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Towards synthetic enzymes based on porphyrins and steroids

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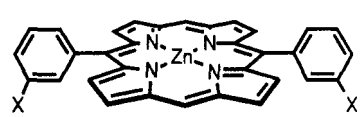
ABSTRACT

The molecular recognition properties of three systems are discussed: a self-associating cyclocholate; a porphyrin–cyclocholate molecular bowl which recognises morphine enantioselectively; and a cyclic porphyrin trimer which efficiently and stereoselectively accelerates a Diels–Alder reaction.

INTRODUCTION

We want to construct model enzymes that are capable of recognition and catalysis. The aim is *not* to mimic any particular natural enzyme. However, inspired by nature, our long-term aim is to understand the principles of how to design and create homogeneous catalytic systems. The justification for this approach, as opposed to using protein engineering or catalytic antibodies, is that the study of 'small', well-defined systems is likely to lead to the discovery of new principles of general utility; this project has already led us unexpectedly to a new understanding of π–π interactions,(1) and of the rôle of templates in synthesis (2, 3).

Model enzymes should have the following features: rapid construction from large, rigid building blocks because the bond-by-bond approach of classical natural product chemistry will be too laborious; built-in spectroscopic probes for geometry, binding and catalysis; convergent binding sites for two or more substrates; and chiral recognition properties. Diaryl porphyrins, **1**, and cholic acid, **2**, each possess most of these features, although neither contains all of them. The diaryl porphyrin unit can be made with a range of different side chains and linkers, has a π-system that tells us spectroscopically about coordination state (UV absorption) and geometry (NMR), and a central metal ion to act as binding site. We use zinc as the central metal ion because it is easy to insert and remove quantitatively, it reliably switches between four- and five-coordinate in porphyrins, and is spectroscopically convenient because it is diamagnetic. Cholic acid is cheap, occurs naturally as a single enantiomer, and is equipped with inward-facing hydroxyls that have the potential to act as binding or catalytic sites.



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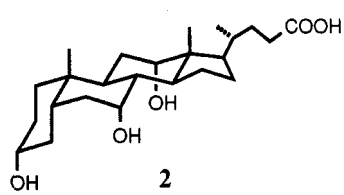

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Many aspects of the synthesis and some of the binding properties of our oligoporphyrins and oligocholates have

been described elsewhere (2–5). We are now moving on to study more subtle aspects of self-assembly, recognition and catalysis. In this article we focus on three aspects of their chemistry: self-associating cyclocholates; the enantioselective recognition of morphine by a porphyrin–cyclocholate molecular bowl; and the *exo*-selective acceleration of a Diels–Alder reaction within the cavity of a cyclic porphyrin trimer.

SELF-ASSOCIATING CYCLOCHOLATES

Cyclic oligomers of cholic acid have the ability to bind a variety of ligands, depending on the functionality attached to the host(4). We wished to expand the potential of these molecules by endowing them with the ability to self-assemble into larger arrays. As a first step (6) they were rendered self-sticky by the introduction of simple cis-amides as complementary hydrogen bond donor/acceptor sites on one rim of the macrocycle as shown in Fig. 1.

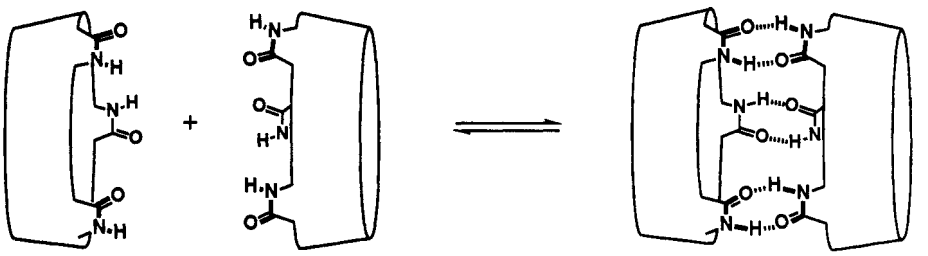


Fig. 1. Schematic representation of self-associating cyclocholates

Various macrocycles such as cyclotrimer **3** were prepared by similar routes(6); they differed only in the type and orientation of solubilizing group. Equilibrium constants for dimerization were determined by ^{1}H NMR of the fast exchange averaged NH resonance as a function of concentration and/or FT IR spectra over the concentration range 5×10^{-6} to ca. 0.5 M in dry carbon tetrachloride at 23–25°C. IR equilibrium constants were derived from the relative and absolute intensities of bands due to monomeric NH stretch (sharp, 3425 cm^{-1}) and dimeric NH stretch (broad multiple absorbances around 3200 cm^{-1}). Experimental data along with best-fit theoretical curves are given below for **3** and the monomeric reference compound **4**. The monomer **4** dimerizes quite weakly, K = 18 l mol^{-1}, while the

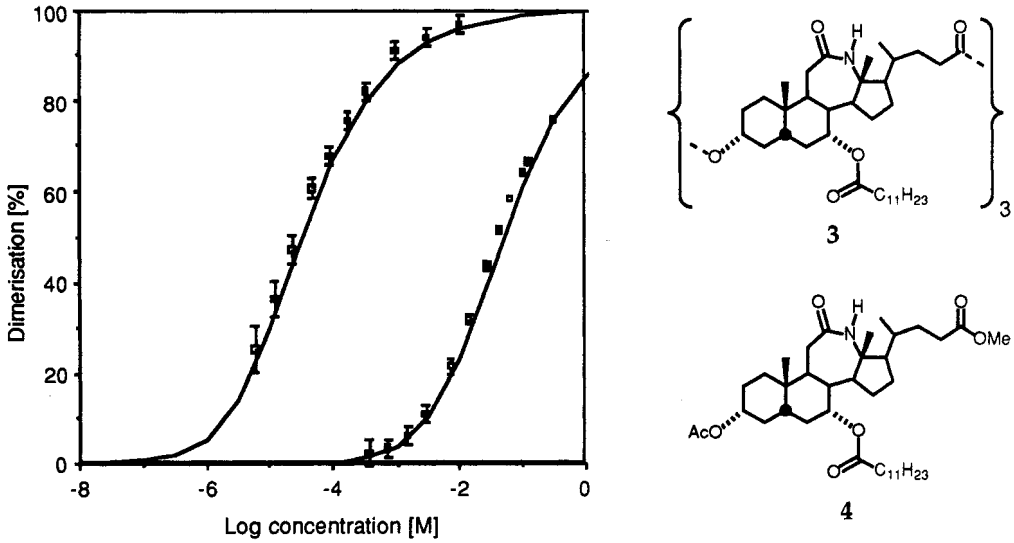


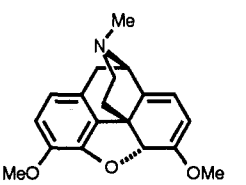
Fig. 2. Self-association of cyclocholate **3** and reference monomer **4**.

triamide **3** self-associates strongly in CCl_4, with $K = 3 (\pm 2) \times 10^4$ l mol^{-1}. The latter value is larger than the cube of the value for the monomer, suggesting some cooperativity in binding. A qualitative study of the self-association by molecular mechanics suggested that a face to face arrangement is the most likely geometry for the dimer, since only in this orientation can enough hydrogen bonds be formed (between four and six) to account for the stability of the complex. However the relative orientation of the amide groups is less than ideal. Energy minimization of the monomer shows that the amide group does not lie in the plane of the steroid, but is angled downwards (into the page as drawn) by about 20°. Thus when two cyclocholate rings come together the steroid subunits have to rotate somewhat to produce more nearly planar amide-amide bridges or form distorted hydrogen bonds, or both. It seems that despite the flexible nature of cyclocholate rings, the strain induced in this process more than offsets the advantage of forming all six possible hydrogen bonds. Indeed the IR spectra of fully associated triamides showed a monomer-like NH stretch at 3430 cm^{-1}, in addition to the more intense dimer bands, implying that there are one or more unbound NHs or 'free ends' present in the dimeric complex. Direct measurement of molecular weight in solution using vapour pressure osmometry eliminated the possibility of non-specific aggregation. Ultimately we hope to be able to extend the work described above to generate larger and kinetically more stable molecular containers and tubes.

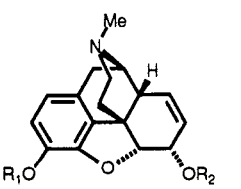
MORPHINE RECOGNITION BY A MOLECULAR BOWL

A design for a molecular bowl, **5**, is shown in Fig. 3: four cholate walls each provide a potential binding or catalytic site, while the porphyrin serves to block one face of the cavity, provide a further binding or catalytic site, and act as spectroscopic reporter. Our first synthesis (7) was based on the left hand disconnection in Fig. 3, building the porphyrin onto a preformed tetracholate. The porphyrin-formation step was extremely inefficient, perhaps due to strain in the intermediate porphyrinogen, making it difficult to accumulate sufficient material for NMR binding studies. More recently (8) we have developed a much improved synthesis based on the right hand disconnection: cholic acid is modified to bear a substituted benzaldehyde at C_7 and that is converted to a porphyrin with four separate pendant cholates. Macrolactonisation proceeds in 18% yield (or 67% per ester linkage) to give reasonable quantities of material which will be used for NMR binding studies.

The main driving force for ligand binding to the bowl is interaction of the basic nitrogen atom with the zinc atom, so the recognition ability is most usefully analysed by comparison with a simple reference porphyrin. Amine-binding to the zinc leads to a shift in the main porphyrin Soret absorption from 422 to 431 nm which is readily monitored on a small scale and analysed to yield binding constants. In the natural series thebaine, **6**, codeine, **7**, and morphine, **8**, the binding constant increases by almost three orders of magnitude on sequentially changing from two OMe to two OH groups. Other alkaloids such as brucine appear unwilling to enter the bowl and are only able to interact with the outside face of the porphyrin.



(–)-6



(–)-7 R_1 = Me; R_2 = H

(–)-8 R_1 = R_2 = H

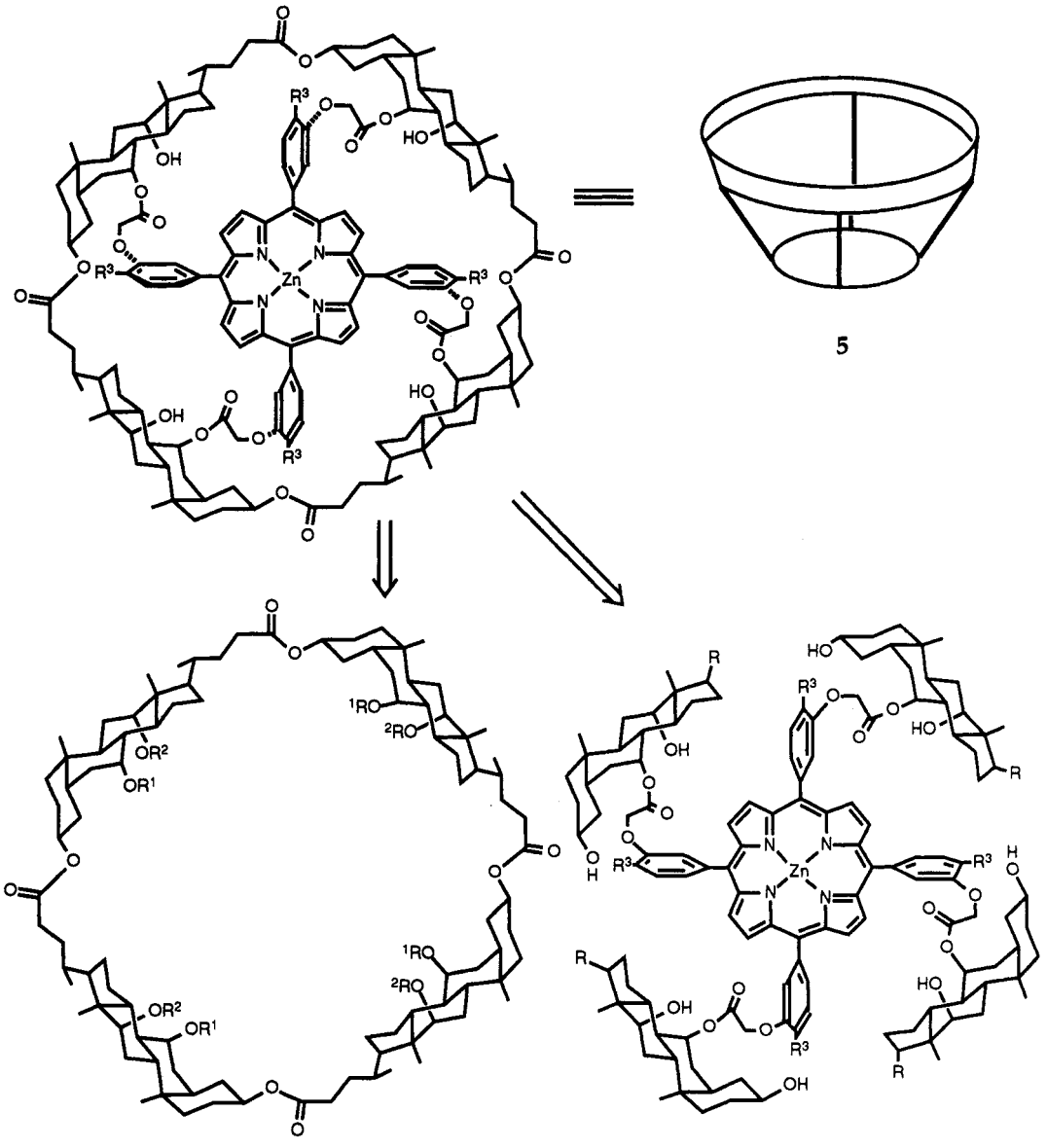


Fig. 3. Two synthetic disconnections from the molecular bowl **5**.

The three-point binding associated with morphine should lead to enantioselectivity, and indeed it does so remarkably effectively. There is no significant selectivity for the two enantiomers of thebaine as there is essentially only a single binding site (the basic nitrogen) and there is plenty of room for either enantiomer to bind and rotate within the cavity. Enantioselectivity for codeine binding is a modest, but genuine, factor of 3.5 in favour of the natural isomer. Morphine can form two strong hydrogen bonds, leading to three-point binding and a selectivity of 43-fold in favour of the natural isomer (Table 1).

Table 1 : Binding constants (l mol^{-1}) for alkaloids to bowl **1** in CH_2Cl_2 solution at 293 K.

Ligands	Natural (–)	Enantio (+)	$\Delta\Delta G$ (kJ mol^{-1})
Thebaine, **6**	420	370	–0.3
Codeine, **7**	1.3×10^4	3.7×10^3	–3.1
Morphine, **8**	1.8×10^5	4.2×10^3	–9.2

The question arises as to the origins of tight binding and enantioselectivity. Models indicate that the diagonal distance across the cavity is *ca* 15 Å, and the cross-cavity separation of two facing cholate hydroxy groups is 11Å, while the cross-section of morphine in the porphyrin-binding orientation is only 7×10 Å. Tight binding appears to be achieved here not so much by a close fit as by the presence of multiple binding opportunities. Once the alkaloid is anchored by metal–nitrogen coordination there are four equivalent sets of hydrogen-bonding sites available to the alkaloid hydroxyls. Model building suggests that the 3-hydroxy group of the natural (–)-isomer binds to the 12-hydroxy of a cholate, while the other alkaloid hydroxy binds to one of the linking ester groups (Fig. 4).

It is more difficult to maintain the third attachment point in (+)-morphine. Once the nitrogen–zinc bond and a hydrogen bond from the 3-hydroxy group have been formed, the remaining hydroxy group faces away from the walls of the bowl into the cavity centre. This picture is supported by the observation that the binding constants for (+)-morphine and (+)-codeine, each of which can make only one hydrogen bond, are virtually identical. The source of the enantioselectivity in codeine binding is unclear: it may result from a weak hydrogen bond to the 3-methoxy group in the natural isomer or from some other, more subtle, factor.

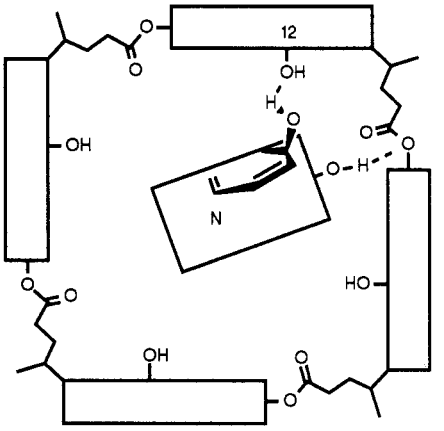


Fig. 4. Schematic view of the binding of (–)-morphine to bowl **5**.

Our observation of efficient enantioselectivity is all the more surprising because the bowl cavity is so large relative to the guest alkaloid. There are two extreme approaches to achieving enantioselectivity in host–guest chemistry: one can either arrange that the van der Waals surfaces of the host and one enantiomer of the guest are highly complementary (i.e. there is a tight fit), or one ensures three point-binding. In practice most synthetic systems employ a combination of these methods (9). Our results indicate that good selectivity can be obtained in cavities which are relatively flexible, allow great rotational freedom and even offer a choice of binding sites.

ACCELERATING THE DIELS–ALDER REACTION

For several years it has been our aim to catalyse reactions within the cavity of a porphyrin oligomer as schematically illustrated for the porphyrin trimer **9** in Fig. 5. Pyridine binding to metalloporphyrins is enthalpically favourable, but entropically unfavourable. When two or three pyridine ligands are bound within the cavity their effective concentration is dramatically increased while at the same time their range of relative orientations is limited by the geometry of Zn–N coordination. The trimer should, therefore, act as an 'entropic trap' and accelerate any reaction whose transition-state geometry matches the relative

orientation of bound ligands. Pericyclic processes which require no catalysis by external functional groups should be ideal candidates, particularly the Diels–Alder reaction which has interesting and perhaps ultimately controllable stereo- and regiochemistry.

The substrates chosen for initial study were the furan-based diene **10** and the maleimide-based dienophile **11**. Binding to the inside of the host holds the reactive ends of the two substrates in close proximity, while the rigidity of the substrates ensures that the high effective concentration produced by their binding is transferred to the reactive site at the heart of the cavity. Furthermore, the Diels–Alder between **10** and **11** is reversible, offering the opportunity of approaching the transition state from both directions.

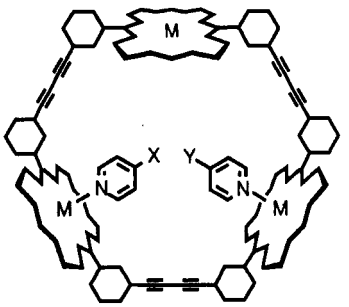


Fig. 4. Schematic view of porphyrin trimer **9** with two reactive ligands bound inside the cavity.

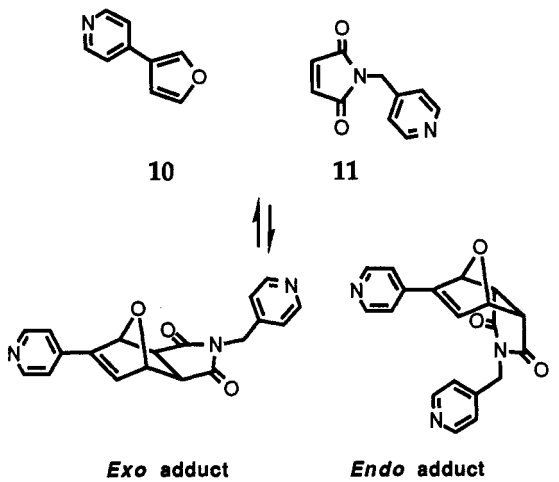


Fig. 6: Reversible Diels–Alder reaction between diene **10** and dienophile **11**.

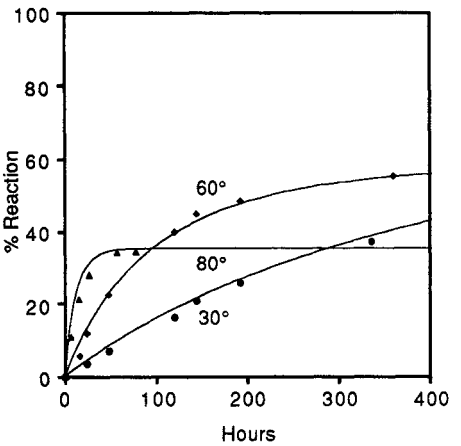


Fig. 7: Progress curves for the Diels–Alder reaction between 9 mM diene **10** and 9 mM dienophile **11** in $C_2D_2Cl_4$ solution. Yields of *endo*- and *exo*- products are summed.

In the absence of any host, the reaction between **10** and **11** (Fig. 6) yields two products, the kinetically favoured *endo*-adduct and the thermodynamically favoured *exo*-adduct which predominates at higher temperatures. Figure 7 shows progress curves for 9 mM reactants at three temperatures: the forward rate for this reaction is of course temperature dependent but the back reaction has a larger temperature dependence so the equilibrium position moves towards starting materials at higher temperatures. We previously reported that the effects of adding one equivalent of trimer are dramatic;(10) at 60° C and 0.9 mM reactants, the rate is greatly increased with more than 95% *exo*-selectivity. The reaction is also effectively inhibited by *s*-tripyridyltriazine, a tridentate ligand which binds strongly within the cavity.

We have since carried out a more thorough kinetic and binding study across a range of temperatures. The binding constants for diene and dienophile to the trimer increase

substantially as the temperature is decreased from 80° to 30° (Fig. 8), so the concentration of productive trimer (with at least one diene and one dienophile within the cavity) should be larger at lower temperatures. We predicted, therefore, that the trimer-accelerated reaction should show a much smaller temperature dependence than the normal reaction: the decreased intrinsic rate at lower temperatures should be offset by the higher fraction bound. This is precisely what is observed, the initial rate of the porphyrin-accelerated reaction being virtually independent of temperature in the range 30–80° (Fig. 9). Note the shorter timescale of the porphyrin-accelerated reaction in Fig. 9 by comparison with Fig. 7, even though the substrates are ten-fold more concentrated in the porphyrin-free reaction.

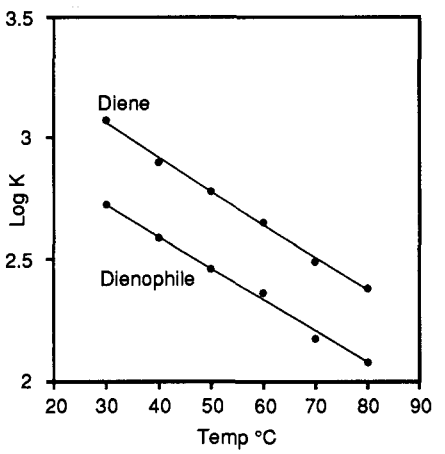


Fig. 8. Temperature dependence of the binding constants of diene **10** and dienophile **11** to trimer **9** in $C_2H_2Cl_4$ solution. The correlation lines are for visual guidance only.

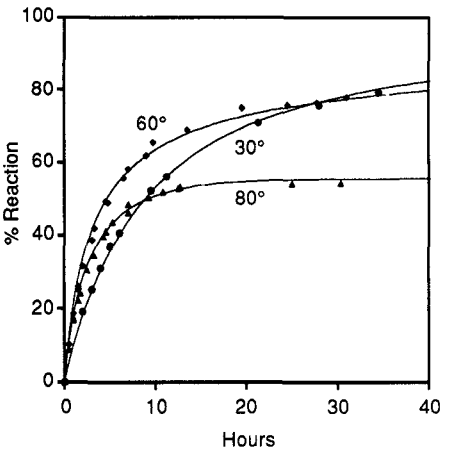


Fig. 9. Temperature dependence of the Diels–Alder reaction between 0.9 mM diene **10** and 0.9 mM dienophile **11** in $C_2H_2Cl_4$ solution in the presence of 0.9 mM porphyrin trimer **9**.

Even though only a small fraction of porphyrin–pyridine complexes is productive, the observed acceleration of *exo*-product formation ranges from 1000-fold at 30° to 80-fold at 80° Computer simulation of the binding and kinetics (solid lines in Fig. 9) have enabled the rate constants for the 'unimolecular' intra-cavity reactions to be calculated. For the forward reaction, these rates correspond to an effective molarity within the cavity of around 20 M at 30°, dropping to 7 M at 80°. The decrease at higher temperatures presumably reflects greater flexibility of the ternary complex. Note that at millimolar concentrations an effective molarity of 20 corresponds to an initial rate enhancement of 2×10^4 fold; although this enhancement is unattainable in practice it is a useful guide to the intrinsic rate acceleration of a productively bound complex. The effect of the trimer on the back reaction is negligible, i.e. the transition state and product are bound equally strongly, so the trimer is product inhibited and shows no catalytic turnover.

The first attempt to accelerate this reaction was preceded by careful design and has been remarkably successful. Preliminary results show that use of 3-maleimido pyridine as dienophile in place of the 4-substituted pyridine reduces the acceleration drastically, as predicted by models. Similarly, hydrogenation of the butadiyne link in **9** to a tetramethylene chain collapses the trimer cavity, reduces the binding affinity for bidentate ligands and abolishes the acceleration. These are encouraging results because they indicate both that macroscopic effects can be achieved by careful microscopic design and that further optimization should be possible.

Much remains to be done: how much faster can we make the Diels–Alder reaction? Can we change its regiochemistry? Can we probe the role of host flexibility in more detail? Can we force efficient turnover so that the porphyrin is catalytic rather than stoichiometric? What other reactions can we accelerate? These are some of the questions we must now address.

Acknowledgements

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