

1

Artificial Enzymes

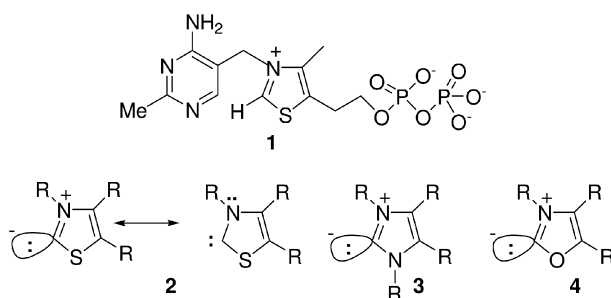
Ronald Breslow

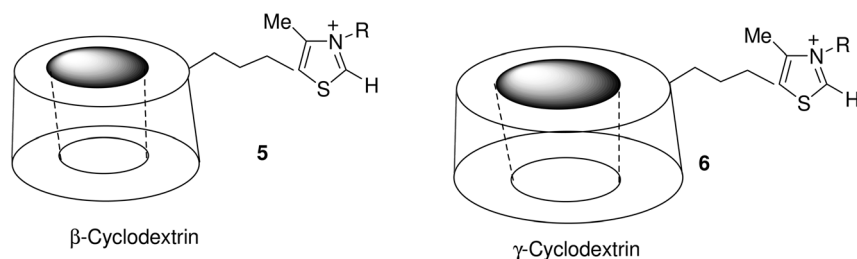
Biomimetic Chemistry, including that involved in the synthesis and study of artificial enzymes, has grown to enormous proportions. Even the part of the field using cyclodextrins as binding groups in synthetic catalysts that mimic enzymes has been the subject of a large review article [1]. Thus in this chapter I will focus mainly, but not exclusively, on work from our own laboratory. Other chapters will help make up for this somewhat narrow focus. I have published several reviews of our work elsewhere [2–51].

1.1

Mimics of Enzymes that use Thiamine Pyrophosphate as a Coenzyme

I have been pursuing enzyme mimics, artificial enzymes that perform biomimetic chemistry, since starting my independent career in 1956. In the first work [52–59] my co-workers and I studied models for the function of thiamine pyrophosphate **1** as a coenzyme in enzymes such as carboxylase. We discovered the mechanism by which it acts, by forming an anion **2** that we also described as a stabilized carbene, one of its resonance forms. We examined the related anions from imidazolium cations and oxazolium cations, which produce anions **3** and **4** that can also be described as nucleophilic carbenes. We were able to explain the structure–activity relationships in this series, and the reasons why the thiazolium ring is best suited to act as a biological



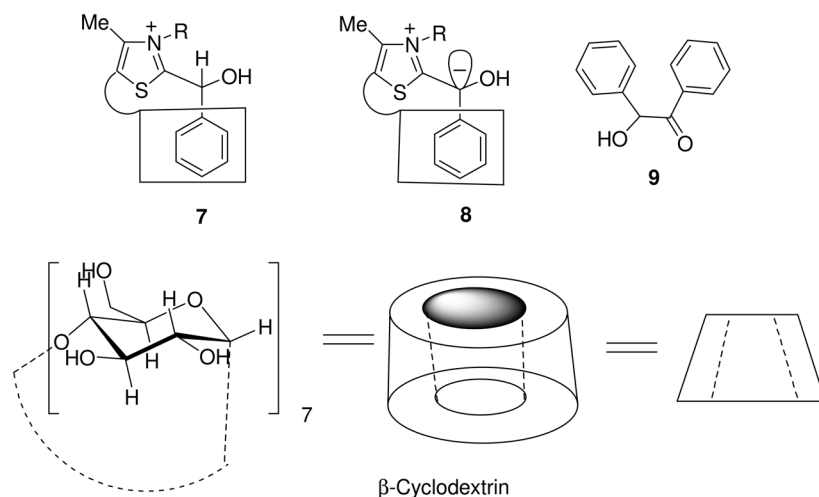


catalyst. Later, we confirmed [60] the thiamine mechanism proposed earlier, for which an alternative had subsequently been proposed [61, 62].

We synthesized artificial enzymes **5** and **6** that incorporated the thiazolium ring of thiamine into a cyclodextrin binding unit [63, 64]. The cyclodextrin imitated the hydrophobic binding pocket typical of many enzymes, but these mimics did not incorporate the catalytic groups that enzymes also use. Thus **5** and **6** showed the substrate selectivity that enzyme binding also achieves, and there was some rate acceleration from binding the substrates in proximity to the catalytic coenzyme group, as in enzymes. However, the rate accelerations were not nearly as large as those in artificial enzymes (*vide infra*) that incorporated more features of natural enzymes.

With β -cyclodextrin, consisting of seven glucose units in a ring, benzaldehyde bound into the cyclodextrin cavity of **5** and was converted into thiazolium adduct **7**, similar to a cyanohydrin [63]. This readily formed the benzylic anion **8** that underwent deuterium exchange and easy oxidation. However, this β -cyclodextrin ring was too small to bind both benzaldehydes – so this artificial enzyme did not catalyze the formation of benzoin **9** significantly better than did a simple thiazolium salt without the attached cyclodextrin binding group.

When the thiazolium unit was attached to the larger γ -cyclodextrin in **6**, with eight glucose units in the ring, benzoin condensation of two benzaldehydes was indeed well



catalyzed, with a rate 150-fold higher than that for a thiazolium salt lacking the cyclodextrin. Interestingly, in this benzoin condensation the rate-determining step – addition of anion **8** to the second benzaldehyde – allowed the benzaldehyde units to bind next to each other in the cavity, but in the product benzoin **9** the extended geometry does not permit this. Thus the benzoin product did not bind strongly to the artificial enzyme **6**, and did not inhibit the process. Our other studies on the benzoin condensation [65, 66] revealed geometries of the transition state and product that support this interpretation.

1.2

Mimics of Enzymes that use Pyridoxamine and Pyridoxal Phosphates as Coenzymes

We also attached pyridoxamine to a cyclodextrin and saw that the resulting enzyme mimics showed good substrate selectivity in the conversion of keto acids into amino acids [67–71]. With a pyridoxamine doubly-linked to the cyclodextrin there was a preference for the hydrophobic *t*-butylphenylpyruvic acid relative to pyruvic acid of at least 15 000-fold. We also made a related system, in which a synthetic macrocycle was attached to the coenzyme mimic [72], that also showed substrate selectivity. In other work we synthesized molecules in which base groups attached to the pyridoxamine could perform transaminations with good stereoselectivity [73–75]. We also made others in which the geometry of the attached base groups could promote different catalyzed processes for pyridoxal, selecting among the various enzymatic processes for which pyridoxal phosphate is a coenzyme [76–80].

These and subsequent artificial enzymes that perform transaminations are described in Chapter 2.

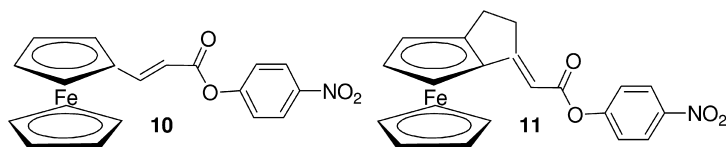
1.3

Artificial Hydrolytic Enzymes

1.3.1

Chymotrypsin Mimics

The field of artificial enzymes has been greatly concerned with mimicking *hydrolytic* enzymes. Since the enzyme chymotrypsin was one of the first to be extensively studied and understood, many laboratories have created artificial peptidases and esterases, including those that use the nucleophilic mechanism like that in chymotrypsin. (However, one chymotrypsin mimic from other laboratories did not have the reported mechanism [81].) The critical requirement is bifunctional catalysis, which in chymotrypsin involves imidazole acting first as a general base, then as a general acid, and the serine hydroxyl group serving as a nucleophile. I have pointed out the special kinetic situation this mechanism implies [82].

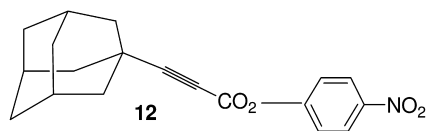


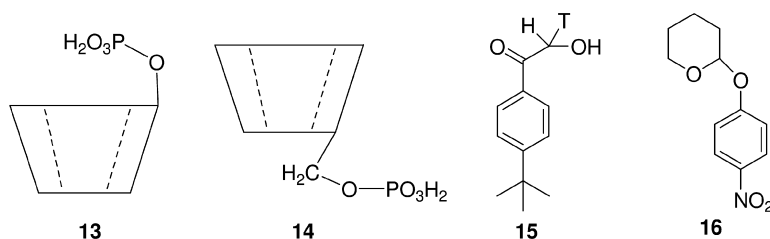
We have made several artificial enzymes that use cyclodextrin to bind a substrate and then react with it by acylating a cyclodextrin hydroxyl group. This builds on earlier work by Myron Bender, who first studied such acylations [83]. We added groups to the cyclodextrin that produced a flexible floor, capping the ring [84]. The result was to increase the relative rate of cyclodextrin acylation by *m*-*t*-butylphenyl acetate from 365 relative to its hydrolysis rate in the buffer to a $k_{\text{complex}}/k_{\text{buffer}}$ of 3300. We changed the substrate to achieve better geometry for the intracomplex acylation reaction, and with a *p*-nitrophenyl ester of ferroceneacrylic acid **10** we achieved a relative rate for intracomplex acylation of ordinary β -cyclodextrin vs. hydrolysis of over 50 000 and a V_{max} comparable to that for hydrolysis of *p*-nitrophenyl acetate by chymotrypsin [85].

Our best combination of the flexible capped cyclodextrin with the well-fitting substrate *p*-nitrophenyl ester **10** gave an acceleration – relative to hydrolysis in the same medium – of over one-million fold, exceeding that achieved by chymotrypsin with *p*-nitrophenyl acetate [86]. An even better fitting substrate (**11**) afforded an acceleration of ca. 80 000 000-fold, and saw a 62-fold increase in enantioselectivity as well [87, 88]. This is an enantiomeric excess of 98.4%.

Substrate binding into the cyclodextrin cavity, which ordinarily is studied in water solution, also occurs in highly polar organic solvents such as DMSO [89]. Furthermore, kinetic studies of our reactions at high pressure were consistent with the geometries proposed for these acylation processes [90]. Molecular modeling showed geometries of the bound substrates and the tetrahedral intermediates that helped explain some of the large rate effects [91].

In the acylation of a cyclodextrin hydroxyl group by a nitrophenyl ester, the preferred geometry requires that the oxyanion of the cyclodextrin attack perpendicular to the plane of the ester carbonyl, so as to form the tetrahedral intermediate. However, the product cyclodextrin ester has the cyclodextrin oxygen *in* the plane of the carbonyl group. Thus, a rapid reaction requires enough flexibility to be present to permit this geometric change to occur rapidly. With very rigid substrates the conversion of the tetrahedral intermediate into the product can be rate determining, and slow. In a study of this question we used substrate **12** in which the ester carbonyl can freely rotate, and saw that this made the formation of the tetrahedral intermediate rate-limiting, and rapid [92].





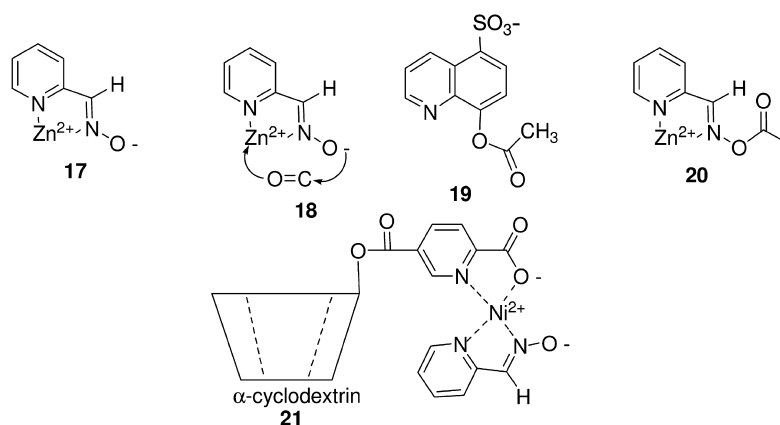
When functional groups are attached to the cyclodextrin ring, new artificial enzymes can result. We have already described this for the attachment of thiazolium rings and pyridoxamine/pyridoxal rings above, and will describe the attachment of metal catalytic groups in the next section. However, one study with attached phosphate groups addressed a general question: Is there a preference for putting such groups on the primary or secondary side of the cyclodextrin ring [93]? In the studies mentioned to this point, the catalytic groups were attached to the primary CH_2 groups of the cyclodextrins, but the acylation reaction occurred on the secondary $\text{CH}-\text{OH}$ groups.

We prepared phosphate esters **13** and **14** of both the secondary hydroxyl and primary hydroxyl groups of β -cyclodextrin, and examined them as general acid and general base catalysts for the reactions of bound substrates [93]. The phosphate anion acted as a general base to catalyze enolization and the resulting hydrogen exchange in a bound tritiated phenacyl ketone **15** when the phosphate was either primary or secondary, showing that the substrate can bind equally well and undergo catalysis when pointed in either direction in the cyclodextrin cavity. However, the general acid-catalyzed hydrolysis of a bound acetal **16** had a preference for the phosphoric acid group on the secondary side. The position of attachment of the catalytic groups can be important.

1.3.2

Metalloenzyme Mimics

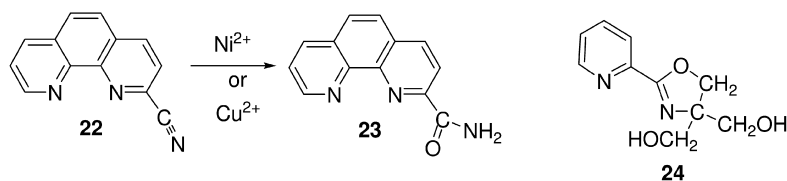
Chymotrypsin is only moderately effective as an enzyme, and much higher rates are seen with metalloenzymes. Zinc is especially important in such hydrolytic enzymes (cf. Ref. 94). For example, the enzyme carboxypeptidase A uses zinc in a typical bifunctional role, at the same time activating a carbonyl for addition by coordinating with its oxygen and activating a water molecule to act as a nucleophile [95–100]. We produced a model for this type of process by using metal complexing as the substrate binding force and a coordinated oxime as the nucleophile (**17**) [101]. The geometry of this compound means that the Lewis acidic zinc and the basic oxime anion can co-exist without quenching each other; the electrons can flow from one to the other only through the bridging carbonyl group of the substrate (**18**). Consequently, the anion of **17** reacted with metal-bound substrate **19** to transfer the acetyl group to the oxime anionic oxygen, and then the intermediate **20** rapidly hydrolyzed. In this process the metal ion is serving multiple functions; it binds the substrate, acidifies the oxime,



coordinates to the carbonyl oxygen of the transferring acetyl group, and then catalyzes hydrolysis of intermediate **20**. It was our first example of such an enzyme-like process, and we built on it further.

Catalyst **17** is effective only with substrates that can bind to the metal ion, so we attached it – coordinated as its Ni^{2+} derivative – to the secondary face of α -cyclodextrin in catalyst **21** [102]. This was then able to use the metallo-oxime catalysis of our previous study, but with substrates that are not metal ligands, simply those that bind hydrophobically into the cyclodextrin cavity. As hoped, we saw a significant rate increase in the hydrolysis of *p*-nitrophenyl acetate, well beyond that for hydrolysis without the catalyst or for simple acetyl transfer to the cyclodextrin itself. Since there was full catalytic turnover, we called compound **21** an “artificial enzyme” – apparently the first use of this term in the literature. The mechanism is related to that proposed earlier for the enzyme alkaline phosphatase [103].

Nitriles can be hydrated enzymatically to form amides. In a model system we showed [104] that **22** can be converted into the amide **23** when metal ions are coordinated into the phenanthroline system. With Ni^{2+} the rate acceleration was 10^7 , while with Cu^{2+} the hydration was accelerated by 10^9 . These are huge rate increases. Much of the driving force is related to the fact that the cyano group is not itself a strong metal ligand in **22**, but the transition state for the hydration is metal coordinated. Also, we used a metal ion to organize the intracomplex reaction of a ligand–ligand reaction [105] in which tris-hydroxymethylaminomethane (Tris) adds to 2-cyanopyridine to form the adduct **24**. Again, the rate was very large and, more to the point, the addition of Tris occurs even though the concentration of water is 10^4 times that of Tris. In the absence



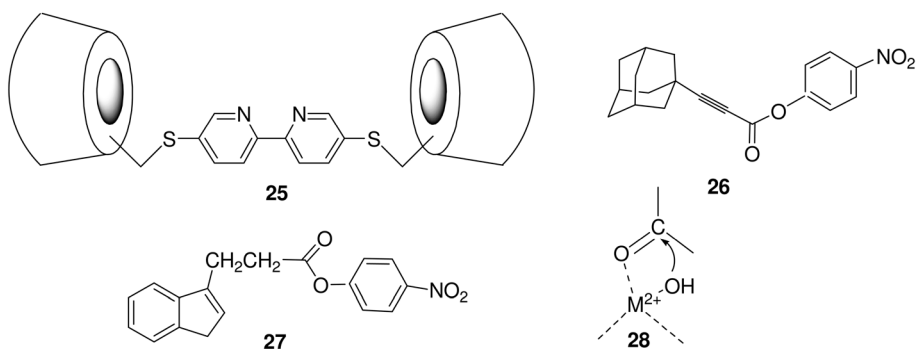
of Tris the metal ion simply catalyzed the hydration of 2-cyanopyridine to its amide, as with **22** above. The coordination of both reactants to the same metal ion has produced selectivity that was induced by the coordination.

In models for carboxypeptidase A we showed the intracomplex catalyzed hydrolysis of an ester by a metal ion and a carboxylate ion [106], which are the catalytic groups of carboxypeptidase A. Some mechanistic proposals for the action of carboxypeptidase involve an anhydride intermediate that then hydrolyzes to the product and the regenerated enzyme. Although we later found convincing evidence that the enzyme does not use the anhydride mechanism in cleaving peptides [96–99], it may well use such a mechanism with esters. In a mimic of part of this mechanism we showed [107], but see also Ref. 108, that we could achieve very rapid hydrolysis of an anhydride by bound Zn^{2+} , which is the metal ion in the enzyme. In another model, a carboxylate ion and a phenolic hydroxyl group, which are in the enzyme active site, could cooperatively catalyze the cleavage of an amide by the anhydride mechanism [109].

Catalysis by Zn^{2+} is ambiguous, since it is not clear whether the metal ion is coordinated to the carbonyl group of the substrate, as it is in the enzyme. Thus, we examined the cleavage of an amide by a combination of Co^{3+} and a carboxylate group. Since Co(III) is “substitution inert,” we prepared a complex in which it was directly coordinated to the carbonyl oxygen of a substrate amide, and in which a neighboring carboxylate ion or phenol group was a potential second catalytic function [110]. Indeed, the phenol group was able to assist the hydrolysis by protonating the leaving amino group, but the carboxylate ion was not effective. The mechanism is intellectually related to that used by the enzyme [97, 98], but the details differ.

By contrast with the absence of catalysis by an internally attached carboxylate ion in the above study, an external carboxylate species and, even more, an external phosphate species were catalysts with the cobalt complexed amide. Thus we examined a process like that just described in which a *phosphonate* group was internally attached to the cobalt complex [111]. In this case it did act as a sequential base/acid catalyst, as the carboxylate ion does in the enzyme, i.e., it first delivered a hydroxide group to the complexed carbonyl, acting as a base, and then the proton that it had accepted was delivered to the leaving group, with the phosphonic acid acting as a general acid. This sequence is indeed like that in the enzyme. We suggested that the phosphonate is more effective because it has a higher $\text{p}K_{\text{a}}$ than the attached carboxylate, and in the enzyme the carboxylate has an abnormally high $\text{p}K_{\text{a}}$. The phosphonate is a better model for the enzyme carboxylate in the artificial enzyme.

These cobalt systems are useful models of enzymatic mechanisms, but they are not turnover catalysts as enzymes are. To achieve turnover we constructed ligand **25** in which a metal-coordinating group links two cyclodextrin rings. As its metal complex it was a good catalyst for the hydrolysis of substrates **26** and **27** that could bind into both cyclodextrins and stretch across the bound metal ion. (Later we will describe the same principle applied to selective oxidation reactions.) Ligand **25** as its Cu^{2+} complex gave as much as a 10^5 -fold rate acceleration in the ester hydrolysis [112, 113]. With an added nucleophile that also binds to the Cu^{2+} ion, the reaction is accelerated by over 10^7 . The mechanism deduced (**28**) – in which the metal ion acts as a Lewis acid by coordination to the substrate carbonyl and also delivers a bound hydroxide ion to the ester carbonyl

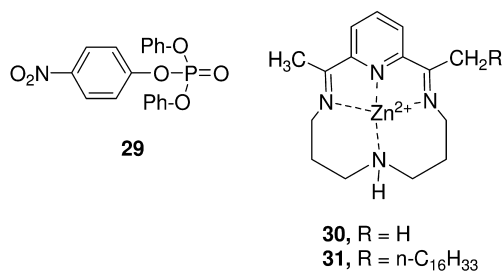


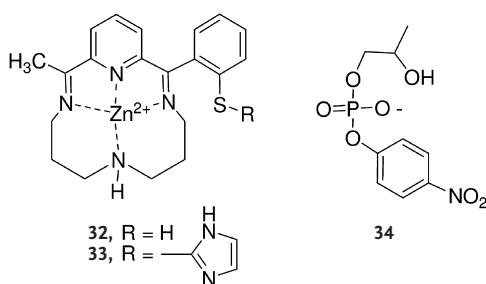
group – is like both that in the enzyme carbonic anhydrase and the one we proposed for histone deacetylase [114, 115]. In those enzymes Zn^{2+} is the catalytic metal ion.

We have pursued such ester hydrolysis by artificial enzymes further. With a cyclodextrin dimer related to **25** we have hydrolyzed an ordinary doubly bound ester, not just the more reactive nitrophenyl esters [116], with catalytic turnovers. Also, with a catalyst consisting of a cyclodextrin linked to a metal ligand carrying a Zn^{2+} and its bound oxime anion, we saw good catalyzed hydrolysis of bound phenyl esters with what is called burst kinetics (fast acylation, slower deacylation), as is seen with many enzymes [117].

Artificial enzymes with metal ions can also hydrolyze phosphate esters (alkaline phosphatase is such a natural zinc enzyme). We examined the hydrolysis of *p*-nitrophenyl, diphenylphosphate (**29**) by zinc complex **30**, and also saw that in a micelle the related complex **31** was an even more effective catalyst [118]. Again the most likely mechanism is the bifunctional $\text{Zn}-\text{OH}$ acting as both a Lewis acid and a hydroxide nucleophile, as in many zinc enzymes. By attaching the zinc complex **30** to one or two cyclodextrins, we saw even better catalysis with these full enzyme mimics [119]. A catalyst based on **25** – in which a bound La^{3+} cooperates with H_2O_2 , not water – accelerates the cleavage of bis-*p*-nitrophenyl phosphate by over 10^8 -fold relative to uncatalyzed hydrolysis [120]. This is an enormous acceleration.

Phosphate ester cleavage can also be achieved with artificial enzymes using both a metal ion and an additional catalytic group, as in the amide and ester hydrolyses described above. In our first example, catalysts **32** and **33** combined a Zn^{2+} with a thiophenol and an imidazole group respectively [121]. The rigid structure prevented the





imidazole or SH coordinating to the metal ion. In electrical terms, they can feed electrons to the metal ion only when there is bridging by a substrate group; the resulting electron flow leads to catalysis. The bifunctional catalysis led to cyclization of substrate 34.

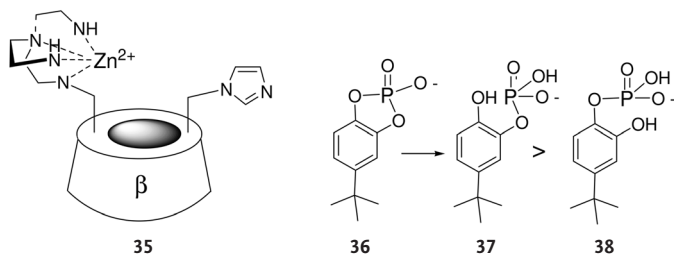
As another example, we prepared disubstituted cyclodextrin 35 in which one substituent was a metal-binding tren group while the other was an imidazole [122]. Zn²⁺ complexed to the tren group gave good rate acceleration in the hydrolysis of bound catechol cyclic phosphate 36, which was fastest when the two catalytic groups were attached to opposite sides of the cyclodextrin so they could not bind each other. The geometry of the complex led to the selective formation of product 37 rather than 38; both are formed equally by ordinary hydrolysis without the catalyst.

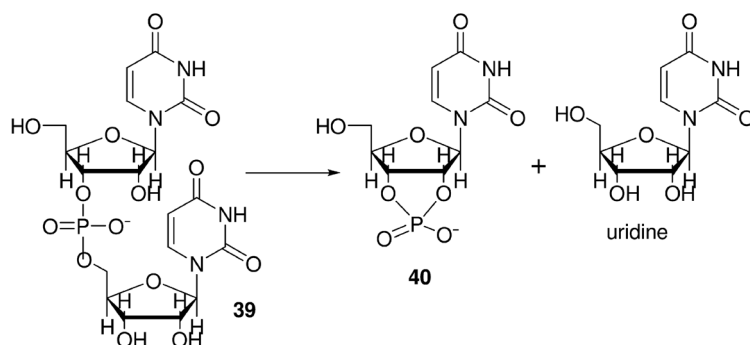
The next section describes further mimics of ribonuclease enzymes. However, we mention here one additional study on the cleavage of ribonucleotides in which metal ions were used [123]. Cyclization of uridyluridine 39, and hydrolysis of the resulting cyclic nucleotides 40, was catalyzed by Eu³⁺ much more effectively than by Zn²⁺, and some added ligands increased the rates.

1.3.3

Artificial Ribonucleases

Ribonuclease A is a member of a group of enzymes that cleave RNA using general acid–base catalysis without a metal ion in the enzyme. In ribonuclease A, such catalysis is performed by two imidazoles of histidine units, one as the free base (Im) and the other, protonated, as the acid (ImH⁺). To mimic this in an artificial enzyme, we prepared β -cyclodextrin bis-imidazoles 41 [124]. The first one was a mixture of the

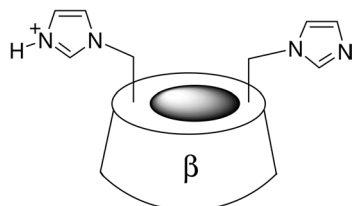




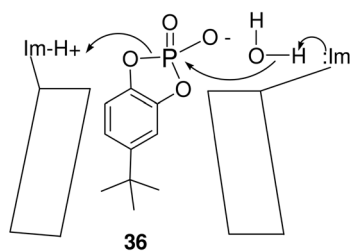
6A,6C and 6A,6D isomers (the seven glucose units of the cyclodextrin are labeled A through G). (As described below, we were later able to prepare as pure catalysts all the isomers of the bis-imidazole cyclodextrin with the imidazole rings on the primary carbons of the ring. The geometric dependence of catalysis indicated the mechanism involved.)

On examining this catalyst mixture in the hydrolysis of the cyclic phosphate of 4-*t*-butylcatechol **36**, we saw a bell-shaped pH rate profile, indicating bifunctional acid–base catalysis of the hydrolysis as in the enzyme ribonuclease. A catalyst carrying only one imidazole showed only base catalysis, by the unprotonated imidazole group Im. Thus, in catalyst mixture **41**, one imidazole was acting as a base – delivering a water molecule to the phosphate group of the bound substrate – while the imidazolium ion of the other catalytic group played a role as a general acid. At the time we thought that this imidazolium ion might be simply protonating the leaving group of the phosphate, as was normally assumed for the enzyme ribonuclease A, but our later work revealed a more interesting role.

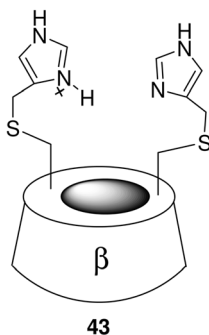
Simple hydrolysis of substrate **36** with base in solution furnishes an essentially equal mixture of **37** and **38**, since the *t*-butyl group is too remote to have any influence. However, catalysis by the artificial enzyme mixture **41** gave **37** only. The geometry of the catalyst–substrate complex directs the attack by water (hydrogen bonded to the imidazole) in a line perpendicular to the ring axis (cf. **42**). When we moved the two imidazole groups out further, in catalyst **43**, they were then able to deliver the water in a



41, a mixture of the A,C and A,D positional isomers, as the mono-protonated species.



42, a possible catalytic bifunctional mechanism for cleavage of **36** by catalyst **41**

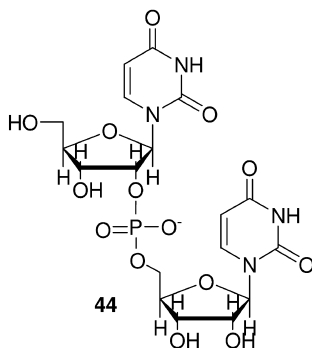


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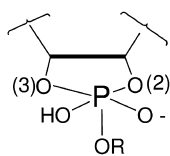
different direction, to afford essentially only **38** [125]. Molecular models were consistent with this change in preferred geometry of attack on the cyclic phosphate.

We developed an assay for the cleavage of uridyluridine (**39**, UpU) by various catalysts, and used it to study the cleavage of this dimeric piece of RNA [126]. We saw that high concentrations of imidazole buffer could catalyze this cleavage, mimicking the high effective local concentrations of imidazole in the enzyme, and concluded that with this buffer there was sequential base, then acid, catalysis [127]. Of course, simultaneous catalysis by two different buffer species by a three-body collision is unlikely unless they are linked in the same catalyst – the enzyme or the artificial enzyme.

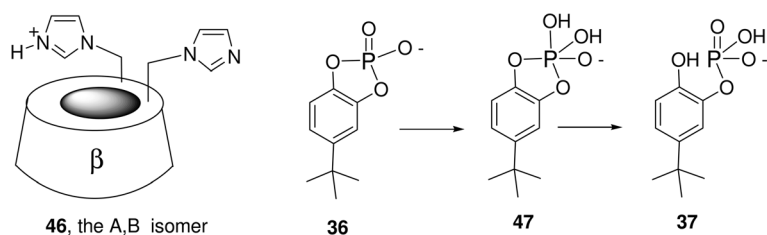
Further work on this simple imidazole catalysis showed that, as well as cyclization of UpU to a cyclic phosphate, 3,5-linked UpU (**39**) also isomerized to the 2,5-linked isomer **44** [128]. Such an isomerization requires a phosphorane intermediate (**45**) and its pseudo-rotation so that the C-3 oxygen can become the leaving group. Our studies showed that in this buffer-catalyzed reaction the ImH⁺ catalyst was not simply protonating the leaving group – it was first protonating the phosphate anion of the UpU so that a phosphorane intermediate could be formed in both the cleavage and isomerization. We suggested that this could also be the mechanism in the enzyme ribonuclease itself, but this is still a matter of controversy. In any case, we soon found evidence that this mechanism – proceeding through a five-coordinate phosphorus, a phosphorane –operated with our artificial enzyme symbolized by **41**.



44



45, a phosphorane intermediate with an apical O-2 and an equatorial O-3. To form **44** it must pseudorotate so the O-3 becomes apical.



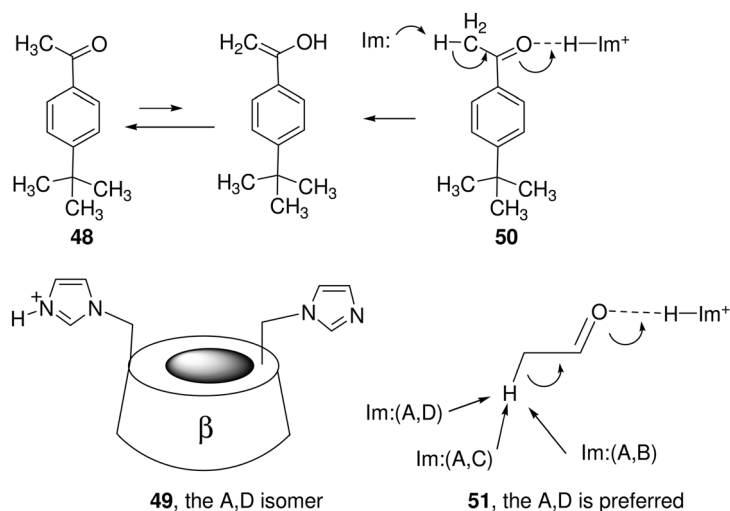
As mentioned above, we first made the cyclodextrin bis-imidazole catalyst with the imidazoles attached to primary carbons that were as far apart as possible (A,D glucose residues) or almost that far (A,C residues). We then made all three isomers selectively – A,D and A,C and A,B – to see how their geometry affected the catalyzed hydrolysis of substrate 36 [129]. All three showed the bell-shaped pH vs. rate curves, indicating that there was both base and acid catalysis, by an Im and an ImH⁺ group. They all catalyzed hydrolysis of the substrate but, remarkably, the best catalyst by far was the A,B isomer 46, i.e., that with the acid and base groups right next to each other. This was completely inconsistent with a mechanism (42) in which a water molecule (bound to an Im) attacks the phosphate while the leaving group departs, assisted by the ImH⁺. Such an in-line mechanism would require the Im and ImH⁺ to be 180° apart, more or less, and so the A,D isomer should have been the best catalyst. Clearly, the ImH⁺ was playing a different role, protonating the phosphate oxyanion to facilitate formation of a phosphorane intermediate (47). This is the mechanism we had deduced for simple Im/ImH⁺ buffer-catalyzed hydrolysis of UpU (vide supra).

By proton inventory, a technique that determines whether acid and base groups act simultaneously, we found that hydrolysis of 36 by artificial enzyme 44 involves two protons moving in the transition state [130]. Thus, ImH⁺ of 46 is hydrogen bonded to a phosphate oxyanion of bound substrate 36; water hydrogen bonded to the Im then attacks the phosphorus, and as the O–P bond forms the ImH⁺ proton transfers (along with the water proton) to produce the phosphorane monoanion 47. This then goes on to the cleaved product in later catalyzed steps before there is time for pseudo-rotation. These general conclusions have been described and summarized in several publications [131–137].

1.3.4

Artificial Enolases and Aldolases

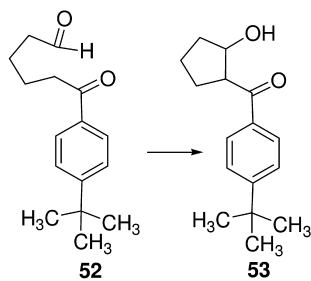
We examined the ability of our bis-imidazole cyclodextrin artificial enzymes to perform other bifunctionally-catalyzed reactions, where again the availability of the A,B and A,C and A,D isomers let us learn mechanistic details. As an important example, we examined three isomeric catalysts' ability to promote the enolization of substrate 48, which binds into the cyclodextrin cavity in water [138]. Here there was again a strong preference among the isomers, but it was the A,D isomer 49 that was the effective catalyst! It was also more effective than a cyclodextrin mono-imidazole that cannot use the bifunctional mechanism.

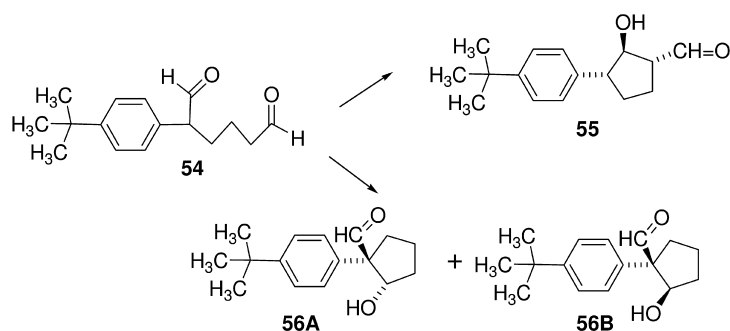


Cyclodextrin bis-imidazole catalyzes enolization by a bifunctional mechanism in which the ImH^+ is hydrogen-bonded to the carbonyl oxygen while the Im removes the neighboring methyl proton (cf. **50**). As expected from this, there was a bell-shaped pH vs. rate profile for the process. In the transition state two protons will move simultaneously, as in the hydrolysis reaction described above. Thus we indeed have a powerful tool to determine the geometric requirements for simultaneous bifunctional catalysis, a tool that could be of quite general use.

With enolization, we were able to understand the preference for the A,D isomer **49** in stereoelectronic terms. Models show that all three isomers can achieve geometries in which the ImH^+ can hydrogen bond to the carbonyl oxygen while the Im can reach the methyl proton, but the direction of attack on that proton differs among the isomers. The preferred isomer, the A,D species, removes the proton by a non-linear attack (cf. **51**), pushing the electrons toward the carbonyl group. This is presumably true for all enolizations, although techniques have not existed before to determine it.

Enolization can be part of an aldol condensation. We examined the aldol cyclization of compound **52** to **53** catalyzed by the bis-imidazole cyclodextrin artificial enzymes, and again saw that the A,D isomer was the preferred catalyst [139]. This was not an obvious result; the rate-limiting step in this case is cyclization of the enol, which is



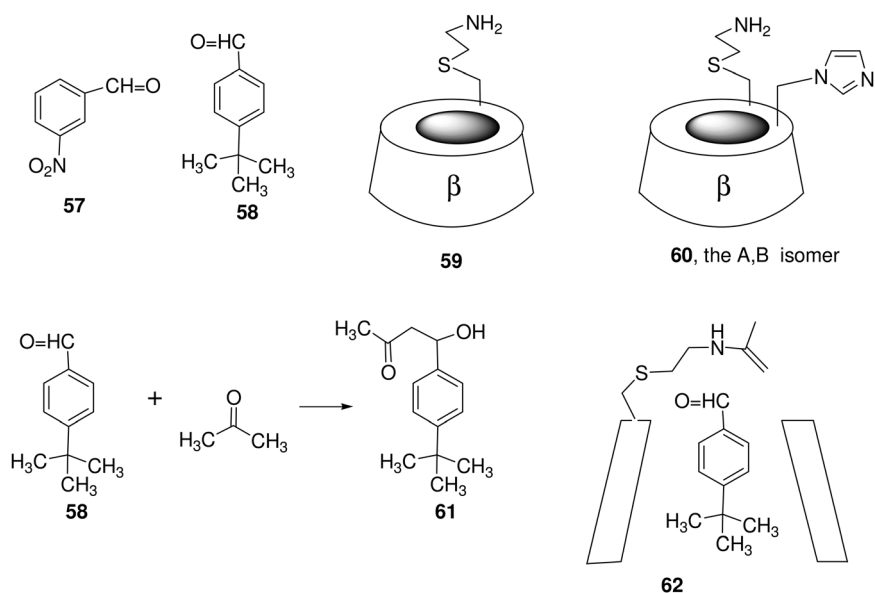


reversibly formed. In the absence of the catalyst, enolization – indicated by deuterium exchange – occurred rapidly next to the more reactive aldehyde group, as expected. However, with catalyst **49** this was reversed, and most of the deuterium exchange occurred next to the ketone group, in reach of the catalytic imidazoles. This enol cyclized with the aldehyde in the slow step of the sequence, but again this step had a preference for the A,D isomer **49** of the catalyst.

The situation is complex. In another study we examined the cyclization of compound **54** catalyzed by cyclodextrin bis-imidazoles [140]. This dialdehyde can perform the intramolecular aldol reaction using the enol of either aldehyde to add to the other aldehyde, forming either **55** or **56**. In solution with simple buffer catalysis both compounds are formed almost randomly, but with the A,B isomer **46** of the bis-imidazole cyclodextrin there was a 97% preference for product **56**. This is consistent with the previous findings that the catalyst promotes enolization near the bound phenyl ring, but in this case the cyclization is most selective with the A,B isomer **46**, not the A,D that we saw previously. Again the enolization is reversible, and the selectivity reflects the addition of an enol to an aldehyde group. The predominant product is a mixture of two stereoisomers, **56A** and **56B**. Both were formed, and were racemic despite the chirality of the cyclodextrin ring.

We have also examined the use of cyclodextrin-derived artificial enzymes in promoting *bimolecular* aldol reactions, specifically those of *m*-nitrobenzaldehyde (**57**) and of *p*-*t*-butylbenzaldehyde (**58**) with acetone [141]. Here, we examined a group of mono-substituted cyclodextrins as catalysts (e.g. **59**), as well as two disubstituted β -cyclodextrins (e.g. **60**) (10 catalysts in all). They all bound the aldehyde components in the cyclodextrin cavity and used amino groups of the substituents to convert the acetone into its enamine. An intracomplex reaction with **58** and hydrolysis of the enamine product then afforded hydroxyketone **61** (cf. **62**). These catalysts imitate natural enzymes classified as Class I aldolases.

Although *m*-nitrobenzaldehyde (**57**) is well bound into the cyclodextrin cavity of our above catalysts, there was essentially no catalysis of its reaction with acetone. The aldehyde group is too inaccessible in the complex. However, with **58** there was good catalysis by a cyclodextrin carrying only one ethylenediamine group on its secondary face, and also by cyclodextrins with two groups on the primary A and B methylenes (e.g. **60**), with imidazoles as base/acid groups and a primary amine to form the acetone



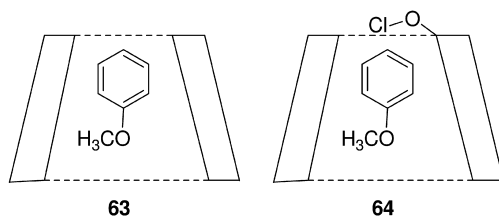
enamine. Some of the artificial enzymes also catalyzed the condensation of **58** with cyclopentanone. In this series we are using the catalytic groups on the cyclodextrin not just to perform acid–base processes but also to bind an external ketone as its enamine and thus promote intracomplex reactions.

1.4 Cytochrome P-450 Mimics

Enzymes exhibit wonderful selectivities, directed by the geometries of the enzyme–substrate complexes. For many practical purposes, learning how to imitate such selectivities is even more important than achieving rate accelerations at the enzyme level. Of course, selectivity is a reflection of relative rates, but a selective rate advantage of only one-hundred-fold could be enough to produce one product rather than either another or a mixture. This is easier to achieve than the huge overall rate accelerations that most enzymes exhibit.

1.4.1 Aromatic Substitution in Cyclodextrin Complexes

In one of our earliest approaches to such biomimetic selectivity, directed by the geometry of the catalyst–substrate complex, we examined the directed substitution of an aromatic ring bound into the cyclodextrin cavity [142, 143]. Anisole was chlorinated by HOCl entirely in the para position when it was bound into α -cyclodextrin, while in



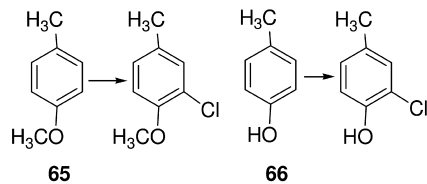
water solution without the cyclodextrin the product was 60% *p*- and 40% *o*-chloroanisole. The simplest idea could be that the cyclodextrin simply blocks the ortho positions in complex **63**, but this is not correct. The cyclodextrin actually accelerates the reaction of the para position, so it is a catalyst, not just a blocker.

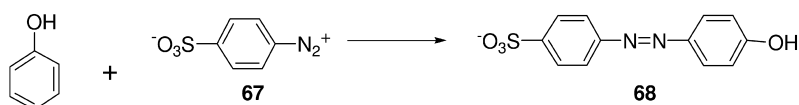
Also, in water solution the chlorination of anisole by HOCl shows second-order kinetics in HOCl; the chlorinating agent is the more powerful Cl_2O , which is in equilibrium with two HOCl molecules [143]. However, when the cyclodextrin is present the chlorination shows only first-order dependence on [HOCl], indicating that a different chlorinating species is involved in the catalytic reaction. We also examined the reaction using β -cyclodextrin instead of α -cyclodextrin. Again, there was no ortho chlorination of anisole in the complex, but now there was no acceleration of the para chlorination. Thus, the catalyzed para chlorination process is faster with the well-fitting α -cyclodextrin than with the more loosely fitting β -cyclodextrin.

From all this we concluded that the catalysis by cyclodextrin involves the reversible formation of a cyclodextrin hypochlorite (**64**), which can transfer chlorine to the anisole para position but cannot reach the ortho position. The reaction is faster when the RO–Cl group is held closer and more rigidly in the smaller α -cyclodextrin.

It is interesting that this artificial enzyme induces such selectivity whereas the enzyme chlorinase does not. The real enzyme produces a 60:40 mixture of para and ortho chloroanisoles, presumably because it generates HOCl that reacts with free anisole in solution. The artificial enzyme is more like a typical enzyme – which would be highly selective in its products – than is this particular natural chlorinating enzyme!

Further insight came from our study of other aromatic substitution reactions. When we blocked the para position of anisole in compound **65**, we saw that ortho chlorination was blocked by binding with α -cyclodextrin, so the only reaction was from the substrate that was in free solution, not that which was bound. However, with *p*-cresol (**66**) there was still, of course, ortho chlorination but now it was *catalyzed* by the α -cyclodextrin. When *p*-cresol binds to the cyclodextrin, the polar phenol or phenoxide group will be out of the cavity, bringing the ortho positions within reach of the cyclo-





dextrin hydroxyl groups. Therefore, in the selective reaction either para or ortho substitution can be catalyzed if the geometry of the complex is suitable.

One important control was the study of the diazo coupling of phenol with **67**. Here para coupling formed **68**, but the reaction was inhibited by α -cyclodextrin. The diazonium group of **67** cannot be delivered by binding to a cyclodextrin hydroxyl group, in contrast to a chlorine atom. This supports the idea that cyclodextrin catalysis of chlorinations involves binding of both the chlorine atom and substrate, holding them together for rapid selective reaction.

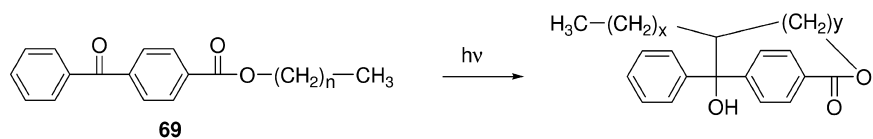
In a further study, α -cyclodextrin that was partially methylated, blocking the OH groups at C-2 and C-6 and leaving the C-3 OH group free [144], was found to catalyze the para chlorination of anisole. Therefore, at least the cyclodextrin C-3 OH can be the catalytic group. Furthermore, the binding of anisole was stronger than that in α -cyclodextrin, probably reflecting the flexible capping of the cavity described earlier [84]. Thus it was possible to achieve 99% para chlorination of anisole (without the flexible capping, there was always some free anisole in solution that led to some ortho chlorination as well). We also synthesized a polymer, consisting of linked α -cyclodextrins, that could be used in a flow mode to produce anisole that was over 99% para chlorinated, because there was essentially no free anisole in equilibrium with the polymeric α -cyclodextrin and the reaction within the complex was completely selective [144].

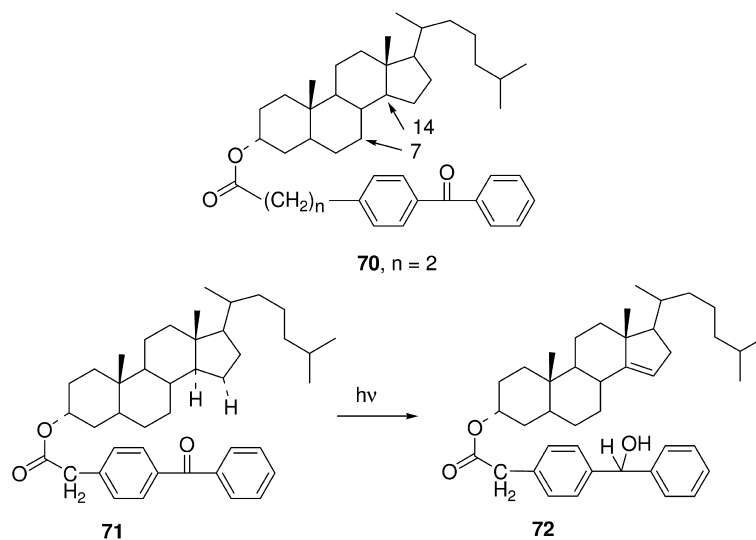
1.4.2

Selective Photochemical Reactions

Our principal target in P-450 mimics was the selective oxidation of saturated carbons directed by geometric control, not by intrinsic reactivity. In our first study, we examined selective geometrically controlled attack on aliphatic C-H bonds by photo-excited benzophenones [145]. In a process we labeled “remote oxidation”, photolysis of a long-chain ester **69** of benzophenone-4-carboxylic acid afforded insertion into CH₂ groups far into the chain.

In such an insertion the benzophenone is excited to its triplet state, resembling a C-O diradical. The oxygen atom of the ketone then removes a hydrogen atom from the methylene group, leading to a carbinyl radical and the hydroxy diphenylmethyl radical formed by hydrogen atom addition to the benzophenone. This pair of radicals couples





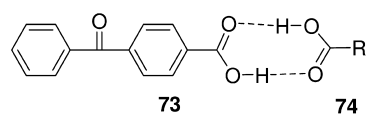
to make the new carbon–carbon bond. There was no great selectivity (with a C₂₀ chain there was appreciable insertion into carbons 11–16) but the method gave an indication of the accessible conformations of the molecule [146]. We later used this method to determine the conformations of the flexible chains in membranes [147, 148], and others have adapted it for photo-affinity labeling in proteins.

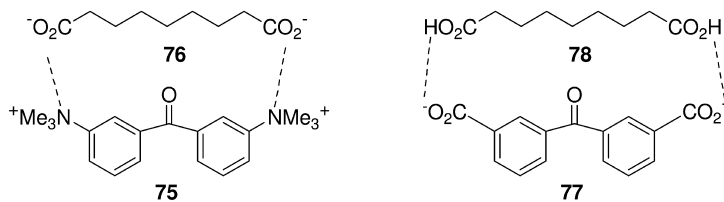
With inflexible substrates, this photochemical functionalization process showed significant selectivity. As our first example, photolysis of steroid ester **70** led to carbonyl insertion into the C–H bonds at C-7 and C-14 [149, 150]. Other steroid esters afforded selective functionalizations in various positions [e.g. 151, 152], and compound **71** directly dehydrogenated the steroid to form the product olefin **72** [150].

Later work gave additional examples, and we were able to elucidate the process by which **72** is formed [153]. After initial removal of the C-14 hydrogen by the oxygen of the benzophenone triplet, the resulting C-14 radical inverts to bring C-15 near the reagent radical; the C-15 α hydrogen is then transferred to produce product **72**. This was demonstrated by use of C-15 α deuterium-labeled steroid. Apparently, in the more rigid **71** such hydrogen transfer in the radical pair is easier than is coupling to form a carbon–carbon bond, whose cyclic product would be strained.

We also saw directed functionalizations when a benzophenone reagent (**73**) was not directly attached to a steroid or long alkyl chain substrate (**74**), but was linked by hydrogen bonding [151].

In all the above studies the benzophenone units were singly attached (or with the previous example, complexed) to the substrates, so that there was intramolecular or





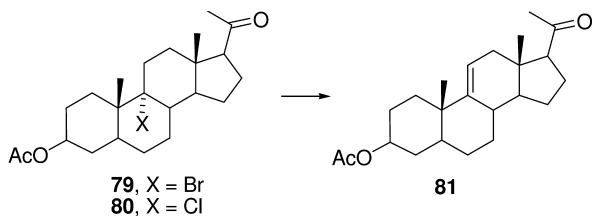
intracomplex functionalization directed by the conformations of freely rotating units. To overcome this flexibility, we studied the functionalization of dicarboxylic acids doubly complexed with disubstituted benzophenones [154]. Two sorts of complexing were used: benzophenone dication **75** ion-paired with substrate dicarboxylate ion **76**, and benzophenone dicarboxylate **77** hydrogen bonded to substrate dicarboxylic acid **78**. We saw high selectivities for attack at the two central (equivalent) carbons with a substrate of the correct length to fit well across the benzophenone, while there was less selectivity with a longer substrate with flexible conformations in the complexes. As described later, such double complexing of substrates was of general interest and general utility in other artificial enzymes.

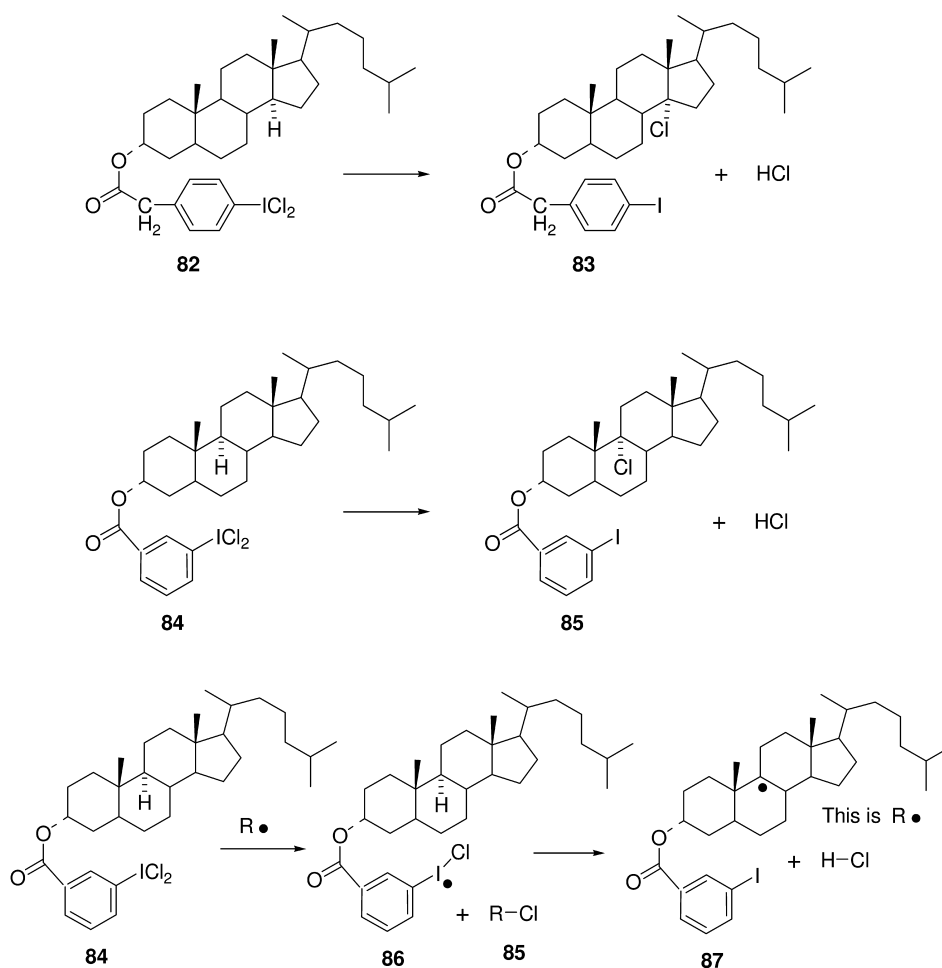
1.4.3

Directed Halogenations

Irradiation of the benzophenone-linked steroids in the presence of CCl_4 solvent gave some chlorination of the steroids. Also, irradiation in the presence of BrCCl_3 afforded no benzophenone products, only bromosteroids from a free radical chain process initiated by the irradiation [155]. In the presence of PhICl_2 we saw only chlorosteroids, again by a radical chain process [155]. In both cases the rather selective hydrogen abstraction led to exclusive substitution of tertiary hydrogens of the steroids, in particular at C-9 and C-14. C-9 bromo- and chlorosteroids **79** and **80** could be dehydrohalogenated to form the 9(11) olefin **81**. This afforded an easy entry into corticosteroids, which have an oxygen atom at C-11 and often, usefully, have a fluorine at C-9.

Such normal free-radical substitution processes might be geometrically directed to otherwise unremarkable positions in substrates, as the benzophenone reactions had been. Consequently, in our first example, we found that intramolecular chlorination could be directed by attachment of a PhICl₂ group to the steroid [156], i.e., compound **82** directed chlorination exclusively to C-14 (**83**), while **84** directed halogenation exclusively to C-9 (**85**). As shown in Scheme 1.1, an intramolecular hydrogen abstraction by

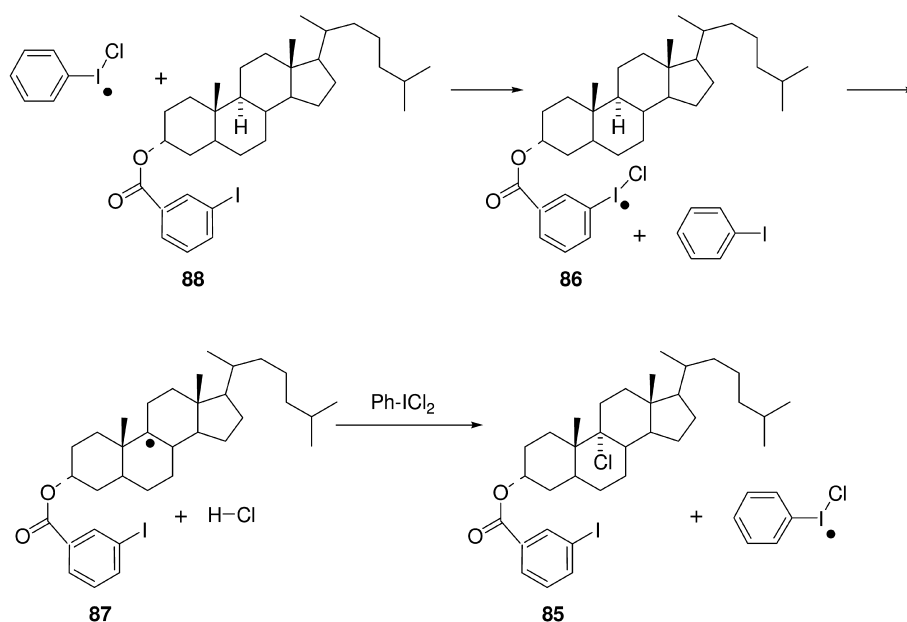




Scheme 1.1

an attached PhICl radical (**86**) to generate (**87**) is followed by an intermolecular chlorine transfer from another substrate to generate its attached radical.

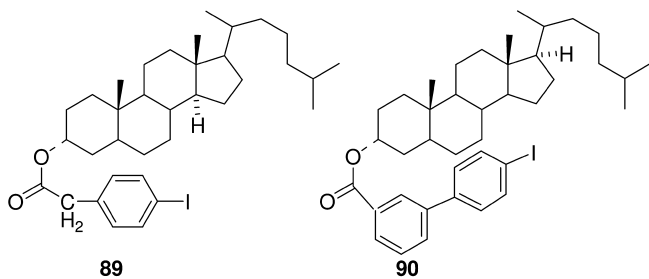
However, because there is only a stoichiometric amount of the chlorinating agent, side reactions mean that some substrate is left unchlorinated. Also, the attached PhICl₂ reagent must be made by adding Cl₂ to the easily produced PhI ester. To better achieve the same results, using chlorine transfer from an external radical to generate the attached PhICl radical, we employed a process we called a Radical Relay Mechanism [157] (Scheme 1.2). Here an external PhICl radical transfers its chlorine atom to the iodophenyl ester group attached to the substrate, and then the intramolecular hydrogen atom abstraction occurs. Thus we can use a slight excess of the PhICl₂ reagent to complete the process, and there is no need to add Cl₂ to the attached iodophenyl group, which acts as a template to steer the chlorinations.

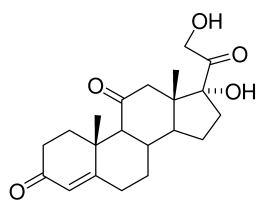


Scheme 1.2

As expected, compound **89** underwent C-14 chlorination and **90** underwent C-17 chlorination. Other chlorinating agents could also be used. In particular, SO_2Cl_2 was able to selectively chlorinate **88–90** by transferring a Cl atom from a ClSO_2 intermediate radical (liberating SO_2), and then generating this radical after Cl transfer to the steroid radical.

Why does this work? Why does an externally generated reagent not directly attack the substrate but instead transfer an atom to the template, which then leads to attack on the C–H bond with geometric control of the attack position? The reason is the same as that which causes many hydrolytic enzymes to cleave a peptide bond in two steps by first forming an ester intermediate with a serine hydroxyl group and then later using water to hydrolyze that ester. By the time the water attacks, with loss of translational entropy, the amine component of the original peptide bond is freely moving and has gained translational entropy. In our radical relay mechanism, the PhI molecule – formed by chlorine atom transfer to the large accessible iodine atom of the template – is freely



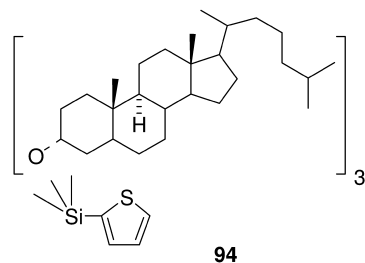
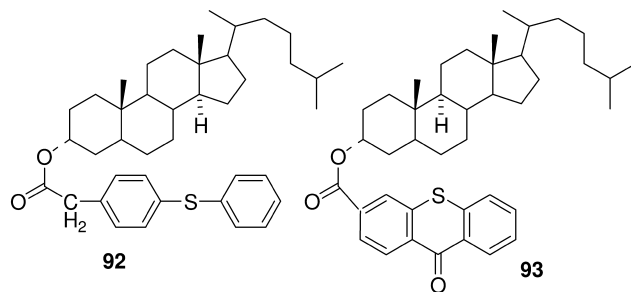
**91**, cortisone

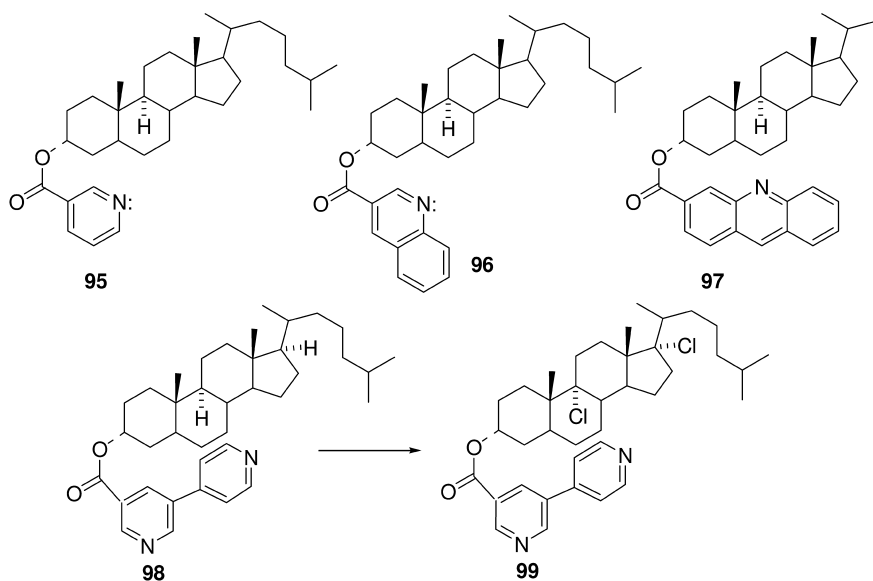
moving by the time the rather tight transition state for hydrogen atom abstraction occurs. As with the enzymatic processes, the advantage is entropy.

We used the radical relay process, chlorinating C-9 and then generating the 9(11) double bond, in a synthesis of cortisone **91** [158]. This is a substitute for manufacturing processes in which C-9 or C-11 are hydroxylated by biological fermentation. Also, with templates that directed the chlorination to C-17 of 3 α -cholestanol, such as that in **90**, we were able to remove the steroid sidechain [159–162]. Using an electrochemical oxidation process, we could direct chlorination by simple chloride *ion* with an iodo-phenyl template [163]. A general review of the processes with iodophenyl templates has been published [164].

The radical relay process also works with other template types. Thus, the thioether unit in **92** directed chlorination of C-14 by SO₂Cl₂ [165]. Also, the sulfur in the thioxanthone template of **93** directed the radical relay process to C-9 [166]. The thiophene sulfur in **94** was able to direct chlorination to C-9 in all three attached steroids [167]. In all these cases, an intermediate is formed with a chlorine atom bonded to sulfur.

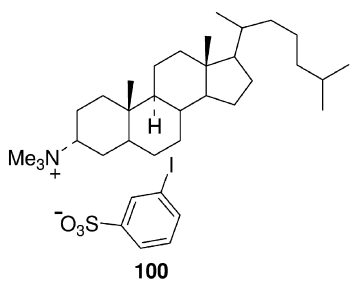
A chlorine atom could also coordinate to the nitrogen of the pyridine template in compound **95**, directing chlorination to C-9 in a radical relay process [168]. Spectro-





scopy and detailed quantum mechanical calculations indicated that the Cl–N bond is a localized three-electron coordination, strong enough to hold the chlorine in place but weak enough to permit it to attack tertiary C–H bonds [169]. Quinoline and acridine templates in **96** and **97**, respectively, could also use this nitrogen coordination of a chlorine atom to direct steroid chlorination [170]. In a particularly interesting example, both nitrogen positions in the template in **98** were used for chlorine coordination, producing the 9,17-dichloroproduct **99** in quantitative yield [171]. We have reviewed the use of heterocycles as radical relay templates [172].

The templates can be simply coordinated rather than attached. For example, complex **100** directed the radical relay chlorination to C-9, although the process was not as clean as with the attached templates [173]. We also used template-directed chlorinations to determine the conformations of flexible chains, just as we had previously with the benzophenone probes [174]. Also, by use of a set of tandem free radical chain reactions we could direct the formation of carbon–bromine and carbon–sulfur bonds, again with geometric control by the attached template [175].



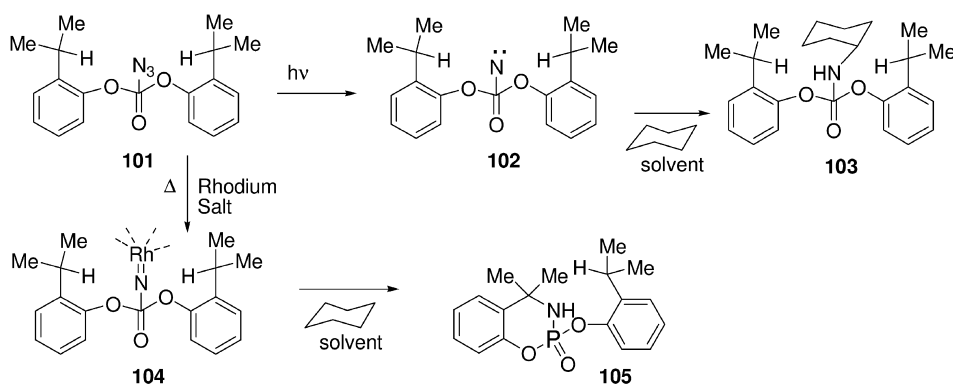
The template-directed steroid functionalizations are remarkably selective. After all, the C-9 and C-14 hydrogens are very close (1,3 diaxial on ring C). In a theoretical paper we discussed this selectivity, and concluded that an extra factor in the selectivity may be more than the ability of a chlorine atom to simply collide with one or the other of these hydrogens [176]. Instead, the trajectory of the collision may be important, requiring a straight C–H–Cl alignment. This would help select among such closely spaced hydrogens.

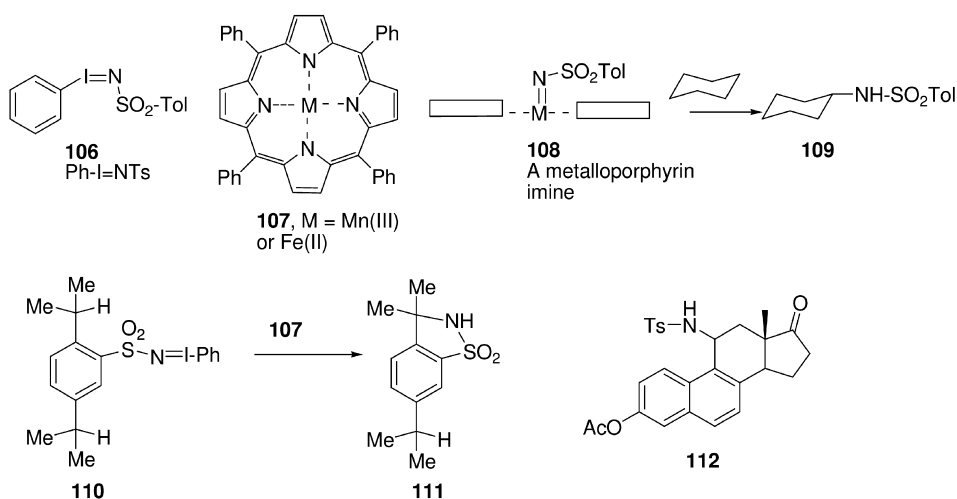
1.4.4

Nitrene Insertions

We briefly explored the use of nitrene insertions directed by geometric placement of an appropriate nitrene precursor, which was generated by photolysis of an azide. Although some nitrenes readily rearrange, we saw a class of phosphoryl nitrenes that performed intermolecular insertions rather than rearrangements [177]. However, when such a nitrene **102** was generated in cyclohexane solution by photolysis of **101** it preferentially attacked the solvent, forming **103**, rather than performing an intramolecular insertion reaction into the neighboring benzylic C–H bond [178]. Intramolecular product **105** was indeed seen when azide **101** was decomposed thermally in cyclohexane in the presence of a rhodium salt. Thus a free nitrene is so reactive that the normal preference for intramolecular reactions is not seen – it reacts on essentially every collision and is surrounded by solvent. Rhodium complexed nitrene **104**, a “nitrenoid”, is less reactive and shows the entropy advantage of an intramolecular reaction with geometric control.

Such metal-complexed nitrenes were also generated by the reaction of (tosyliminoiodo)benzene (**106**) with Mn(III)- or Fe(II)-tetraphenylporphyrin, **107**, in a mimic of cytochrome P-450 but with a tosylimino group instead of an oxygen atom on the metals (**108**) [179]. It was able to functionalize cyclohexane solvent, by nitrogen insertion into a C–H bond to form **109**. Furthermore, the metalloporphyrins also catalyzed an intramolecular nitrogen insertion converting **110** into **111** [180].





Cytochrome P-450 can perform both intramolecular and intermolecular nitrene insertions with **106** in water solution [181]. However, the intermediate metallo-nitrene (**108**) also hydrolyzed to some extent; so the enzyme performed a hydroxylation as well as an amidation. Although we have also performed an intermolecular amidation of a steroid, using such a metalloporphyrin reaction with **106** to form **112** [182], we have not yet extended it to the kinds of directed functionalizations described above with either benzophenones or chlorinations.

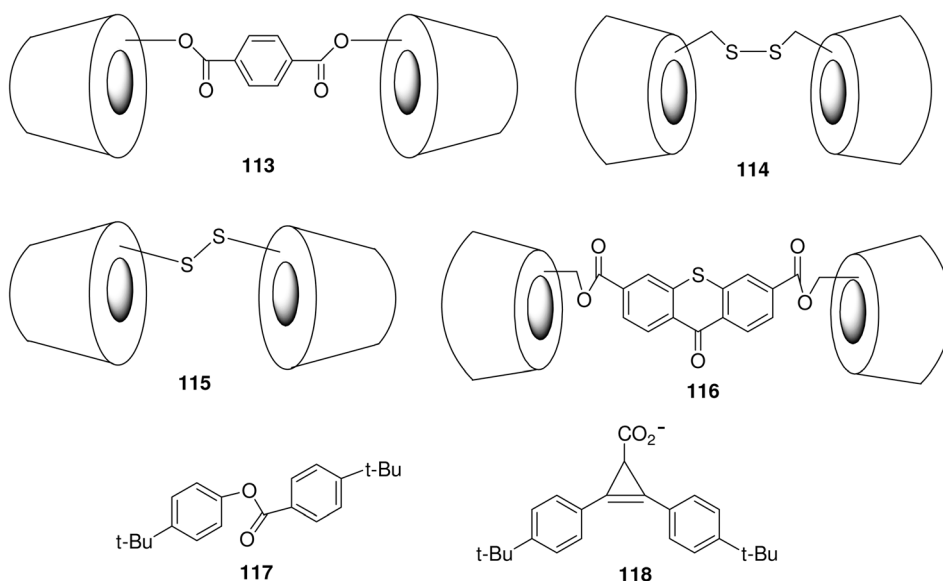
1.4.5

Binding by Cyclodextrin Dimers

To create artificial enzymes that could bind substrates in water solution with defined geometry, we examined dimers of cyclodextrins. As mentioned above, we used such dimers in mimics of hydrolytic enzymes [119, 120]. Now we wished to use them for mimics of cytochrome P-450.

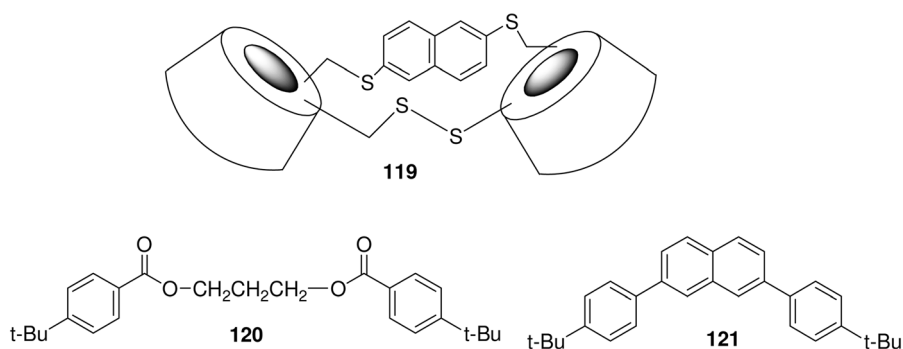
Initially, we prepared β -cyclodextrin dimers **113–116** [183], which were examined for substrate binding with two *p-t*-butylphenyl groups. With ester **117** and cyclopropene **118** we saw binding constants as high as 10^8 M^{-1} . This is double the free energy of binding of simple *p-t*-butylphenyl groups into a single cyclodextrin – binding constants are generally about 10^4 M^{-1} – so the free energies of binding were additive with substrates that could fit well.

Even stronger dimeric binding, due to the entropy advantages of chelate binding, was seen with cyclodextrin dimer **119**, which was doubly linked, restricting its rotational freedom [184]. The flexible substrate **120** gave a binding constant to dimer **119** of 10^{10} M^{-1} , while with the rigid substrate **121** the binding constant was even larger. Interestingly, although the arguments for special effects in chelate binding normally involve *entropy* advantages, a study we did of several such chelate binding situations



with cyclodextrin dimers and double-ended substrates showed that the special chelate effect was reflected in the *enthalpies* [185]. Entropy–enthalpy compensation is not an unusual phenomenon. We performed a number of such studies, including one in which we were able to quantitate the binding energy that could be ascribed to the hydrophobic effect [186].

We also investigated chelate binding by dimers of a synthetic hydrophobic macrocycle, in place of the cyclodextrins [187]. In the systems examined the chelate effect was weaker than that seen with the cyclodextrin dimers. We also studied the strong binding of cholesterol by some cyclodextrin dimers and a cyclodextrin polymer, and saw that the large sterol could occupy parts of two binding cavities [188].

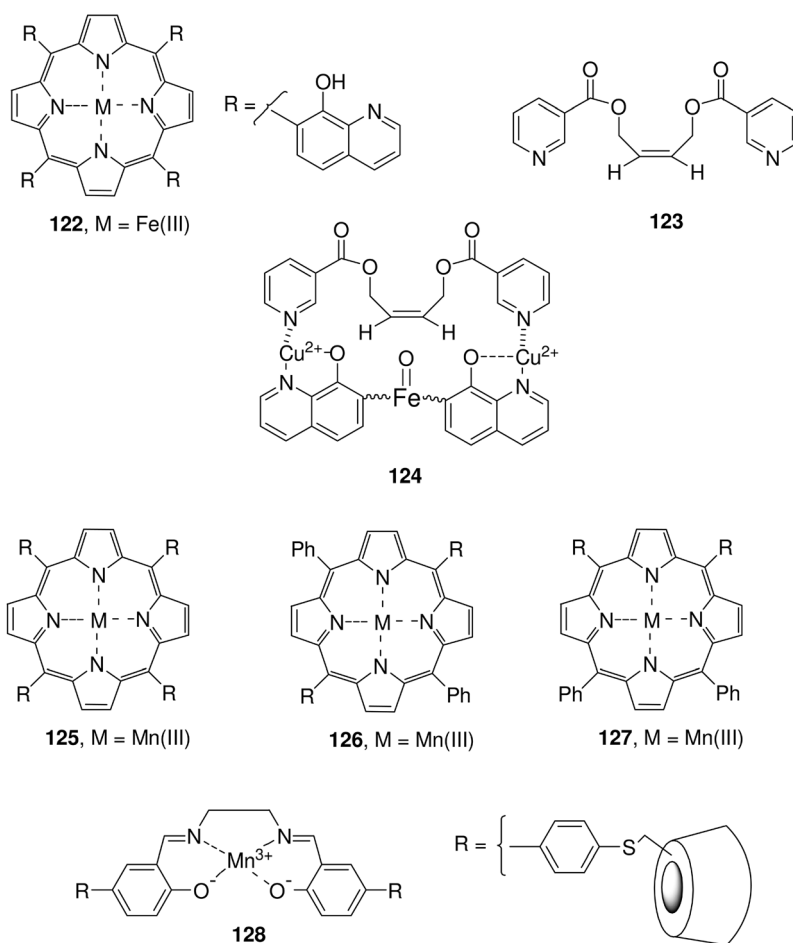


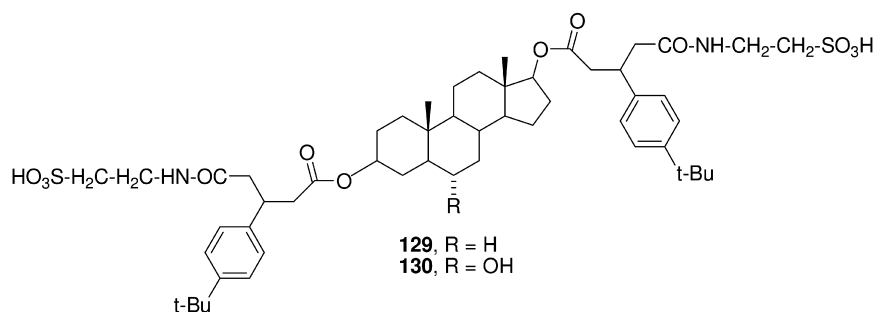
1.4.6

Hydroxylations by Artificial P-450 Enzymes

Metalloporphyrins can catalyze the hydroxylations of solvent species such as cyclohexane. From our studies with cyclodextrin dimers, we concluded that by attaching cyclodextrin rings to metalloporphyrins we should be able to bind substrates in water and achieve selective hydroxylations directed by the geometries of the complexes. This was successful.

Initially, instead of cyclodextrin complexing, we used metal coordination [189]. With Fe(III) porphyrin **122** carrying 8-hydroxyquinoline groups we epoxidized substrate **123** using Cu^{2+} as a bridge (cf. **124**). A metallosalen catalyst was not as effective. We then synthesized a series of Mn(III) porphyrins, **125**–**127**, as well as a Mn(III) metallosalen **128**, all carrying attached β -cyclodextrin groups [190]. We examined their ability to epoxidize the double bonds of substrates that could bind into two of the cyclodextrin



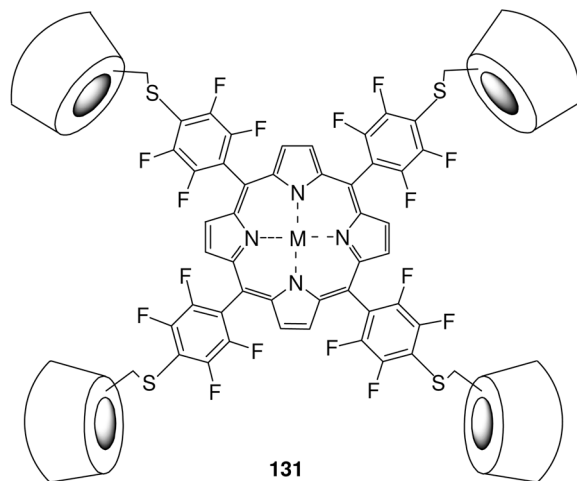


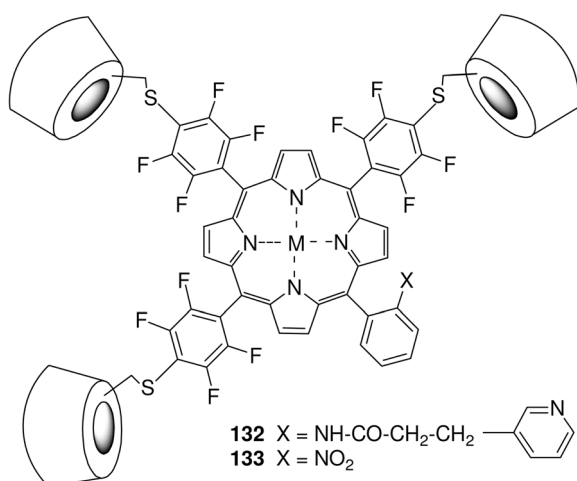
groups, using iodosobenzene as the oxidant, and saw sensible variations in the relative rates of epoxidation with structure, indicating that the porphyrins that could bind the substrates across the face of the metalloporphyrin core were the most effective.

Building on these results, we examined the capability of manganese porphyrin **125** in hydroxylating steroid substrate **129**, again using iodosobenzene as oxidant [191, 192]. Indeed, the reaction was successful, and a single product was produced in which the C-6 equatorial hydrogen of substrate **129** was replaced with a hydroxyl group in product **130**. There was even some turnover catalysis, but with only 4 turnovers or so before the catalyst was oxidatively destroyed.

The turnover problem was solved with porphyrin derivative **131**, in which the fluorines on the phenyl groups greatly stabilized the catalyst against oxidative destruction [193]. Again with iodosobenzene as the reagent, catalyst **131** converted substrate **129** into its 6-hydroxy derivative **130**, but now with 187 turnovers. We have since produced catalysts with even higher turnovers for this process [194].

In our catalytic hydroxylations described above we added some pyridine to the solution to coordinate with one face of the manganese porphyrin, so as to direct an oxygen atom and the bound substrate to the same face. To avoid this, we synthesized catalyst

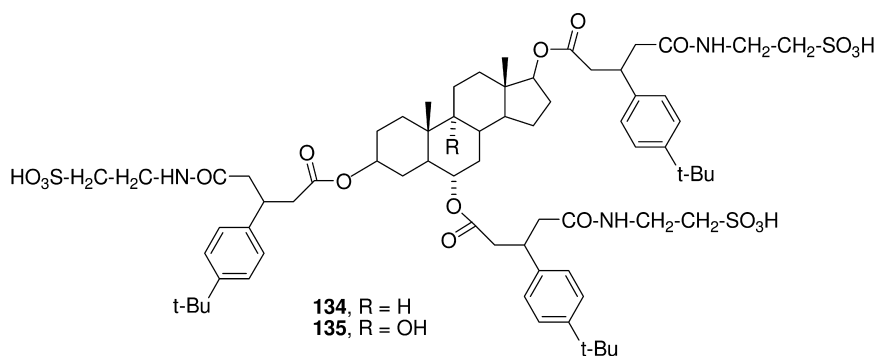


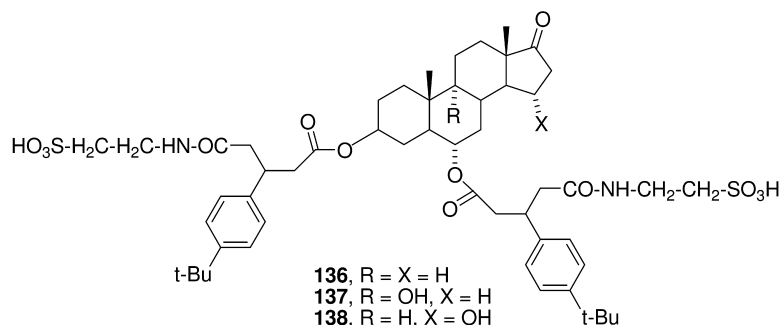


132 with three cyclodextrins and a linked pyridine ring; no extra pyridine was needed with this catalyst [194]. As a bonus, this catalyst hydroxylated the C-6 position with 2000 turnovers. Catalyst **132** was synthesized through intermediate **133**, with a nitrophenyl group, and this performed the C-6 hydroxylation with 3000 turnovers (although it still needed the added pyridine in solution). Such very high turnovers make these catalytic hydroxylations potentially practical.

While the selective hydroxylations at C-6 are certainly examples of geometrically directed functionalizations, imitating the enzyme cytochrome P-450, C-6 is not the most attractive target for such selectivity. We wanted to perform a selective multi-turnover hydroxylation of C-9 in the steroid. This would imitate the hydroxylations that take place in fermentations, and would let us convert the product into the 9(11) olefin, as we had done in our directed chlorinations described above.

Computer molecular modeling indicated that the hydroxylations at C-6 occurred because, with only two binding points for the substrate (into the two cyclodextrins on opposite sides of the porphyrin), the substrate could rotate so as to point its edge toward the Mn=O species that performs the hydroxylation. To solve this, we



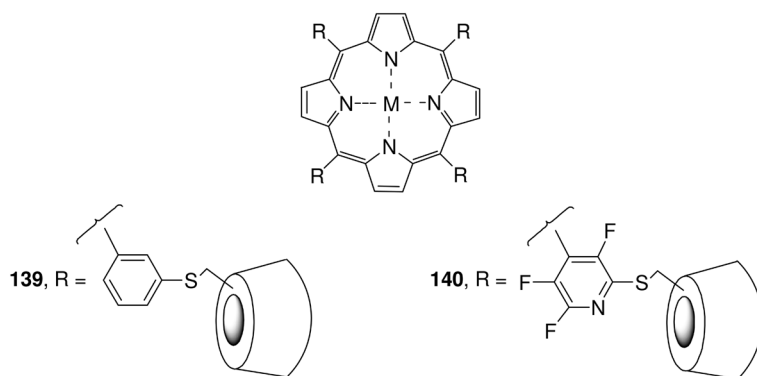


added an additional binding ester group to the substrate, in **134** [195]. This made *three* binding interactions with the catalyst **131**, and the hydroxylation product was now indeed the C-9 hydroxy compound **135**. Our computer models made it clear why this was successful [195]. Some of this work has been reviewed [196].

The third binding interaction introduced in the hydroxylation of **134** made the substrate present its face, not its edge, to the Mn=O of catalyst **131**, but it seemed likely that *double* binding of the substrate could also achieve this if the binding groups were attached to C-3 and C-6 rather than C-3 and C-17 as in **134**. Thus we synthesized a new substrate, **136**, and examined its hydroxylation by catalyst **131** [197]. We saw that indeed there was hydroxylation at C-9 to form **137**, but there was also some hydroxylation at C-15 to form **138**. With the lack of a binding interaction at C-17, apparently the substrate had slipped a little to the left, moving C-15 into position to be attacked.

To move the substrate back in place, by shortening the distance from a cyclodextrin to the Mn=O group, models suggested that the cyclodextrin be attached to the meta rather than para position of the phenyl rings in the catalyst. Thus we synthesized catalyst **139**, with such a meta attachment, and indeed it did cleanly convert substrate **136** into the C-9 hydroxy product **137**, with no hydroxylation at C-15 [197]. However, as there was no fluorine in the phenyl linkers, the catalyst was destroyed after only 2.5 turnovers.

This was solved by replacing the phenyl linkers with trifluoropyridine rings in catalyst **140** [197]. The compound was easily made, and it performed the C-9 hydroxylation of substrate **136** to form product **137** with 90 turnovers.



We have also synthesized a catalyst related to **131** in which the cyclodextrin rings were replaced with synthetic macrocyclic binding groups [196]. Also, we have examined catalysts related to **131** in which substrate binding involved metal ion coordination, not hydrophobic binding into cyclodextrins or macrocycles [198].

1.5

Future Prospects

As Philip Ball has pointed out, in biomimesis we take principles from Nature, not blueprints [199]. That is, we adopt the style of natural chemistry, but do not simply reproduce the same enzymes by which Nature achieves selectivity. His analogy is very apt: “A jumbo jet is not just a scaled-up pigeon.” We learned the principle of wings from the birds, but not the details of how to use them and power the flight. Thus, in our work we have adopted the principles of reversible bonding and geometrically directed selectivity characteristic of natural enzymes, but used very different structures in our artificial enzymes. Our goal is to “liberate chemistry from the tyranny of functional groups” [194].

This new style of synthetic catalysis will of course not replace all normal synthetic methods. For many purposes, the standard methods and rules – e.g. aldehydes are more easily reduced than are ketones – will continue to dominate organic synthesis. However, when we require a synthetic transformation that is not accessible to normal procedures, as in the functionalization of unactivated carbons remote from functional groups, artificial enzymes can play a role. They must compete with natural enzymes, and with designed enzyme mutants, but for practical large-scale industrial synthesis there can be advantages with catalysts that are more rugged than proteins.

Our work in the development of artificial enzymes, described here and in Chapter 2, has established that catalysts can be made that will achieve excellent defined geometrically directed functionalizations and, furthermore, that the combination of binding groups with coenzyme analogs leads to powerful catalysts for some reactions. We expect the field of biomimetic chemistry to continue to grow, as we combine lessons from Nature with the ingenuity of chemists.

We will learn to produce mimics of enzyme clusters, imitating natural clusters such as gene transcription assemblies. We will learn to produce artificial enzymes that show induced fit, and allosteric control by analogs of hormones. Then we will move to mimics of cells themselves, with their components of many enzymes, to achieve chemical processes more complex than those done by a single enzyme. The biochemistry of life is impressive, but the role of chemistry is not just to admire it. As humans were impelled to invent ways to fly after observing birds, we will learn to create a new area of chemistry – biomimetic reaction chemistry – adding both to our understanding and to our practical abilities.

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