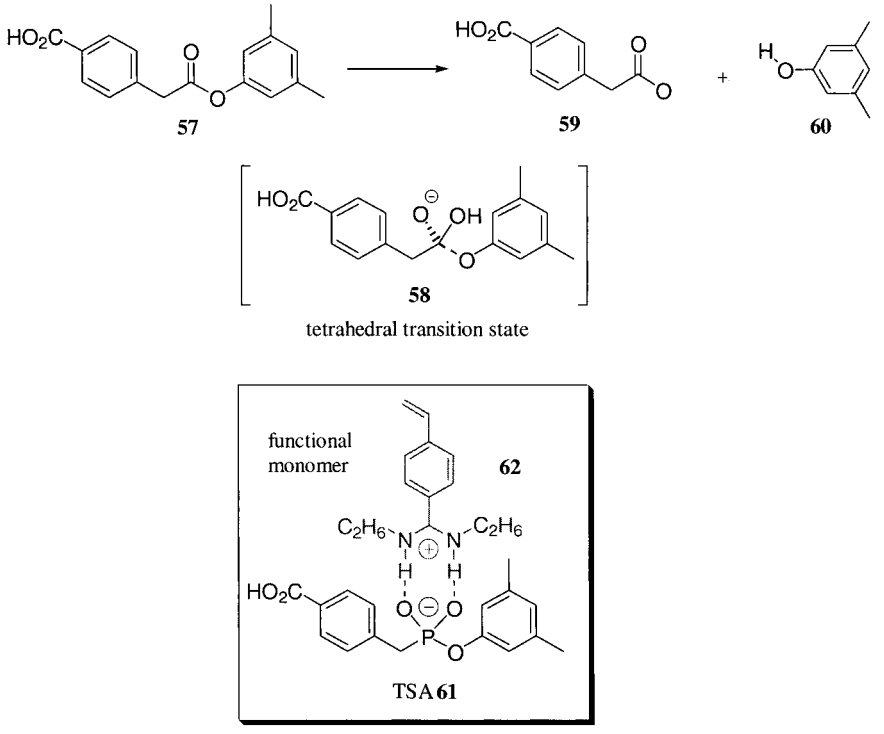
Scheme 12.

An elegant example is the catalysis of the Diels–Alder reaction of tetrachlorothiophene S,S-dioxide **52** and maleic anhydride **53** reported by Mosbach et al. (Scheme 12).[60] The challenge of designing an artificial enzyme to catalyse the Diels–Alder reaction is that the product very often resembles the transition state and can bind to the active site inhibiting turnover. The reaction above avoids this problem since spontaneous extrusion of sulfur dioxide leads to a product sufficiently distinct from the imprinted TSA to be expelled from the active site.

Methacrylic acid (functional monomer), ethylene-glycol dimethacrylate (crosslinker) polymers imprinted with the TSA **56** selectively rebound the imprint molecule and accelerated the rate of reaction with a k_{cat}/k_{uncat} 27. The rate accelerations were corrected to the reaction in the presence of a non-catalytic polymer produced in the absence of the imprint molecule. This rate acceleration is modest compared to the catalytic antibody equivalent,[38] but nevertheless demonstrates the feasibility of this technique.

Promise for future progress comes from the work of Wulff and co-workers[56,57] who recently reported a successful hydrolytic MIP. In recognition that TSA binding alone may not be enough to confer catalytic activity they designed their system to contain an appropriately positioned amidine catalytic group **62** (Scheme 13).[56] This group mimics the active arginine residue in the catalytic antibody equivalent and was shown to bind strongly to phosphonic acid monoesters such as the TSA **61**. Phosphonic monoesters such as **61** are well established mimics of the transition state of ester hydrolysis representing the tetrahedral geometry of the ester intermediate **58**.

The MIPs formed with **61** as imprint molecule caused a 100 fold rate acceleration of the hydrolysis of ester **57** with



Scheme 13.

62 63

Scheme 14.

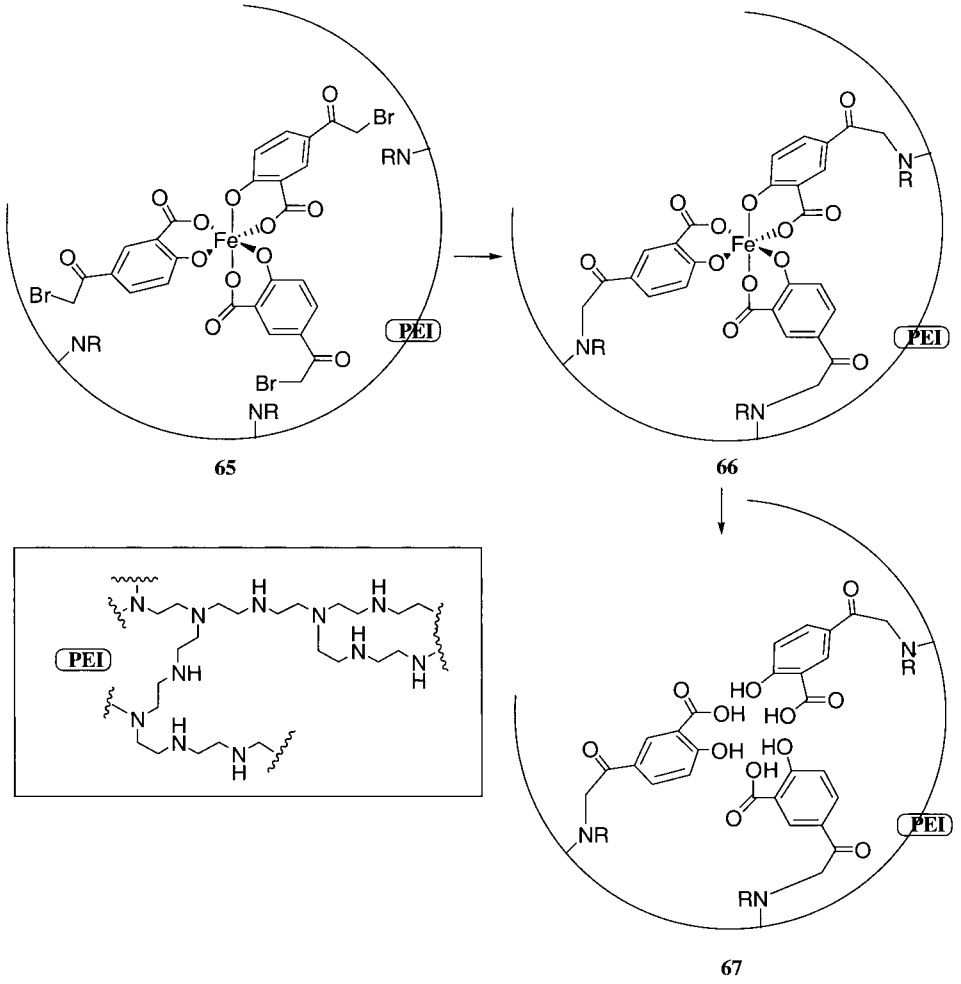
typical Michaelis–Menten saturation kinetics. When a similar interaction to form an amidine–phosphate complex, was combined with the use of suspension polymerisation, the resultant MIPs were able to accelerate the rate of hydrolysis of aryl cabonate **63** and aryl carbamate **64** (Scheme 14) by a factor of 588 and 1435 respectively.[57] Interestingly, the non imprinted polymer, randomly functionalised with the amidine monomer, was also a significantly active catalyst: the rate enhancements corrected to the reference polymer were between 6–24, in marked contrast to the fact that the amidine functional monomer in solution showed negligible catalysis at the reaction pH. It is clear that the effects of local environment are far from being predictably understood. Nevertheless, this is by far the largest rate acceleration for ester hydrolysis achieved with the MIP technique and, by analogy with the trend in catalytic antibody technology, is illustrative of the benefits to be gained by ensuring that an enzyme mimicking catalytic group is incorporated into enzyme mimic TSA binding.

A further confirmation of the benefits of incorporating a catalytic group into TSA binding comes from the MIP chymotrypsin mimic,[59] which incorporated a histidine group into monomer–chiral template binding (Entry 9, Table 1). The resultant polymers were capable of enantioselective ester hydrolysis towards the matching substrate enantiomer.

The majority of applications for MIPs are analytical. However, several other synthetic applications of MIPs which are not strictly enzyme mimics have been reported. MIPs have been used as microreactors containing reagents for selective reductions,[64] as specific adsorbents capable of shifting the equilibrium of a thermodynamically unfavourable enzymatic reaction,[65] and as 'protecting groups' using an external reagent.[66] In all cases, catalysis is not however the goal and stoichiometric quantities of MIP are required.

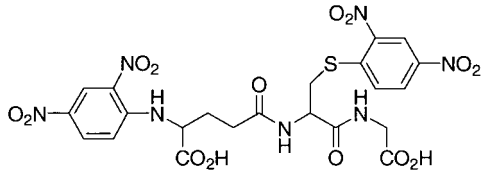
4.3. Imprinting an artificial proteinase

Several other approaches to enzyme mimic's using 'imprinting' have been reported. J. Suh et al. attached an Fe(III) salicylate derivative complex **65** to a polyethylene imine (PEI) backbone **66** (Scheme 15).[67] Subsequent removal of the Fe(III) ion led to a polymer with three salicate groups in close proximity **67**.



65 66 67

Scheme 15.

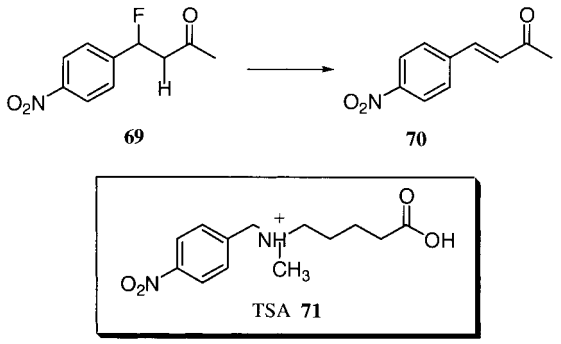


68

Figure 11.

The polymer thus contained regions of high functionality with carboxylate groups, which might be expected to mimic glutamic acid or aspartic acid side chains, and three phenolic hydroxyls as cogeners of tyrosine. The polymer **67** acted as an artificial proteinase in the cleavage of bovine serum albumin γ-globulin Gbn, and displayed substantially higher activity than a randomly functionalised PEI cogener. Both the heavy and light chains of Gbn were cleaved into peptides smaller than 5 kDa at pH7 and 50°C with a half life of 1h, which is in comparison to a half life of amide bonds of ~1000 years[68] in free solution at pH7 and 25°C. Comparable data for the reaction at 50°C or for the randomly functionalised PEI standard was not available.

This approach proved successful in a similar model system which incorporated PEI in a similar strategy.[69]



69 70

TSA **71**

Scheme 16.

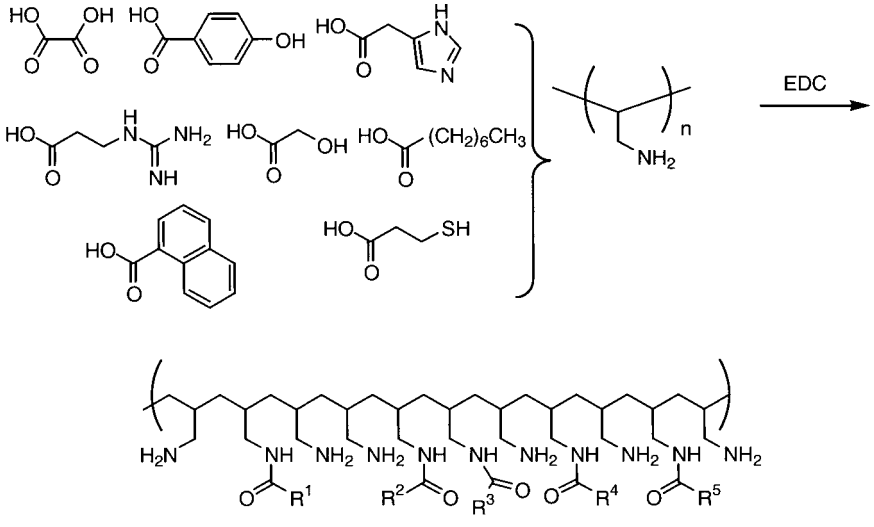
4.4. Bioimprinting

In a different approach, Guimin Luo et al. reported the bioimprinting of a glutathione peroxidase (GPX) mimic.[70] The natural GPX enzyme active site contains a selenocysteine catalytic group and a binding site for the cofactor glutathione (GSH). Using this information Luo et al. 'imprinted' a denatured egg albumin protein with GSH mimic **68** (Fig. 11) and cross-linked the conformation in basic glutaraldehyde solution. The GSH derivative **68** was then removed by dialysis and the protein was treated with NaHSe to create active selenocysteine residues in the binding site. The protein was then purified and tested for activity.

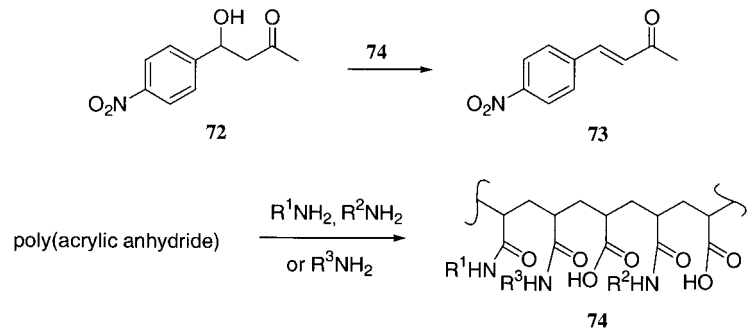
The imprinted protein catalysed the reduction of hydrogen peroxide to water in the presence of co-factor GSH with an 80 fold increase in activity compared to egg albumin treated in the absence of the imprint molecule **68**. Competitive inhibition with the imprint molecule was observed for the imprinted protein whilst addition of **68** had no effect on the reference treated protein, indicating that specific binding sites for GSH were involved.

In a related approach utilising a TSA rather than a co-factor mimic, the bio-imprinting of bovine serum albumin (BSA) to produce a catalyst for a dehydrofluorination reaction was reported.[71,72] The TSA **71** used was expected to interact with a complementary basic group in the protein which would be subsequently suitably placed to abstract the β proton from substrate **69** (Scheme 16). Although the rate accelerations observed were modest k_{cat} 3.3, this experiment demonstrated that it was possible to recruit enzyme activity into a non-catalytic protein.

The various imprinting methods above all share the advantage that the enzyme mimics produced are relatively easy and quick to assemble. In most cases synthesis is a matter of days and systems can be tested for activity immediately. However, at this stage in the development of the subject, rate accelerations which rival catalytic antibodies are still rare.[67]



Scheme 17.



Scheme 18.

5. The 'catalytic activity-selection approach'

Although some ingenious solutions to the design and synthesis of artificial enzymes have so far been described, all of the methods above have the common failing that the desired property of catalysis is not in the screening criteria. As a direct consequence of such an approach effective catalysts may often fail to be detected.

Combinatorial chemistry[73] has witnessed an explosive growth in popularity in recent years and it is now recognised that applications can extend much further than the creation of libraries for medicinal chemistry. In particular, it is now realised that combinatorial methods can be a useful tool in the discovery of effective catalysts.

5.1. Combinatorial polymers as enzyme mimics

In a highly original approach to artificial enzymes, Menger et al. have developed the combinatorial derivatisation of pollyallylamine.[74,75] The basic idea was to attach various combinations of carboxylic acids to polyallylamine backbones and then screen for catalysis in the presence of a metal ion (Scheme 17). The idea that a vast number of molecules can be generated from a very restricted number of initial partners is of particular in interest in terms of evolutionary chemistry.

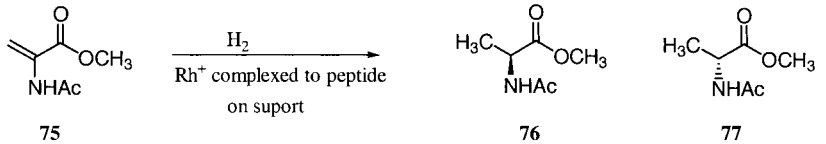
Regions of local organisation are expected on the basis that once a particular residue has attached to the backbone it may influence subsequent neighbouring substitutions. For example, a hydrophobic residue might be expected to favour addition of another neighbouring hydrophobic residue. However, in general the composition of the polymer will be combinatorial with each individual polymer containing a range of metal ion sites. Thus, not only would each polymer made vary from the others in composition, each polymer in itself represents a combinatorial range of sites. As such it seems unlikely that 'the ensemble of countless variations' will ever be deconvoluted. Nevertheless, if the primary goal is catalysis, such a method has proved to be successful in more than one model. Both phosphotase[74] catalysis (k_{cat}/k_{uncat} 10^3–10^4) and the reduction of benzylformic acid ($PhCOCO_2^-$) to mandelic acid ($PhCH(OH)CO_2^-$)[75] have been reported.

Using the same principles in the derivatisation of poly(acrylic anhydride), a combinatorial polymer capable of catalysing the biologically relevant dehydration of β-hydroxy ketone **72** with a k_{cat} 920 over the background reaction was identified[76] (Scheme 18).

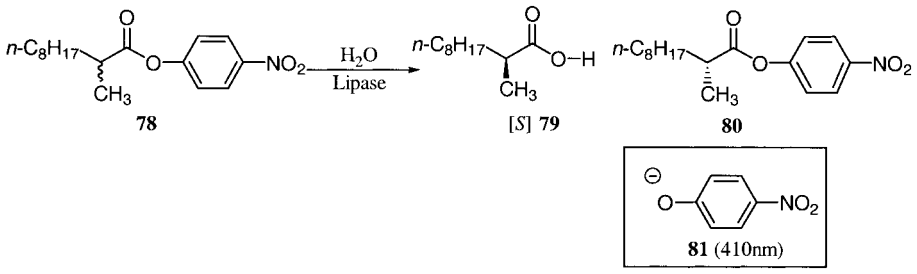
5.2. Combinatorially developed peptide catalysts

In a more conventional application of combinatorial chemistry Gilbertson and co-workers have described the synthesis of a library of phosphine containing peptides.[77] A variable sequence of four to five amino acids including two phosphine derivatised amino acids was incorporated into a basic Ac-Ala-Aib-Ala-[ ]-Ala-Aib-Ala-NH_2 peptide. The peptides were expected to form a helical conformation presenting the two donor phosphine ligands in an appropriate orientation for metal co-ordination. Rh was complexed to the functionalised peptides whilst they were attached to a resin support and each member was screened for it's ability to catalyse the asymmetric hydrogenation of the simple enamide **75** (Scheme 19).

Although the enantiomeric excesses observed were modest (ca 10%), some correlations between positional substitution in the peptide and stereoselectivity were observed, thus demonstrating the potential of this technique for developing catalysts. In a similar manner Hoyveda and Snapper have studied a library of Schiff's base peptides for their ability to catalyse the titanium promoted addition of CN to a variety of imines.[78] The results were impressive; yields and enantiomeric excesses were in the range of 90%, but it seems unlikely that the 'representational search' strategy employed will be applicable to large artficial enzymes since the number of possible permutations of amino acids involved is prohibitively large. The discovery of effective catalysts using such a combinatorial chemistry approach has



Scheme 19.



Scheme 20.

been the subject of several useful reviews, all of which deal with the subject in considerable depth.[79]

5.3. The in vitro evolution of artificial enzymes.

Another application of the 'catalytic activity-selection approach' is the in vitro evolution of enzymes. This method, as in catalytic antibodies, represents the cross-over between molecular biology and chemistry and several examples of artificial enzymes have been reported using this protocol.[80] A recent example is the enantioselective hydrolysis of racemic *p*-nitrophenyl-2-methyldecanoate **78**. The wild type lipase from the bacterium *Pseudamonas aeruginosa* shows an enantioselectivity of only 2% for the (*S*)-configurated acid **79** (Scheme 20). This poorly enantiospecific enzyme was deliberately chosen by the Reetz group for studies into the in vitro evolution of enzyme substrate selectivity.[81]

Using the error prone polymerase chain reaction (epPCR) the lipase gene was subjected to random mutagenesis under conditions such that statistically one to two base substitutions per lipase were introduced. The modified genes were then expressed in a suitable expression vector, amplified in *E. coli* and transformed into *Pseudamonas aeruginosa*. Around 1000 lipase mutants expressed by these bacteria were isolated and screened for enantioselectivity in the test reaction. In order to allow for rapid parallel screening a photometer was developed which could measure simultaneously the absorption of the *p*-nitrophenolate anion **81** at 410 nm over time, in each cell of a 96 well plate. The lipase mutants were thus added to either the (*R*) or (*S*) enantiomer of starting material **78** in a 96 well plate and the level of hydrolysis for each measured. Candidates which favoured the hydrolysis of the (*S*) enantiomer of **78** to give **79** (Scheme 20) were subjected to further mutagenesis and after only four generations an artificial enzyme which hydrolysed the racemic **78** to give an 81% ee of the (*S*) acid **79** was obtained. The screening method used to detect catalytic activity in such an approach is of course a vital element (vide infra). Several other groups have also put the in vitro evolution strategy into practice to produce artificial esterases[82] and artificial cytochrome P450 monooxygenases[83,84] amongst others.[80]

In a related approach to the directed evolution of enzymes, which may also be applicable to antibody libraries, Schultz et al have reported a novel screening method for the catalytic activity selection approach.[85,86] The basic principle is outlined in Fig. 12. In these studies all the enzymes are expressed on solid phase bound phages where the linker used represents the substrate for a desired enzyme catalysed cleavage reaction. Catalytic activity by the enzyme results in cleavage of the phage from the solid support whilst non catalytic counterparts remain bound, thus the population eluted from the solid phase has an enhanced level of enzyme activity for the desired transformation.

In the example described, the phage expressed enzyme is attached to the solid support using a linker containing an oligonucleotide. The enzyme expressed in the model system is SNase, a Ca^{2+} dependent enzyme capable of cleaving single or double stranded DNA or RNA at A-U or A-T rich regions. It should thus be capable of cleaving the oligonucleotide linker and releasing the SNase expressing phage. The group was able to demonstrate the feasibility of the approach; the percentage of phages displaying the SNase catalytic activity was enhanced, relative to the control, in the population released from the solid support.[86] However, the development of such a system is a very involved process and in situations where greater quantities of catalyst are available for screening there are a range of more accessible techniques emerging (vide infra).

5.4. Screening methods for the identification of catalysts in combinatorial mixtures

The detection of catalytic activity in a combinatorial system is not trivial. Much of the progress described above in catalytic selection approaches has been a direct result of technological and analytical advances. Recently many ingenious solutions to the detection of catalysts in combinatorial

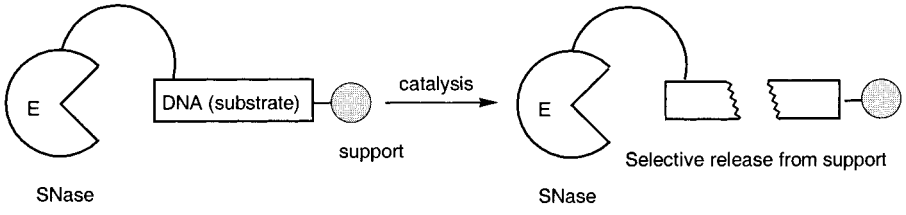
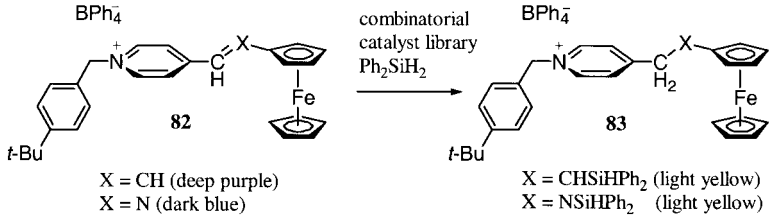


Figure 12.



Scheme 21.

chemistry have been developed and are highlighted in a recent review.[79b]

Whilst the desirability of directly screening for catalysis is evident, how this is achieved quickly, in a parallel combinatorial fashion, is another matter. It is obvious that screening of the classical 96 well plate of artificial enzymes using HPLC is a time consuming and usually rate limiting process. In order to facilitate rate studies and analysis in selection approaches to enzyme mimics, substrates are often designed to release a UV active product, as applied in the evolution of an artificial esterase outlined in Scheme 20,[81] or undergo a colour change upon reaction.[84] This latter screening method is one of the simplest to monitor and has been used with success by Crabtree et al. to detect catalytic activity in the hydrosilylation of alkenes and imines (Scheme 21).[87]

Similarly reactions have been monitored by fluorescence[83] or by colour dependent pH indicators.[82] However, the imposition of such monitoring prerequisites restricts the choice of reaction for study, and recently several ingenious methods which have a broader reaction scope have been developed,[88] particularly mass spectrometry methods,[89] which have even been developed to screen for enantioselectivity,[90] as well as the use of IR thermography.[91,92]

A significant development in both the field of combinatorial catalyst development and artificial enzymes is the time resolved IR thermography of Reetz et al.[93] The technique uses an IR camera to monitor, in parallel, a series of test reactions run in a 96 well plate. The camera provides a spatially addressable picture of the system and is able to identify even modest changes in temperature due to either exothermic or endothermic processes, thus the cells which contain catalytically active components are identified by the camera as 'hot (or cold) spots' respectively. It can be used to monitor a typical 96 well plate of reactions run in parallel and is broad in scope; reactions that can be monitored in this fashion range from lipase mediated acylation reactions to the chiral metal mediated hydrolysis of epoxides.[93] However, the limitation of this method at present, is that it is not capable of quantitative analysis, although the potential speed of parallel detection makes it an attractive concept for future research.

The 'catalytic activity-selection approach' is by far at present, the least studied area of enzyme mimics. This is reflected in the scarcity of examples using this approach. However, the improvements in combinatorial methods and the development of techniques such as IR thermography and improved automation in mass spectrometry suggest that it will rapidly become a growing field of research.

6. Dynamic combinatorial libraries (DCLs)

Since molecular recognition is an important aspect of enzyme action, it seems relevant to discuss some of the recent advances in the combinatorial generation of receptors. Many receptors have been generated by traditional combinatorial methods[94] but several recent examples have made use of dynamic combinatorial libraries (DCLs),

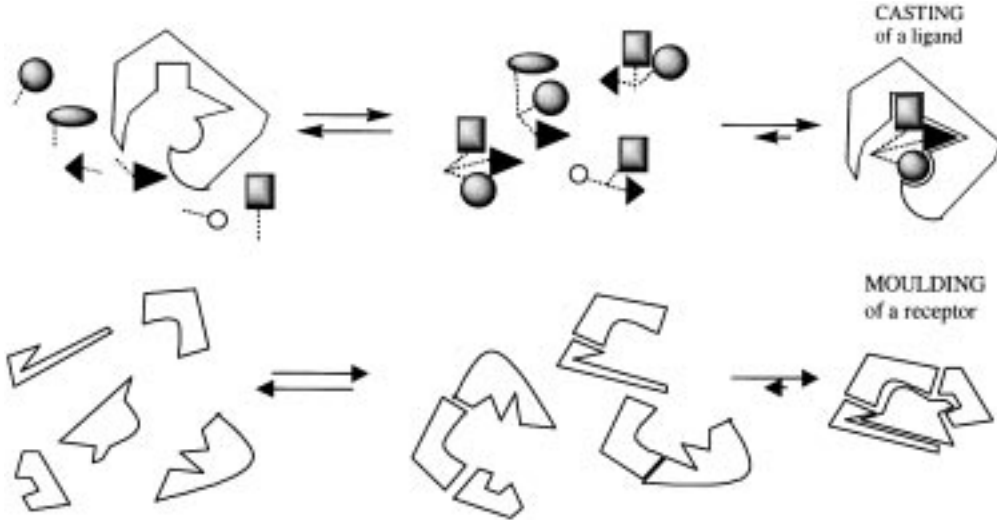


Figure 13.

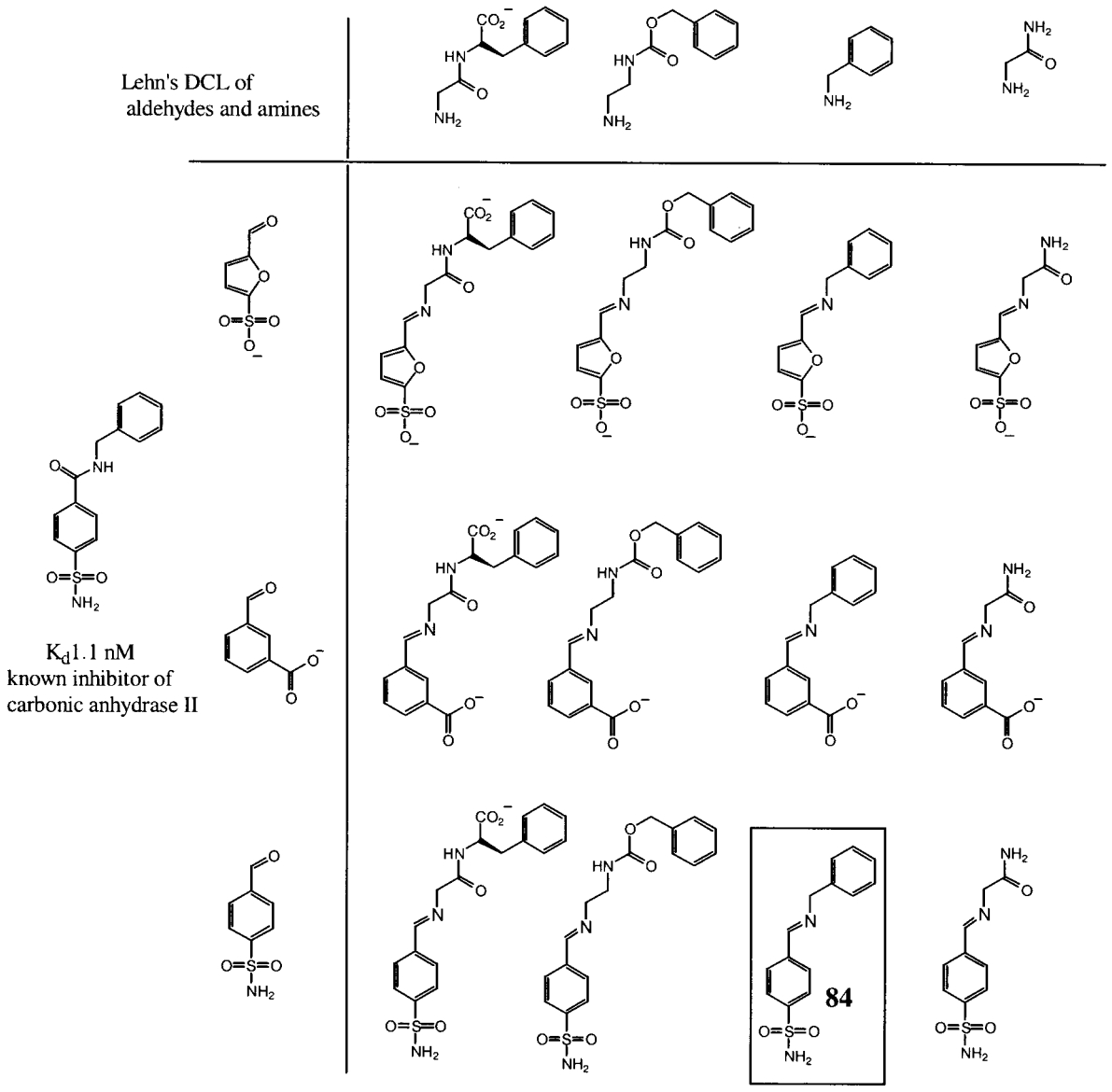


Figure 14.

sometimes referred to as virtual combinatorial libraries (VCLs).[95] In DCLs the library members are homogeneous in solution and are allowed to equilibrate reversibly in the presence of a molecule to which binding is desired. This molecule can be either a receptor or a ligand leading to 'casting' or 'moulding' respectively (Fig. 13).

The freezing out of the desired receptor or ligand requires termination of the reversible association process and can be achieved by a change in temperature,[96] or pH,[96,97] by simply switching off irradiation,[98] or by chemical methods.[99–105] The advantage of this technique is that of all the possible permutations of products possible in the library only the formation of those with the desired properties is thermodynamically favoured. On the down side, the choice of a suitable reversible library is not trivial. Although a recent in depth review of DCLs lists possible reversible reactions,[95] and much research has been carried out on DCLs including reversible libraries of hydrazones,[105,106] alkenes,[100–103] imines based on *o*-aryl and *o*-alkyl oximes,[96] cinchona alkaloids,[107] catenanes,[101] and peptides[108] only a few examples of thermodynamically driven selection have thus far been reported.[109]

Lehn et al. employed an imine–aldehyde exchange reaction to reversibly associate a library of ligands for the receptor carbonic anhydrase II (CAII).[99] *para*-Substituted sulfonamides were known to be effective inhibitors of CAII, thus the library precursors were chosen with this in mind. After equilibration, the library was frozen chemically by the addition of $NaBH_3CN$. Subsequent analysis of the library members showed an increase in **84** relative to the receptor free library which is in accord with previous studies of inhibitors of CAII (Fig. 14).

Using the same concept in the quest for receptors Still[103] used the thiol disulfide exchange reaction to achieve reversible assembly of a small library of receptors. The influence of a solid phase bound tri-peptide on the relative proportions of the receptors in solution was then evaluated.

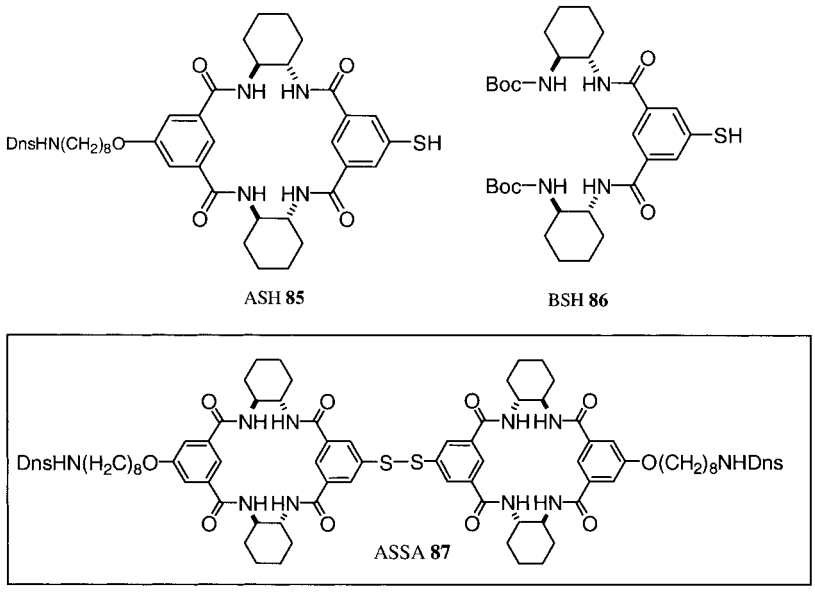


Figure 15.

The receptor ASSA **87** (Fig. 15) was synthesised with a fluorescent sensor and exposed to an encoded combinatorial library of 3375 different *N*-acetyl tri-peptides on polystyrene beads. The peptide Ac(D)-Pro-(L)-Val-(D)-Val-PS was found to bind favourably with a binding constant of the order 10^4–10^5.

A mixture of ASH **85** and BSH **86** was then equilibrated in the presence and absence of the resin bound peptide (Table 2). As can be seen from the table, an equilibrium shift in favour of ASSA **87** was observed when the tripeptide **88** was present. This receptor **87** was conveniently isolated from the beads in >99% purity, allowing easy identification, a detail which will become important if this technique is applied to systems where the structure of the receptor is not known in advance.

Similarly Nicolau has recently reported the target-accelerated combinatorial synthesis of vancomycin dimers.[102] Binding to the Lys-(D)-Ala-(D)-Ala-(D) fragment of the growing peptidoglycan biosynthetic precursor is known to be vital to vancomycin's antibacterial activity. When a range of vancomycin derivatives were dimerised in the presence of the Lys-(D)-Ala-(D)-Ala-(D) fragment, rate enhancements for the formation of favoured dimers correlated well with the observed biological activity. Molecular amplification in dynamic combinatorial libraries has also been observed by the groups of Sanders,[106] Eliseev,[98] and Timmerman and Reinhoudt.[104]

Table 2. Disproportionation of A-SS-B in the presence of Ac(D)-Pro-(L)-Val-(D)-Val-PS **88**

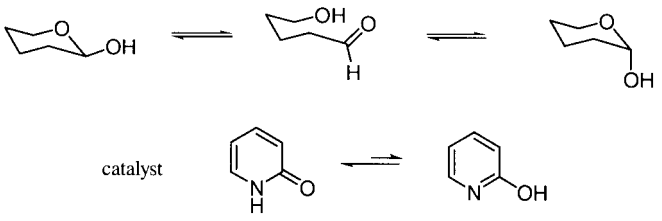
	A-SS-B	⇆	B-SS-B	A-SS-A
Absence of resin bound tripeptide **88**:	43%		57%	
Presence of resin bound tripeptide **88**:	15%		85%	
(i) solution phase	13%		85%	10%
(ii) resin phase	2%		0%	75%

In all these cases, however, the DCL chosen was small and the aim of the research was more to demonstrate the potential of the technique rather than to use it as a tool for identifying useful macromolecules. The use of this approach for the discovery of novel ligands or receptors, as opposed to confirming predicted trends, will now become an experimental challenge.

7. Conclusions

The field of artificial enzymes is a rapidly evolving subject. As the barriers between chemistry and biology become less distinct a range of new methods, which combine expertise from both areas, are developing. In recognition of both the fact that the de novo design approach can be time consuming, and that a tiny miscalculation will be catastrophic, a trend in all these recent techniques is the use of 'selection approaches'. The natural process of selection and amplification is after all, the way in which enzymes have evolved their sophisticated function.

The basic problem in artificial enzyme synthesis is choosing the selection event. Experimentally, selection based on binding affinity is the easiest method to use. However, many methods which have employed binding criteria, using TSAs as the ligands, have stopped short of producing the rate accelerations required to rival natural enzymes. This is perhaps understandable, since the electrostatic and geometric fidelity of a TSA to the real transition state cannot be entirely complete. To a certain extent, the effect of this problem can be alleviated by introducing an element of design into the system. Using information available on the natural enzyme mechanism and catalytic groups, several researchers have improved the rate accelerations available with TSA methods by incorporating catalytic groups into



Scheme 22.

TSA binding as we have seen above with catalytic antibodies and MIPs.

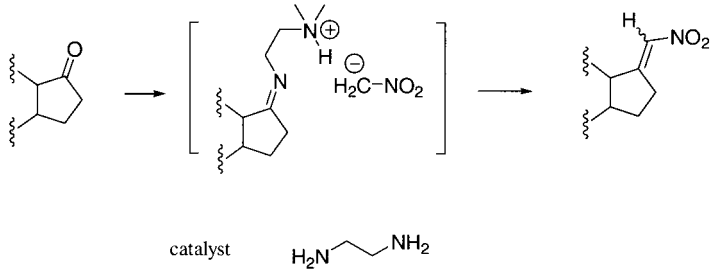
A problem with chemical applications of a binding-selection process can be the vast array of permutations of structures possible. This has been a problem in polymer approaches causing heterogenearity of binding sites, and can hinder combinatorial methods because identification of the desired binder may not be possible with so little material. This latter problem is not so prominent in biochemical equivalents where amplification of the selected candidate is routinely performed. The emergence of dynamic selection approaches in combinatorial chemistry, such as DCLs may solve some of the chemical problems of isolating macromolecular receptors from complex combinatorial libraries. In this case, theory predicts that of all the possible library molecules, only those which favour binding will be produced in significant quantities. This should make deconvolution and identification a much easier process. Moreover, the concept of equilibrating a reversible library of compounds in the presence of a resin bound ligand (TSA), then freezing the reaction, filtering off the solution and isolating a solid phase bound receptor(s) synthesized under thermodynamic control is intellectually attractive. However, the problem with this method, and all others using a TSA strategy, remains the fact that the property required; catalytic activity, is not involved in the selection criteria.

Recent molecular biology approaches (and some combinatorial chemistry methods) have focussed on using catalytic activity as the selection criteria. This strategy has been employed with success in the field of in vitro evolution and has necessitated the development of experimental methods, also common to combinatorial chemistry, which allow the simultaneous screening of many reactions for catalysis. IR thermography and improved automation in mass spectrometry represent important developments in this respect and will probably be influential in future research into artificial enzymes. Whilst in vitro evolution methods use catalytic selection directly it seems likely that catalytic antibody and chemical approaches to artificial enzymes will continue to maintain an element of TSA binding and design. In any event, as always throughout the history of organic chemistry, the inspiration of Nature will continue to challenge the efforts of the chemist at the bench. Much useful information has been gleaned, and more remains to be discovered, perhaps using concepts such as DCLs, but it is clear that rational a priori design of a tailored artificial enzyme for any given reaction will remain as a formidable challenge for some time to come.

Finally, although the foregoing overview has been based on recent advances which have evolved through consideration of Nature's principles, it is interesting, especially in an era of increasing specialisation, to reflect on the field of catalysis in a more general way. The word itself is clearly a highly evocative and attractive one for organic chemists, as witnessed for example by the desperation of free radical chemists to 'catalyse' reactions by the addition of azo-bis-isobutyronitrile (AIBN)!

At the simplest level, however, it is never to be forgotten that, irrespective of whether the objective is to increase the rate of a reaction or to engender a high degree of stereospecificity for a given transformation, a successful catalyst operates by giving a 'helping hand' through initial association with the substrate. Many classic examples such as the influence of 2-pyridone on the mutarotation of tetramethylglucose (Scheme 22),[110] or the bifunctional catalysis involved in ketone nitromethane reactions (Scheme 23)[111] can still certainly repay in this respect.

Within the apparently differing areas of enantioselective transformations induced by enzyme-like mimics or by transition metal catalysts, many of the most fundamental principles of design should still apply to both. The vocabulary and emphasis used by each specialist group is such however, that effective interaction and transmission of useful ideas and concepts is almost discouraged, even though techniques such as combinatorial searches are now used by both. At present, when the transition state or intermediate in a catalysed reaction may involve partial formation and



Scheme 23.

cleavage of several bonds, and such factors as shape, polarity match, electrostatic potential and acidity and basicity as a function of molecular environment all have to be factored in, it would seem timely to devise some new pictorial way of representing and calculating the relative incremental value of these factors for various alternatives. In reality however, although an enormous data set is available, and spectacular advances have been made, catalysis still remains a delightful area of discovery.

Acknowledgements

We are grateful to the Leverhulme Trust for the provision of a grant which has allowed us to begin our exploration of this area.

References

1. Pauling, L. *Chem. And Eng. News* **1946**, 1375–1377.
2. Jencks, W. P. *Catalysis in Chemistry. Enzymology*, McGraw-Hill: New York, 1969.
3. For leading references see: (a) Breslow, R.; Dong, S. D. *Chem. Rev.* **1998**, *98*, 1997–2011.
4. (a) Avalle, B.; Friboulet, A.; Thomas, D. *Journal of Molecular Catalysis B: Enzymatic* **2000**, *10*, 39–45. (b) Wentworth, P.; Janda, K. D. *Current Opinion in Chemical Biology* **1998**, *2*, 138–144. (c) Smithrud, D. B.; Benkovic, S. J. *Current Opinion in Biotechnology* **1997**, *8*, 459–466. (d) Kirby, A. J. Acta Chem. *Scand.* **1996**, *50*, 203–210. (e) Schultz, P. G.; Lerner, R. A. *Science* **1995**, *269*, 1835–1842. (f) Schultz, P. G.; Lerner, R. A. *Acc. Chem. Res.* **1993**, *26*, 391–395.
5. (a) Suh, J. *Acc. Chem. Res.,* **1992**, *25*, 273–278. (b) Chin, J. *Current Opinion in Chemical Biology* **1997**, *1*, 514–521.
6. Menger, F. M. *Biochemistry* **1992**, *31*, 5368–5373.
7. Mader, M. M.; Bartlett, P. A. *Chem. Rev.* **1997**, *97*, 1281–1301.
8. Fersht, A. *Enzyme Structure and Mechanism*, 2nd ed.; W. H. Freeman and Co.: New York, 1985.
9. Lemieux, R. U. *Acc. Chem. Res.,* **1996**, *29*, 373–380.
10. Dunitz, J. D. *Science* **1994**, *264*, 670.
11. Williams, D. H.; Searle, M. S.; Mackay, J. P.; Gerhard, U.; Maplestone, R. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 1172–1178.
12. Fersht, A. R.; Shi, J. P.; Knill-Jones, J.; Lowe, D. A.; Wilkinson, A. J.; Blow, D. M.; Brick, P.; Carter, P.; Waye, M. M. Y.; Winter, G. *Nature* **1985**, *314*, 235–238.
13. Street, I. P.; Armstrong, C. R.; Withers, S. G. *Biochemistry* **1986**, *25*, 6021–6027.
14. Teague, S. J.; Davis, A. M. *Angew. Chem. Int. Ed. Engl.* **1999**, *38*, 736–749.
15. Garcia-Tellado, F.; Goswami, S.; Chang, S.-K.; Geib, S. J.; Hamilton, A. D. *J. Am. Chem. Soc.* **1990**, *112*, 7393–7394.
16. Fan, A.; Van Arman, S.; Kincaid, S.; Hamilton, A. D. *J. Am. Chem. Soc.* **1993**, *115*, 369–370.
17. Rockwell, A.; Melden, M.; Copeland, R. A.; Hardman, K.; Decicco, C. P.; DeGrado, W. D. *J. Am. Chem. Soc.* **1996**, *118*, 10337–10338.
18. (a) Ma, J. C.; Dougherty, D. A. *Chem. Rev.* **1997**, *97*, 1303–1324. (b) Minoux, H.; Chipot, C. J. *J. Am. Chem. Soc.* **1999**, *121*, 10366–10372.
19. Molecular Recognition. Gellman, S. H., Ed. *Chem. Rev.* **1997**, *97 (5)*, 1233–1734.
20. (a) Kirby, A. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 707–724. (b) Kirby, A. *Acc. Chem. Res.* **1997**, *30*, 290–296.
21. (a) Breslow, R.; Schmuck, C. *J. Am. Chem. Soc.* **1996**, *118*, 6601–6605. (b) Breslow, R.; Anslyn, E. *J. Am. Chem. Soc.* **1989**, *111*, 8931–8932.
22. Breslow, R.; Zhang, B. *J. Am. Chem. Soc.* **1992**, *114*, 5882–5883.
23. Breslow, R.; Zhang, X.; Huang, Y. *J. Am. Chem. Soc.* **1997**, *119*, 4535–4536.
24. Breslow, R.; Brown, A. B.; McCullogh, R. D.; White, P. W. *J. Am. Chem. Soc.* **1989**, *111*, 4517–4518.
25. Breslow, R.; Zhang, X.; Xu, R.; Maletic, M. *J. Am. Chem. Soc.* **1996**, *118*, 11678–11679.
26. Breslow, R.; Huang, Y.; Zhang, X.; Yang, J. *Proc. Natl. Acad. Sci. USA.* **1997**, *94*, 11156–11158.
27. (a) Diederich, F.; Habicher, T. *Helv. Chim. Acta.,* **1999**, *82*, 1066–1095. (b) Diederich, F.; Mattei, P. *Helv. Chim. Acta.* **1997**, *80*, 1555–1589.
28. Diederich, F.; Jiminez, L.; Chang, S.-W. T. *Helv. Chim. Acta.,* **1993**, *76*, 2616–2639.
29. Kang, J.; Santamaria, J.; Hilmersson, G.; Rebek, Jr., J. *J. Am. Chem. Soc.* **1998**, *120*, 7389–7390.
30. (a) Lauschat, S. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 289–291. (b) Ichihara, A.; Oikawa, H. *Biosci. Biotechnol. Biochem.,* **1997**, *61*, 12–18.
31. (a) Rebek, Jr., J. *Acc. Chem. Res.,* **1999**, *32*, 278–286. (b) Conn, M. M.; Rebek, Jr., J. *Chem. Rev.* **1997**, *97*, 1647–1668.
32. (a) Kang, J.; Rebek, Jr., J. *Nature* **1997**, *385*, 50–52. (b) Kang, J.; Hilmersson, G.; Santamaria, J.; Rebek, Jr., J. *J. Am. Chem. Soc.* **1998**, *120*, 3650–3656.
33. (a) Hudlicky, T.; Butora, G.; Fearnley, S. P.; Gum, A. G.; Perschini, III, P. J.; Stabile, M. R.; Merola, J. S. *J. Chem. Soc. Perkin. Trans. I* **1995**, 2392–2398. (b) Chung, W.-S.; Wang, J.-Y. *J. Chem. Soc. Chem. Commun.* **1995**, 971–972. (c) Schneider, H.-J.; Sangwan, N. K. *Angew. Chem. Soc.* **1980**, *102*, 7817–7818.
34. (a) Marty, M.; Clyde-Watson, Z.; Twyman, L. J.; Nakash, M.; Sanders, J. K. M. *J. Chem. Soc., Chem. Commun.* **1998**, 2265–2266. (b) Sanders, J. K. M.; Aderson, H. L.; Walter, C. J. *J. Chem. Soc., Chem. Commun.* **1993**, 458–460.
35. For leading references see Ref. 20 (a) and (a) Sanders, J. K. M. *Chem. Eur. J.* **1998**, *4*, 1378–1383. (b) Rowan, S. J.; Sanders, J. K. M. *Current Opinion in Chemical Biology* **1997**, *2*, 483–490. (c) Murikami, Y.; Kikuchi, J.-I.; Hisaeda, Y.; Hayashida, O. *Chem. Rev.* **1996**, *96*, 721–758.
36. Tramontano, A.; Janda, K. D.; Lerner, R. A. *Science* **1986**, *234*, 1566–1570.
37. Pollack, S. J.; Jacobs, J. W.; Schultz, P. G. *Science* **1986**, *234*, 1570–1573.
38. Hilvert, D.; Hill, K. W.; Narel, K. D.; Auditor, M.-T. M. *J. Am. Chem. Soc.* **1989**, *111*, 9261–9262.
39. Li, T.; Janda, K. D.; Ashley, J. A.; Lerner, R. A. *Science* **1994**, *264*, 1289–1293.
40. Hasserodt, J.; Janda, K. D.; Lerner, R. A. *J. Am. Chem. Soc.* **1997**, *119*, 5993–5998.
41. Hasserodt, J.; Janda, K. D.; Lerner, R. A. *J. Am. Chem. Soc.* **1996**, *118*, 11654–11655.
42. Li, T.; Janda, K. D.; Lerner, R. A. *Nature* **1996**, *379*, 326–327.

43. Romesburg, F. E.; Flanagan, M. E.; Uno, T.; Schultz, P. G. *J. Am. Chem. Soc.* **1998**, *120*, 5160–5167.
44. Driggers, E. M.; Cho, H. S.; Lui, C. W.; Katzka, C. P.; Braisted, A. C.; Ulrich, H. D.; Wemmer, D. E.; Schultz, P. G. *J. Am. Chem. Soc.* **1998**, *120*, 1945–1958.
45. Zhou, Z. S.; Flohr, A.; Hilvert, D. *J. Org. Chem.,* **1999**, *64*, 8334–8341.
46. Stewart, J. D.; Liotta, L. J.; Benkovic, S. J. *Acc. Chem. Res.* **1993**, *26*, 396–404.
47. (a) Barbas, III, C. F.; Heine, A.; Zhong, G.; Hoffman, T.; Gramatikova, S.; Bjornestedt, R.; List, B.; Anderson, J.; Stura, E. A.; Wilson, I. A.; Lerner, R. A. *Science* **1997**, 2085–2092. (b) Zhong, G.; Hoffmann, T.; Lerner, R. A.; Danishefsky, S.; Barbas, III, C. F. *J. Am. Chem. Soc.* **1997**, *119*, 8131–8132. (c) Bjornestedt, R.; Zhong, G.; Lerner, R. A.; Barbas, III, C. F. *Science* **1995**, *270*, 1797–1800.
48. (a) Morris, A. J.; Tolan, D. R. *Biochemistry* **1994**, *33*, 12291–12297. (b) Lai, C. Y.; Nakai, N.; Chang, D. *Science* **1974**, *183*, 1204–1206.
49. Gao, C.; Lavey, B. J.; Lo, C.-H. L.; Datta, A.; Wentworth, Jr., P.; Janda, K. D. *J. Am. Chem. Soc.* **1998**, *120*, 2211–2217.
50. (a) Whitcombe, M. J.; Alexander, C.; Vulfson, E. N. *Synlett* **2000**, 911–923. (b) Cormack, P. A. G.; Mosbach, K. *Reac. and Func. Polymers* **1999**, *41*, 115–124. (c) Takeuchi, T.; Matsui, J. *Acta Polymer.* **1996**, *47*, 471–480. (d) Wulff, G. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1812–1832.
51. Beach, J. V.; Shea, K. J. *J. Am. Chem. Soc.* **1994**, *116*, 379–380.
52. Matsui, J.; Nicholls, I. A.; Karube, I.; Mosbach, K. *J. Org. Chem.* **1996**, *61*, 5414–5417.
53. Robinson, D. K.; Mosbach, K. *J. Chem. Soc. Chem. Commun.* **1989**, 969–970.
54. Ohkubo, K.; Urata, Y.; Hirota, S.; Honda, Y.; Sagawara, T. *J. Molecular Catalysis* **1994**, *87*, L21–L24.
55. Toorisaka, E.; Yoshida, M.; Veza, K.; Goto, M.; Furusaki, S. *Chem. Lett.* **1999**, 387–388.
56. Wulff, G.; Gross, T.; Schonfeld, R. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1962–1964.
57. Strikovsky, A. G.; Kasper, D.; Grun, M.; Green, B. S.; Hradril, J.; Wulff, G. *J. Am. Chem. Soc.* **2000**, *122*, 6295–6296.
58. Sellergren, B.; Shea, K. J. *Tetrahedron: Asym.* **1994**, *5*, 1403–1406.
59. Selergren, B.; Karmalkar, R. N.; Shea, K. J. *J. Org. Chem.* **2000**, *65*, 4009–4027.
60. Liu, X.-C.; Mosbach, K. *Macromol. Rapid Commun.* **1997**, *18*, 609–615.
61. Liu, X.-C.; Mosbach, K. *Macromol. Rapid Commun.* **1998**, *19*, 671–674.
62. Santora, B. P.; Larsen, A. O.; Gagne, M. R. *Organometallics* **1998**, *17*, 3138–3140.
63. Polborn, K.; Severin, K. *J. Chem. Soc. Chem. Commun.* **1999**, 2481–2482.
64. Bystrom, S. E.; Borje, A.; Akermark, B. *J. Am. Chem. Soc.* **1993**, *115*, 2081–2083.
65. Ye, L.; Ramstrom, O.; Mansson, M.-O.; Mosbach, K. *J. Molecular Recognition* **1998**, *11*, 75–78.
66. Alexander, C.; Smith, C. R.; Whitcombe, M. J.; Vulfson, E. V. *J. Am. Chem. Soc.* **1999**, *121*, 6640–6651.
67. Suh, J.; Hah, S. S. *J. Am. Chem. Soc.* **1998**, *120*, 10088–10093.
68. (a) Bryant, R. A. R.; Hansen, D. A. *J. Am. Chem. Soc.* **1996**, *118*, 5498–5499. (b) Rudzicka, A.; Wolfenden, R. *J. Am. Chem. Soc.* **1996**, *118*, 6105–6109.
69. Jang, B. B.; Lee, K.-P.; Min, D.-H.; Suh, J. *J. Am. Chem. Soc.* **1998**, *120*, 12008–12016.
70. Liu, J.; Luo, G.; Gao, S.; Zhang, K.; Chen, X.; Shen, J. *J. Chem. Soc. Chem. Commun.* **1999**, 199–200.
71. Ohya, Y.; Miyaoka, J.; Ouchi, T. *Macromol. Rapid. Commun.,* **1996**, *17*, 871–874.
72. Slade, C. J. *J. Molecular Catalysis B.: Enzymatic* **2000**, *9*, 97–105.
73. (a) Gordon, E. M.; Kerwin, J. F. *Combinatorial Chemistry and Molecular Diversity in Drug Design*, John Wiley & Sons, 1998. (b) Wilson, S. R.; Czarnik, A. W. *Combinatorial Chemistry: Synthesis and Applications*, John Wiley & Sons, 1997.
74. Menger, F. M.; West, C. A.; Ding, J. *J. Chem. Soc., Chem. Commun.* **1997**, 633–634.
75. Menger, F. M.; Eliseev, A. V.; Mingulin, V. A. *J. Org. Chem.* **1995**, *60*, 6666–6667.
76. Menger, F. M.; Ding, J.; Barragan, V. *J. Org. Chem.* **1998**, *63*, 7578–7579.
77. (a) Gilbertson, S. R.; Wang, X. F. *Tetrahedron* **1999**, *55*, 11609–11618. (b) Gilbertson, S. R.; Wong, X. *Tetrahedron Lett.* **1996**, *37*, 6475–6478. (c) Gilbertson, S. R.; Wong, X. *J. Org. Chem.* **1996**, *61*, 434–435.
78. Krueger, C. A.; Kuntz, K. W.; Dzierba, C. D.; Wirschun, W. G.; Gleason, J. D.; Snapper, M. L.; Hoveyda, A. H. *J. Am. Chem. Soc.* **1999**, *121*, 4284–4285.
79. (a) Kuntz, K. W.; Snapper, M. L.; Hoveyda, A. H. *Current Opinion in Chemical Biology* **1999**, *3*, 313–319. (b) Jandeleit, B.; Schaefer, D. J.; Powers, T. S.; Turner, H. W.; Weinberg, W. H. *Angew. Chem. Int. Ed. Engl.* **1999**, *38*, 2494–2532. (c) Bein, T. *Angew. Chem. Int. Ed. Engl.* **1999**, *38*, 323–329. (d) Shimazu, K. D.; Snapper, M. L.; Hoveyda, A. H. *Chem. Eur. J.,* **1998**, *4*, 1885–1889. (e) Gennari, C.; Nestler, H. P.; Piarulli, U.; Salom, B. *Leibigs Ann. Recueil* **1997**, 637–647.
80. Reviews and leading references on in vitro evolution: (a) Reetz, M. T.; Jaeger, K.-E. *Chem Eur, J.* **2000**, *6*, 407–412. (b) Olsen, M.; Iverson, B.; Georgiou, G. *Current Opinion in Biotechnology* **2000**, *11*, 331–337. (c) Griffiths, A. D.; Tawfick, D. S. *Current Opinion in Biotechnology* **2000**, *11*, 338–353. (d) Arnold, F. H.; Volkov, A. A. *Current Opinion in Chemical Biology* **1999**, *3*, 54–59. (e) Bornscheuer, U. T. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 3105–3108. (f) Kuchner, O.; Arnold, F. H. *Tibtech.* **1997**, *15*, 523–530.
81. Reetz, M. T.; Zonta, A.; Shimossek, K.; Liebeton, K.; Jaeger, K.-E. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2831–2832.
82. Bornscheuer, U. T.; Altenbuchner, J.; Meyer, H. H. *Bioorg. Med. Chem.* **1999**, *7*, 2169–2173.
83. (a) Joo, H.; Lin, Z.; Arnold, F. H. *Nature* **1999**, *399*, 670–673. (b) Joo, H.; Aniswara, A.; Lin, Z.-L.; Arnold, F. H. *Chemistry and Biology* **1999**, *6*, 699–706.
84. Li, Q.-S.; Schwaneberg, U.; Fischer, P.; Schmid, R. D. *Chem Eur., J.* **2000**, *6*, 1531–1536.
85. Lui, D.; Schultz, P. *Angew. Chem. Int. Ed. Engl.* **1999**, *38*, 37–54.
86. Pederson, H.; Holder, S.; Sutherlin, D. P.; Schwitter, U.; King, D. S.; Schultz, P. G. *Proc. Natl. Acad. Sci USA* **1998**, *95*, 10523–10528.
87. Cooper, A. C.; McAlexander, L. H.; Lee, D.-H.; Torres,

M. T.; Crabtree, R. H. *J. Am. Chem. Soc.* **1998**, *120*, 9971–9972.
88. For general reviews and references see Ref. 79.
89. Enjalbal, C.; Martinez, J.; Aubagnac, J.-L. *Mass Spectrometry Reviews* **2000**, *19*, 139–161.
90. Reetz, M. T.; Becker, M. H.; Klein, H.-W.; Stockigt, D. *Angew. Chem. Int. Ed. Engl.* **1999**, *38*, 1758–1761.
91. Taylor, S. J.; Morken, J. P. *Science* **1998**, *280*, 267–270.
92. For a general text: Gaussorges, G. *Infrared Thermography*, Chapman and Hall: London, 1994.
93. (a) Reetz, M. T.; Becker, M. H.; Liebl, M.; Furstner, A. *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 1236–1239. (b) Reetz, M. T.; Becker, M. H.; Kuling, K. M.; Holsworth, A. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 2647–2650.
94. (a) Davies, M.; Bonnat, M.; Guilier, F.; Kilburn, J. D.; Bradley, M. *J. Org. Chem.* **1998**, *63*, 8696–8703. (b) Loewik, D. W. P. M.; Weingarten, M. D.; Broekema, M.; Brouwer, A. J.; Still, W. C.; Liskamp, R. M. J. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 1846–1850. (c) Bonnat, M.; Bradley, M.; Kilburn, J. D. *Tetrahedron Lett.* **1996**, *37*, 5409–5412. (d) Still, W. C. *Acc. Chem. Res.* **1996**, *29*, 155–163. (e) Wennemers, H.; Yoon, S. S.; Still, W. C. *J. Org. Chem.* **1995**, *60*, 1108–1109.
95. Lehn, J.-M. *Chem. Eur. J.,* **1999**, *5*, 2455–2463.
96. Polyakv, V. A.; Neelen, M. I.; Nazapack-Kandlousy, N.; Ryabov, A. D.; Eliseev, A. V. *J. Phys. Org. Chem.* **1999**, *12*, 357–363.
97. Cousins, G. R. L.; Poulsen, S.-A.; Sanders, J. K. M. *J. Chem. Soc. Chem. Commun.* **1999**, 1575–1576.
98. Eliseev, A. V.; Nelen, M. I. *Chem. Eur. J.* **1998**, *4*, 825–834.
99. Huc, I.; Lehn, J.-M. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 2106–2110.
100. Giger, T.; Wigger, M.; Audetat, S.; Benner, S. A. *Synlett* **1998**, 688–691.
101. Hamilton, D. G.; Feeder, N.; Teat, S. J.; Sanders, J. K. M. *New J. Chem.* **1998**, *10*, 1019–1021.
102. Nicolau, K. C.; Hughes, R.; Young Cho, S.; Winssinger, N.; Smethurst, C.; Labischinski, H.; Endermann, R. *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 3823–3828.
103. Hioki, H.; Still, W. C. *J. Org. Chem.,* **1998**, *63*, 904–905.
104. Cardullo, F.; Crego Calama, M.; Snellink-Ruel, B. H. M.; Weidmann, J.-L.; Bielejewska, A.; Fokens, R.; Nibbering, N. M. M.; Timmerman, P.; Reinhoudt, D. N. *J. Chem. Soc. Chem. Commun.* **2000**, 367–368.
105. Cousins, G. R. L.; Poulsen, S. A.; Sanders, J. K. M. *J. Chem. Soc. Chem. Commun.* **1999**, 1575–1576.
106. Furlan, R. L. E.; Cousins, G. R. L.; Sanders, J. K. L. *J. Chem. Soc. Chem. Commun.* **2000**, 1761–1762.
107. (a) Rowan, S. J.; Lukeman, P. S.; Reynolds, D. J.; Sanders, J. K. M. *New J. Chem.* **1998**, *10*, 1015–1018. (b) Rowan, S. J.; Sanders, J. K. M. *J. Chem. Soc. Chem. Commun.* **1997**, 1407–1408.
108. Swann, P. G.; Casanova, R. A.; Desai, A.; Frauenhoff, M. M.; Urbancic, M.; Slomczynska, U.; Hopfinger, A. J.; Le Breton, G. C.; Venton, D. L. *Biopolymers* **1996**, *40*, 617–625.
109. Refs. 102, 104, 106; For further examples and a recent review on thermodynamically driven selection in combinatorial chemistry: (a) Ganesan, A. *Angew. Chem. Int. Ed Engl.* **1998**, *37*, 2828–2831. (b) Ref. 95
110. Swain, C. G.; Brown, J. F. *J. Am. Chem. Soc.* **1952**, *74*, 2538–2543.
111. Barton, D. H.; Motherwell, W. B.; Zard, S. Z. *Bull. Soc. Chim. Fr.* **1983**, *3-4*, 61–65.

Biographical sketch

William B. Motherwell received his BSc and PhD degrees from the University of Glasgow, completing his doctoral thesis in 1972 as a Carnegie scholarship holder under the direction of Dr James S. Roberts. He was then awarded an ICI Fellowship for independent university research and in 1975 moved to Imperial College as a Schering-Plough postdoctoral fellow with Professor Sir Derek Barton. Two years later, he moved with Barton to the Institut de Chimie des Substances Naturelles in Gif-sur-Yvette, where he was *Chargé de Recherche* during a highly creative period which saw the invention of free radical chain reactions using Barton esters and the birth of the Gif system for the oxidation of saturated hydrocarbons. In 1983 he returned to Imperial College, first as lecturer and then as reader, prior to moving to University College London in 1993 as the first incumbent of the Alexander Williamson chair of chemistry. His research interests concentrate on the invention and discovery of new reactions and reagents for Organic Synthesis, and include such apparently diverse areas as transition metal catalysis and organometallic carbenoids, selective fluorination reagents and protocols, and free radical chemistry. He has previously held visiting professorships at the Universities of Auckland, Paris-Sud at Orsay, and Bordeaux, and been a Merck-Frosst lecturer in Canada as well as the recipient of the Royal Society of Chemistry's Bader award, and Corday-Morgan and Tilden medals.

Matilda Bingham was born in Southport, UK. She graduated from Imperial College in 1996 before moving to UCL where she carried out her PhD studies under the supervision of Professor W. B. Motherwell. Her research focused on the development of approaches to the design and synthesis of artificial enzymes. In 2000, she joined the group of Professor S. Z. Zard in Gif-sur-Yvette where her postdoctoral research has focused on the chemistry of dithiocarbonates and oximic esters.

Yvan Six was born in 1970 in Sète, France. He completed his PhD under the supervision of Professor Jean-Yves Lallemand at the Ecole Polytechnique, Palaiseau, France, where he worked on an approach towards the total synthesis of Clerodin, an insect antifeedant. In 1997, he joined Professor W. B. Motherwell's group at University College London as a postdoctoral student to work on the design of polymer catalysis using the molecular imprinting technique. He then returned to France in 1999 to complete another postdoctoral period in Professor S. Z. Zard's group at the Institut de Chimie des Substances Naturelles in Gif-sur-Yvette, where he worked mainly on tandem reactions using the radical chemistry of dithiocarbonates. Dr Six entered the Centre National de la Recherche Scientifique in 2000. He currently works on methodology based on the chemistry of organotitanium compounds.