Synthetic and biological catalysts in chemical synthesis: how to assess practical utility

Enzymes, synthetic catalysts, and catalytic antibodies can all be used to perform asymmetric reactions, but their practical utility is not always easy to evaluate. Criteria with which to compare such catalysts are proposed and illustrated for asymmetric epoxidation, a reaction to which all three approaches have been applied.

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As synthetic chemists continue to show that even the most complex natural products are accessible to total synthesis (see [1–3] for some recent dramatic examples), attention is shifting away from the question of whether a molecule can be made synthetically towards the issue of how it can be made in a truly practical manner. Reactions that perform difficult chemical transformations with high selectivity and efficiency, are cheap, and generate minimal amounts of reaction by-products and waste, are therefore much in demand. Selective catalysis is often the best way to achieve such reactions.

Enantioselective synthesis is a particularly important application for selective catalysis. This type of synthetic chemistry allows optically active compounds to be generated directly, either from precursors that are not chiral, or from racemic mixtures. For most biologically active compounds, the correct absolute stereochemistry is crucial. As a result, the search for useful asymmetric catalyst systems has become one of the most active areas of research in organic and bioorganic chemistry.

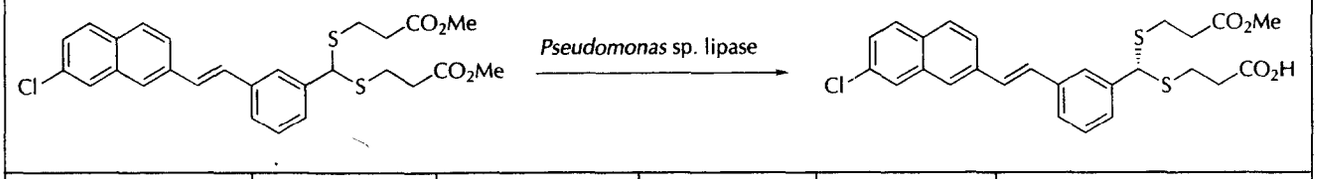
There are currently three major approaches to asymmetric catalysis, employing fundamentally different strategies. Natural enzymes can either be used directly, in whole cell-systems or as a purified preparation, or may be modified in several different ways [4]; purely synthetic catalysts can be constructed [5]; and biological catalysts can also be generated *de novo*, an approach typified by catalytic antibodies [6]. All three approaches have received considerable attention and have been extensively reviewed. Despite this, issues that are critical in determining whether an approach is practically useful are often not the focus of attention. Here, we will assess the current status of these three classes of asymmetric catalysts from a pragmatic synthetic perspective. Since all three approaches have been applied successfully to the asymmetric epoxidation of unfunctionalized olefins, we will use this type of reaction to compare these approaches directly.

Enzymes

Enzymes accomplish an array of remarkable, often almost magical, chemical transformations with high selectivity. For many types of reaction, there are no known synthetic catalysts or reagents that come close to emulating the selectivity that can be achieved with enzymes. A particularly impressive application of an enantioselective lipase reaction was developed by the Merck process group for the synthesis of an LTD_4 antagonist (Fig. 1; [7]). This reaction afforded product in 92 % yield and 98.5 % enantiomeric excess or ee (% ee = % major enantiomer – % minor enantiomer). The carbon in the diester starting material that becomes the stereogenic center in the product is four atoms removed from the reaction site, a huge distance over which to relay stereochemical information. Yet the enzyme accomplishes the necessary asymmetric induction to give very high yield of product in nearly enantiomerically pure form. This reaction illustrates one of the most impressive features of enzymes: they can catalyze extremely difficult reactions with high stereoselectivity. They are usually efficient in energy use, give little unwanted by-product, and their reaction rates are generally rapid. But they also have limitations. Enzymes are usually highly specific for their substrates, so that they can only be used with a limited substrate pool, and they often show severe product inhibition. Perhaps more important, they have unalterable absolute stereochemistry, so that only one product enantiomer can be made. Furthermore, our ability to control the structure and reactivity of an enzyme is limited. It is nevertheless possible to make some useful modifications to natural enzymes; for example, site-directed mutagenesis has recently been used to give a remarkable improvement in enantioselectivity in a sulfoxidation reaction catalyzed by horseradish peroxidase [8].

Synthetic catalysts

Synthetic asymmetric catalysts are an important alternative to enzymes, because the limitations of one are in many ways the advantages of the other. Several chiral synthetic catalysts that give high enantioselectivity but can also be used with a remarkable range of substrates



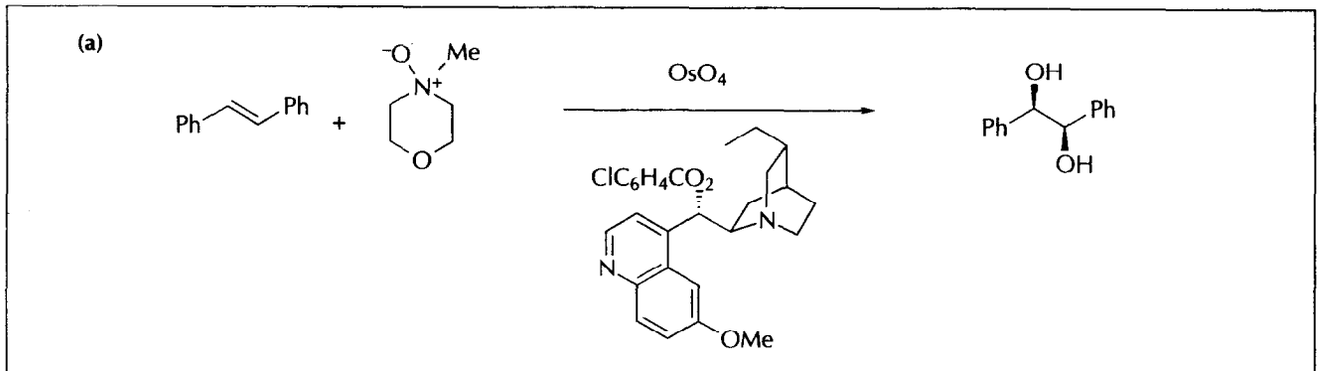
Weight of substrate	Weight of catalyst	Weight of product	Yield	ee	Volumetric productivity
5.27 g	0.020 g	4.85 g	92%	98.5%	≈10 g l^{-1}

Fig. 1. Selective hydrolysis of a diester by *Pseudomonas* lipase [7]. An achiral substrate was converted almost quantitatively into the correct enantiomer of product. Reaction parameters are summarized.

have been developed over the last several years. The osmium-catalyzed asymmetric dihydroxylation reaction discovered and developed by Sharpless and co-workers [9,10] is a particularly good example (Fig. 2). Synthetic catalysts can offer enormous advantages with regard to cost, design flexibility, and stability. Most synthetic catalysts developed to date can be made in either enantiomeric form, allowing control over the absolute stereochemistry of the product. They can usually be used in non-aqueous media and tolerate a variety of reaction conditions. Perhaps most important, they can be designed to effect reactions for which Nature has no counterpart. Synthetic catalysts can rarely match the selectivity routinely attained by enzymes, however, and for applications such as the selective transformation of highly functionalized, water-soluble substrates, synthetic catalysts will probably never be as good as enzymes. Researchers at Scripps have recently illustrated the relative strengths of each class of catalyst by applying both types in tandem in an elegant synthesis of carbohydrates [11].

Catalytic antibodies

At least from a conceptual standpoint, catalytic antibodies combine many of the best features of synthetic and enzymatic catalysts. Like enzymes, they are generated by a living system, and thus make use of the ability of living systems to generate a diverse pool of molecules from which the desired catalyst can be selected. Unlike enzymes, but like



Weight of substrate	Weight of catalyst	Weight of product	Yield	ee	Volumetric productivity
180.25 g	23.25g + 1 g OsO_4	222.7 g (155–162 g)	104 % (72–75%)	90 % (99+ %)	≈250 g l^{-1}

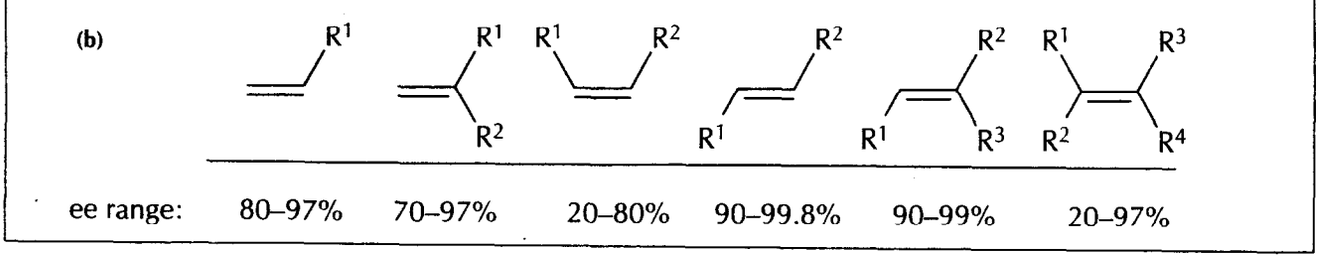
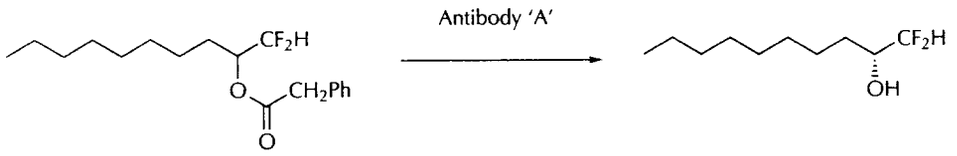


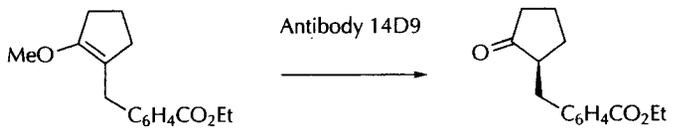
Fig. 2. Osmium-catalyzed asymmetric dihydroxylation has a remarkable substrate range. **(a)** Reaction scheme for osmium-catalyzed dihydroxylation [9,10]. Reaction parameters for *trans*-stilbene are summarized (weight, yield and ee after recrystallization are given in parentheses). **(b)** Enantioselectivity ranges for the optimized reaction using different olefin classes.

(a)



Weight of substrate	Weight of catalyst	Weight of product	Yield	ee	Volumetric productivity
6.25 g	3.6 g	1.79 g	92 %	98 %	≈30 g l^{-1}

(b)



Weight of substrate	Weight of catalyst	Weight of product	Yield	ee	Volumetric productivity
0.500 g	0.400 g	0.370 g (0.310 g)	78 % (65 %)	89–91 % (86 %)	≈6 g l^{-1}

Fig. 3. Synthesis of macroscopic quantities of product by catalytic antibodies. **(a)** Hydrolysis of a fluorinated substrate by antibody 'A' [13] with summarized reaction parameters. **(b)** Hydrolysis of an enol ether catalyzed by antibody 14D9 [16]. Parameters for a single round of reaction are given. Weight, yield, and ee of product after purification and recrystallization are given in parentheses. The reaction was repeated five times with the same batch of antibody to give 1.50 g total product.

synthetic catalysts, the specificity of catalytic antibodies can be controlled by chemical design. The first step in producing a catalytic antibody is to devise and prepare a synthetic molecule that chemically resembles the transition state of a particular reaction [12]. Provoking an immune response against this molecule (often called a hapten) generates some antibodies that bind to it tightly. The transition state stabilization theory for enzyme action then predicts that such antibodies should accelerate the reaction [6]. They do; over the past few years, this strategy has been shown to have remarkably general application, and researchers in this field have thus been able to generate monoclonal antibodies that accelerate both natural reactions, such as hydrolytic processes [13], and unnatural reactions, such as cycloadditions [14,15]. So far, catalytic antibody technology has been used to synthesize macroscopic quantities of product in only two cases [13,16], however, and in both cases extremely large amounts of antibody relative to substrate were required (Fig. 3).

Criteria for the comparison of asymmetric catalytic methods

Each of the three strategies outlined above — enzymatic, synthetic, and antibody catalysis — has been applied to a wide assortment of asymmetric chemical reactions, and each approach has been touted highly for its practical potential. The emphasis on practicality is certainly appropriate: little argument can be made for synthetic methods that have no chance of being synthetically useful. Yet a direct comparison between enzymatic, synthetic, and catalytic antibody approaches is anything but straightforward. This may be attributed, at least in part, to the fact that some catalysts are described in the literature from a chemical perspective, while others are analyzed using the language of enzymology. That a language barrier exists is illustrated by the fact that the concept of 'turnover number', which is at the heart of any catalytic process, has entirely different definitions in enzymology and in chemistry:

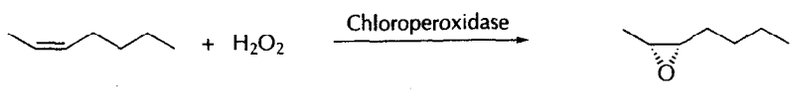
Enzymology: turnover number = k_{cat}, in $v = \frac{k_{cat}[E][S]}{[S] + K_M}$

Chemistry: turnover number = $\frac{\text{total \# moles of substrate consumed}}{\text{total \# moles of catalyst used}}$

It is common practice to discuss the reactivity of biological catalysts using Michaelis–Menten kinetic parameters. The rate accelerations are frequently described in terms of k_{cat}/k_{uncat}, where k_{uncat} is the rate constant corresponding to the background reaction in the absence of catalyst [17]. Although this parameter provides some insight into the reactivity of the enzyme–substrate complex, k_{cat} is not a direct measure of the reaction rate, and the use of this ratio thus obscures the issue of how much faster the reaction runs with catalyst than without. This is a critical consideration for an enantioselective reaction; as the background reaction gives a 50:50 mixture of enantiomeric products, it must be at least 50 times slower than the catalyzed reaction if high selectivities are to be attained.

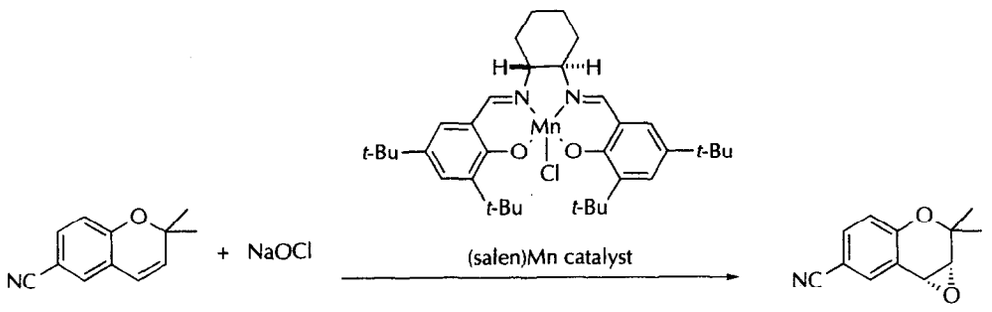
Recently, all three catalysis strategies have been applied to the asymmetric epoxidation of unfunctionalized olefins (Fig. 4; [18–21]). Although the reports on these reactions focus primarily on the catalyst reactivity and substrate scope rather than on practical, preparative aspects, it is still possible to compare the three reactions. We propose the following

(a)



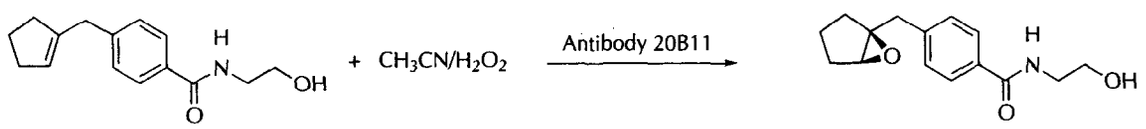
Weight of substrate	Weight of catalyst	Weight of product	Yield	ee	Volumetric productivity
49 mg	9.6 mg	47 mg	82 %	96 %	≈1 g l^{-1}

(b)



Weight of substrate	Weight of catalyst	Weight of product	Yield	ee	Volumetric productivity
25 g	3.1 g	26 g (22.1 g)	96 % (81 %)	97 % (99+%)	≈35 g l^{-1}

(c)



Weight of substrate	Weight of catalyst	Weight of product	Yield	ee	Volumetric productivity
0.015 mg	1.4 mg	0.005 mg	31.8 %	71 %	≈13 mg l^{-1}

Fig. 4. Three approaches to the asymmetric epoxidation of olefins. Catalysis by **(a)** chloroperoxidase [18], **(b)** a synthetic catalyst [19,20] and **(c)** antibody 20B11 [21], with summarized reaction parameters (recrystallized product weight, yield, and ee given in parentheses in (b); weight of product and yield in (c) estimated by assuming quantitative product yield based on consumed substrate). For antibody 20B11, the uncatalyzed reaction rate is significant compared to the catalyzed rate; approximately 30 % of the overall product mixture is calculated to be due to the background reaction. When the racemic product from the background reaction is subtracted from the total, the theoretical enantioselectivity of the antibody-catalyzed reaction is found to be > 98 %.

five criteria for the analysis of asymmetric catalytic methods that purport to be useful: enantioselectivity; the amount of product that can be generated with a given amount of catalyst; catalyst accessibility and cost; substrate scope; and how the method compares with alternative strategies.

Enantioselectivity

A widely accepted standard for a highly enantioselective synthetic reaction is one that provides product with > 90 % ee (that is, > 95:5 ratio of enantiomeric products). Although it is obviously desirable that the product consist of only one enantiomer (> 99 % ee), it is often not essential that the reaction leading to it be quite so selective. It is often possible to increase the enantiomeric purity of an enantiomerically enriched product by recrystallization, with only minor sacrifice in yield (see, for example, [9,19–21]). Synthetic sequences often include crystallization steps anyway, so such purification by recrystallization usually does not pose significant practical problems.

Enantioselectivities reported with all three epoxidation catalyst systems are high. In the case of antibody 20B11, however, the rate of uncatalyzed epoxidation (the background rate) was similar to the catalyzed rate. Thus, to calculate the enantioselectivity of the antibody-catalyzed reaction it was necessary to subtract the racemic product expected from the background reaction (approximately 30 %) from the overall product mixture of 71 % ee. This provided a calculated antibody enantioselectivity of > 98 %. As the products of catalyzed and uncatalyzed

epoxidation are not separable, however, this distinction is entirely artificial. Correcting enantioselectivity data for the rate of the background reaction may be useful in examining the catalytic mechanism, but is not helpful for a discussion of synthetic utility.

How much product can be made with a given amount of catalyst?

This most basic question is surprisingly difficult to answer from the information provided in many papers on catalytic synthetic methods. Several issues are involved, such as product yield, degrees of substrate conversion and product inhibition, total catalyst turnover number (using the chemistry definition, see above), the molecular weight of the catalyst, and the volumetric productivity of the process.

Comparison of the reactions diagrammed in Fig. 4 shows that the (salen)Mn-catalyzed epoxidation in Fig. 4b provides the most product by weight relative to catalyst, and the highest volumetric productivity of the three catalytic reactions. It is significant that the turnover numbers for the (salen)Mn- and the antibody-catalyzed epoxidations were similar (33 versus 3.5, respectively), yet the weight ratios were dramatically different (substrate/catalyst = 8 and 0.01 w/w, respectively). The chloroperoxidase enzyme gave higher turnover numbers (~ 2 000), but also required more catalyst than the (salen)Mn reaction (substrate/catalyst = 5 w/w). These data highlight a key advantage of low molecular weight synthetic catalysts.

The performance of catalytic antibodies, as exemplified by the study on antibody 20B11, is often based only on Michaelis–Menten parameters calculated at low substrate conversion (< 5 %) using high catalyst loadings. The problem with this approach is that it fails to address product inhibition and does not reveal the true chemical yield of a reaction. It is thus difficult to assess the practical synthetic potential of this catalyst.

Catalyst accessibility and cost

A complete experimental protocol describing the preparation, isolation, and/or purification of a catalyst should, in theory, provide the reader with an accurate idea of its cost and accessibility. Unfortunately, this critical information can be surprisingly difficult to deduce from many papers. Other considerations, such as whether a catalyst can be recovered from the reaction and whether it is toxic, also affect its cost.

Chloroperoxidase is produced in high concentrations and excreted in almost pure form from cells of the fungus *Caldariomyces fumago* [22]. It is commercially available, but expensive (about $10 per mg crude, $100 per mg purified, from Sigma). The (salen)Mn catalyst is available commercially on both small and bulk scale at relatively low cost (< $1 per gram in bulk), and its preparation is straightforward [23]. The accessibility of antibody 20B11 was not specifically addressed, but macroscopic quantities of antibodies raised against closely related haptens have been produced and purified [16]. In general, scale up of monoclonal antibodies is labor-intensive and very expensive [12], although practical advances in this technology are certain to occur.

Substrate scope

Next to reaction enantioselectivity, substrate scope is probably the best documented aspect of most reported asymmetric catalytic systems. Nonetheless, there is a perhaps inevitable tendency for papers to focus only on the very best substrates, and to downplay (or not even to mention) poor substrates. This naturally makes it difficult to predict how a never-before-tested substrate will perform, creating a significant psychological barrier to using the catalyst.

It is usually true in selective catalysis that synthetic catalysts have a much wider substrate range than their biological counterparts, and the epoxidation reactions described here are no exception. Almost all classes of conjugated olefins undergo highly enantioselective epoxidation in the presence of the (salen)Mn catalysts, giving high yields [19,20,24–26]. Non-conjugated, unfunctionalized olefins are very poor substrates for this and all other known synthetic asymmetric epoxidation catalysts. In contrast, chloroperoxidase can catalyze this reaction with a limited yet significant pool of non-conjugated olefins, showing high enantioselectivity; again, the synthetic and biological catalysts complement one another. Enantioselective epoxidation with antibody 20B11 was limited to a small number of trisubstituted olefins, but it seems very likely that this substrate scope could be expanded by re-designing the transition state analog and eliciting new antibodies.

Comparison with alternative strategies

No matter how good a new catalyst, it will not be used in a synthetic procedure if a better route to the same target involving more accessible substrates or different catalysts already exists. However, the concept of 'better' is surprisingly subjective; it can depend on such non-scientific variables as patent considerations, local regulatory policies, and the research background of the person performing the experiment. The existence of effective competing strategies thus does not always mean that a new method cannot be useful.

One competing strategy, at least for the example of olefin epoxidation, is to use indirect methods. Several multiple-step procedures for the synthesis of epoxides involving asymmetric catalysis have been reported recently [27–29] and methods involving olefin dihydroxylation and carbonyl reduction using synthetic catalysts seem likely to be particularly useful in providing practical access to certain terminal epoxides. Despite the requirement for multiple synthetic steps before the enantiomerically enriched epoxide is produced, these procedures offer appealing alternatives to the existing direct epoxidation methods.

Conclusions and prospects

The example of epoxidation helps to show that, by any standard, this is an incredibly exciting time for research in

selective catalysis. In under 5 years, several promising new technologies for asymmetric epoxidation have emerged, and for certain substrate classes truly practical asymmetric catalytic epoxidation has already been achieved. It is clear that new and better synthetic methods will continue to be required. Recent advances in protein engineering, chemical analysis, and combinatorial synthesis should help in the discovery of novel catalysts and the improvement of known systems.

As a field in which chemistry and biology meet and overlap, research on the development of selective catalysts for synthetic application will clearly benefit from a multidisciplinary perspective. Although it may be difficult to devise a fully universal language for the field, we propose that disclosures of new selective catalytic reactions which aspire to be synthetically useful should describe their reactions using as many of the criteria discussed above as possible, and should at the very least provide the information given in the summary figures in this review. Without such information, it will continue to be difficult to compare approaches directly, and hence to learn from one another.

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