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Enzymes as Catalysts in Synthetic Organic Chemistry

New Synthetic Methods (53)

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Enzymes have great potential as catalysts for use in synthetic organic chemistry. Applications of enzymes in synthesis have so far been limited to a relatively small number of large-scale hydrolytic processes used in industry, and to a large number of small-scale syntheses of materials used in analytical procedures and in research. Changes in the technology for production of enzymes (in part attributable to improved methods from classical microbiology, and in part to the promise of genetic engineering) and for their stabilization and manipulation now make these catalysts practical for wider use in large-scale synthetic organic chemistry. This paper reviews the status of the rapidly developing field of enzyme-catalyzed organic synthesis, and outlines both present opportunities and probable future developments in this field.

1. Introduction

Synthetic organic chemistry now faces important challenges derived from, and concerning, biology. The stunning recent advances in molecular and cellular biology—in genetics, immunology, endocrinology, neurobiology, and receptor and membrane biochemistry—have outlined broadly the course of many processes important in life. Understanding these processes in full molecular detail and using this understanding to produce chemically well-defined compounds useful in medicine, agriculture, and biology requires the preparation of classes of substances that have traditionally not been the major foci of activity in organic synthesis. Proteins, polypeptides, sugars, oligosaccharides, polysaccharides, nucleic acids, and combinations, analogues, and derivatives of these species are all centrally important in molecular biology. Their syntheses have received a relatively small fraction of the creative effort of modern synthetic organic chemistry. For chemistry to participate fully in molecular biology, it must be able to make these classes of compounds with the same high degree of skill with which it now makes hydrocarbons, heterocycles, and related classes of compounds. For molecular biology to contribute most effectively to society, its discoveries must be reduced to molecular detail and manipulated in ways that produce useful substances. Improved techniques from synthetic organic chemistry for the synthesis of biologically relevant compounds—especially those useful in modern molecular biology and biotechnology—would be of broad benefit.

The community of synthetic organic chemists has moved relatively slowly to explore many of the important synthetic problems posed by molecular biology for four reasons:

1) Many of the substances important in biology have physical properties which are unfamiliar and inconvenient. These compounds are often soluble in water, charged, nonvolatile, and high molecular weight. They may be unstable, impractical to obtain in quantity, and difficult to purify. Spectroscopy may be of limited utility in defining their structures.

2) Certain of the important generic problems encountered in the synthesis of these substances—e.g., the formation of amide, phosphodiester, and glycosidic linkages in the repetitive processes required to make macromolecules, or the manipulation of carbohydrates—have been considered in some senses to be "chemically uninteresting."

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3) Classical synthetic organic technology does *not* provide the best approach to certain of these problems—e.g., fermentation, tissue culture, and recombinant DNA technology are more useful than chemical techniques for the production of proteins, and fermentation is presently the only practical route to polysaccharides—and for many others the relative value of classical chemical and biologically derived synthetic methods remains undefined.

4) These problems have not been fashionable within the synthetic community.

Thus, for organic synthesis to function most effectively in the preparation of biologically relevant substances, modifications are required in the experimental techniques it uses, in the types of scientific problems it addresses, and in the scientific community it serves. These modifications to the scope and style of synthesis are substantial. Nonetheless, the central challenge—the synthesis of pure, well-defined molecular entities—is one at which synthetic chemists have (at least in the past) always been preeminent.

This article reviews one of the areas of synthesis which lies at the border between organic chemistry and biochemistry: the use of enzymes as catalysts in organic synthesis.[1-12] This area is the one which is the most "chemical" or "molecular" of the biologically derived synthetic technologies. Chemists comfortably use platinum as a catalyst; they will eventually use lipase with no more hesitation. Enzymology provides a class of catalysts genuinely useful in the synthesis of biologically relevant substances, and requires a less drastic change in experimental approach than does, for example, fermentation using obligate anaerobes or mammalian cell culture. Enzymology is also, in some sense, the most fundamental of the synthetic techniques derived from biology: the less familiar (to chemists) synthetic technologies based on living cells also, of course, ultimately depend on enzymes. We emphasize that enzymatic synthesis (whether biologically based or abiological) is not necessarily the most useful synthetic technique for the solution of an arbitrarily chosen biologically derived synthetic problem,[9,10] and one function of this article is to try to define the areas of synthesis in which enzymatic methods are those of choice.

2. Areas of Current Application

Enzymes are catalysts which have already demonstrated high utility and potential in certain areas of current importance in organic and pharmaceutical chemistry.

2.1. Preparation of Chiral Synthons

The first area in which enzymes have found important, broad application is in the preparation of chiral synthons, commonly by kinetic resolution. The practical importance of this area to the pharmaceutical industry is based in part on the desire for efficient syntheses and in part on regulatory constraints: regulatory pressures increasingly favor the production of enantiomerically pure substances, rather than racemates. Chiral synthesis using optically active transition-metal catalysts is also, of course, an area of current intense activity,[13-17] and the competition between biological and transition-metal-based methods is one of real strategic interest. To date, from a practical point of view, biologically based routes to chiral substances have proved more broadly useful than catalytic methods using transition metals.

2.2. Specialty and Fine Chemicals

Enzymes have established utility in the manipulation of sugars, nucleic acids, amino acids, and lipids, and in fact the major applications of enzymology in process chemistry all lie within these areas.[18] The quantities of materials produced using enzymatic catalysis establish that, in appropriate circumstances, enzymes can be practical catalysts for large-scale processes:[9] for example, high-fructose corn syrup is produced on a scale of $> 10^5$ t per year in the USA, and aspartic acid, malic acid, and guanosine 5′-phosphate (GMP), which is used as a flavoring, are all produced in substantial quantity by enzymatic methods.

A wide variety of substances used for research in biochemistry, metabolism, and pharmacology have been best produced using enzymatic methods.

2.3. "Biological" Pharmaceuticals

One of the areas of greatest activity in modern pharmaceutical research is concerned with complex, biologically derived substances—especially proteins, polypeptides, and related materials obtained by, or stimulated by, the techniques of molecular genetics.[7-9] Enzymology is now playing an increasingly important role in modification of materials derived from fermentation synthesis: for example, peptidases are used to cleave selectively (and, perhaps, to form selectively) peptide links, and other enzymes to attach oligosaccharide moieties to proteins or to modify those of glycoproteins. The recent commercialization of a process for conversion of porcine to human insulin by Novo Industrie provides the first large-scale example of this type of application.

2.4. Biotechnology[9,10]

Many of the range of applications of biology subsumed in the word "biotechnology" require chemical manipulation of complex, biologically derived or biologically related substances. Clinical analysis, biologically derived sensors, and the preparation of flavor and odor compounds, for example, involve compounds which may require enzymatic synthesis or modification.

We note explicitly that the area of enzymatic catalysis in organic synthesis is, as are most areas of practical catalysis, fundamentally a process chemistry. That is, its principal objective is the efficient and economical (both in terms of money and time) synthesis of specific compounds (Fig. 1). In this sense it differs from a substantial amount of the work in organic synthesis presently carried out in universities, in which the compound that is the specific objective of the synthesis is less important than is the value of the individual steps (or the overall synthetic strategy) in demon-

strating new and often broadly generalizable chemical principles.

Synthesis

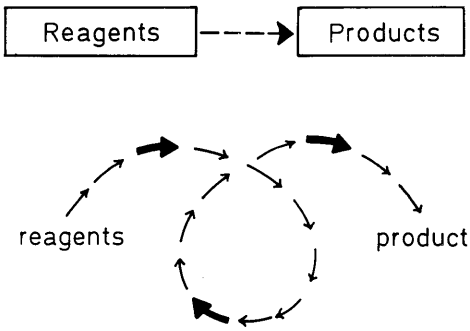


Fig. 1. Two approaches to synthesis. One (upper) is concerned with the development of practical, efficient routes to specific products. The second (lower) uses synthesis primarily to illustrate principles of reactivity and synthetic strategy. Enzyme-catalyzed synthesis (together with much of catalysis) is concerned primarily (although not exclusively) with the former objective.

3. Characteristics of Enzymes as Catalysts

Enzymes have three distinguishing characteristics as catalysts:

1) They accelerate the rates of reactions.

2) They are selective: the rate of reaction of a particular substance may be accelerated dramatically, while that of a structurally closely related substance is not.

3) They may be subject to regulation: that is, catalytic activity may be strongly influenced by the concentrations of substrates, products, or other species present in solution.

The selectivity of enzymes is the basis for much of their utility in organic synthesis. Enzymes offer the opportunity to carry out highly selective transformations—a feature of great value in working with chiral and polyfunctional molecules. The feature of regulation is critically important to the functions of enzymes in metabolic systems, but is most commonly an inconvenience in applications in organic synthesis. The most important manifestation of regulation for applications of enzymes in organic synthesis is product inhibition: that is, a decrease in the catalytic activity of an enzyme when the concentration of a product of the reaction catalyzed by that enzyme reaches a certain value.[19,20] Overcoming product inhibition may require removing the product as it is formed[20] (often an inconvenience), accepting low concentrations of product in the final reaction mixture (thereby complicating workup), or using large quantities of enzymes (with attendant increases in cost). No close analogy to regulation exists in transition-metal-based catalysis, although poisoning by product or starting material represents a loose analogy.

Enzymes have certain other characteristics which are important in considering their applications in organic synthesis:

1) Their availability, cost, and lifetime in use vary widely. A typical enzyme will contain one active site per 20000–50000 molecular weight. This high-molecular-weight equivalent per active site may be balanced by a high catalytic turnover number (moles of product produced per mole of enzyme per unit time), but is not always. The economics of enzyme use depend upon a number of factors: the cost of the enzyme, its specific activity, and its operating lifetime. Table 1 summarizes costs of research quantities of representative, commercially available enzymes useful in syn-

Table 1. Specific activities and costs of some commercially available enzymes of use in organic synthesis [a].

Enzyme [b]	E.C. Number	Specific Activity [U/mg protein]	Cost [US $ 1000 U] [c]
Horse Liver ADH	1.1.1.1	2	50
Yeast ADH	1.1.1.1	400	0.2
ADH (*T. brockii*)	1.1.1.2	15	53
Glycerol DH (*Cellulomonas sp.*)	1.1.1.6	50	56
L-Lactic DH	1.1.1.27	1400	0.5
D-Lactic DH	1.1.1.27	300	26
Malic DH	1.1.1.37	3000	1.3
6-Phosphogluconate DH	1.1.1.44	50	280
Glucose DH (*B. cereus*)	1.1.1.47	250	160
Glucose-6-phosphate DH	1.1.1.49	400	15
Glucose Oxidase	1.1.3.4	200	0.4
Galactose Oxidase	1.1.3.9	300	20
Formate DH	1.2.1.2	3	667
Aldehyde DH	1.2.1.5	25	140
Glutamic DH	1.4.1.3	40	2.5
Leucine DH	1.4.1.9	80	1750
L-Amino Acid Oxidase	1.4.3.2	1	500
D-Amino Acid Oxidase	1.4.3.3	1	50
Catalase	1.11.1.6	20000	0.00005
Horseradish Peroxidase	1.11.1.7	330	1.8
Tyrosinase	1.14.18.1	4000	0.1
Galactosyl Transferase	2.4.1.22	5	5000
Hexokinase	2.7.1.1	300	2.7
Phosphoribulokinase	2.7.1.19	4	50
NAD Kinase	2.7.1.23	20	85
Glycerokinase	2.7.1.30	150	33
Pyruvate Kinase	2.7.1.40	500	0.6
Acetate Kinase	2.7.2.1	600	50
Polynucleotide Phosphorylase	2.7.7.8	35	428
Pig Liver Esterase	3.1.1.1	100	10
Pig Pancreatic Lipase	3.1.1.3	50000	0.00002
Phospholipase A_2	3.1.1.4	1500	5.3
Cholesterol Esterase (Bovine Pancreas)	3.1.1.13	3	200
Pancreatin [d]			
α-Amylase (*B. lichiniformis*)	3.2.1.1	500	0.01
Amylo-1,6-glucosidase	3.2.1.3	70	0.004
NADase	3.2.2.5	3	667
Chymotrypsin	3.4.21.1	50	0.1
Trypsin	3.4.21.4	100000	0.00009
Papain	3.4.22.2	40	1.5
Carboxypeptidase A	3.4.17.1	50	8
Protease [e]			
Acylase (*Aspergillus sp.*)	3.5.1.14	150	0.006
Penicillinase	3.5.2.6	2000	4
Aldolase (Rabbit Muscle)	4.1.2.13	20	5
Sialic Acid Aldolase	4.1.3.3	1	5000
Tryptophanase	4.1.99.1	1	200
δ-Aminolevulinate Dehydrase	4.2.1.24	2	7500
Phenylalanine Ammonia Lyase	4.3.1.5	3	6670
UDP-Galactose 4-Epimerase	5.1.3.2	15	1330
Glucose Isomerase	5.3.1.5	30	0.005
Phosphoglucoisomerase	5.3.1.9	800	3

[a] Prices vary widely with source and quantity. [b] Abbreviations: ADH, Alcohol Dehydrogenase; DH, Dehydrogenase. [c] 1000 U of enzymatic activity will generate approximately 1 mol of product per day under favorable conditions. [d] Pancreatin is a crude, inexpensive (US$ 20/kg) mixture of enzymes which often provides the least expensive source of activities for lipases, cholesterol esterase, and certain proteases and glycosidases. [e] A variety of relatively crude proprietary proteases (Pronase, Thermolysin, Subtilisin Carlsberg, Subtilisin BPN, Newlase, Papaya, and others) are available at low cost (US$ 0.1–1/1000 U).

thesis, together with the specific activities of these enzymes. For reference, one International Unit (IU) of catalytic activity is defined as that amount which generates 1 μmol of product per mg of enzyme per min; 700 U of enzymatic activity produces ca. 1 mol of product per day. The last column of Table 1 thus indicates roughly the cost of a quantity of enzyme sufficient to generate a mole of product per day when operating under ideal—that is, V_{max}—conditions. (In fact, few synthetic reactions are run under ideal conditions, and the quantities of enzyme required to achieve this level of synthetic productivity may be greater than the theoretical by factors of between 2 and 10^2.)

2) Enzymes may require cofactors for activity. The economics of consumption or regeneration of the cofactor may be as important as that of the enzyme itself.

3) Most enzymes are water-soluble, and are most active in aqueous solution at pH 7–8 and room temperature. The importance of water solubility in limiting the applicability of enzymes in organic synthesis has probably been overestimated by organic chemists. While most enzymes are not active with truly water-insoluble substrates, it is often possible to carry out enzyme-catalyzed reactions in heterogeneous reaction mixtures in which only a small amount of the organic substrate is present in the aqueous solution containing the enzyme.[21–24] In certain cases enzymes will tolerate modest concentrations of organic cosolvents such as ethylene glycol, glycerol, dimethyl sulfoxide (DMSO), and dimethylformamide (DMF).[25–27]

4) Several enzymes can often be used in combination to carry out processes involving sequential or parallel enzyme-catalyzed transformations. This characteristic of enzymes stems from the common environment in which they function in vivo. The development of corresponding multicatalyst systems for non-enzymatic catalysts is less common.

5) Enzymes may be degraded under relatively mild conditions. Chemists are accustomed to storing metallic catalysts for long periods of time without loss in activity. Enzymes may denature on storage by a number of mechanisms:[28–33] for example, destruction by small amounts of proteases present as contaminants in the enzymatic preparation, temperature- or medium-induced changes in conformation, microbial contamination, autoxidation. All of these characteristics render the handling of enzymes, both on storage and in use, a relatively more delicate process than the handling of many other types of catalysts.

4. Enzymatic Kinetics and Mechanisms: Relevance to Synthesis

The subject of the mechanism of action of enzymes is, of course, a major field in its own right. Knowledge of characteristic kinetic constants for enzymatic reactions (especially Michaelis-Menten and inhibition constants and pH dependencies)[19,20] is very useful although not required for synthetic application. In fact, one of the major advantages of enzymatic catalysis (relative to, for example, homogeneous and heterogeneous catalysis by metals) is that a relatively small investment of time in obtaining kinetic constants for an enzyme-catalyzed reaction yields a very large return in terms of ability to control rationally the activity of that enzyme in synthetic applications. Figure 2 and Equations (a) and (b) outline essential terms. For the simplest case in which a substrate S is converted into a product P by an enzyme E in a process following Michaelis-Menten kinetics [Eq. (a)], without inhibition by products or other species, the rate of reaction is given by the familiar Equation (b).

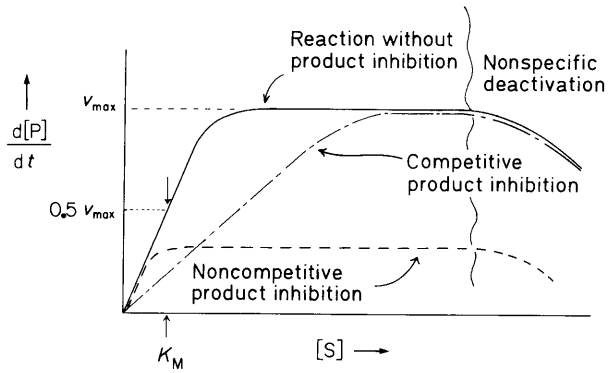


Fig. 2. Schematic representation of the relation between the reaction rate d[P]/dt and the substrate concentration [S] for a simple reaction following Michaelis-Menten kinetics (——) in the absence of inhibition by product. Curves for competitive (— — —) and noncompetitive (– – –) inhibition by product illustrate the influence of an arbitrary fixed concentration of product on the relation between d[P]/dt and [S], relative to that observed in the absence of inhibition. The slope of the curve at low [S] for an enzyme subject to competitive inhibition, and the height of the plateau for that subject to noncompetitive inhibition are determined by the concentration of the product and the inhibition constants. At high concentrations of substrate, enzymatic reactivities commonly decrease due to nonspecific deactivation.

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} S \cdot E \xrightarrow{k_2} P + E \qquad (a)$$

$$\frac{d[P]}{dt} = \frac{k_2[E]}{1 + \frac{K_m}{[S]}} = \frac{V_{max}}{1 + \frac{K_m}{[S]}} \qquad (b)$$

From the vantage of synthetic application, for a given quantity of enzyme, the rate of reaction increases as [S] is increased until all of the available enzyme active sites are occupied. Further increase in [S] does *not* increase the rate of reaction. In fact, if the substrate concentration is increased sufficiently, most enzymes are eventually deactivated (as the character of the solvent becomes markedly different from that of water). Maximum efficiency of use of the enzyme (usually the most expensive component of the reaction mixture) is achieved in the region of substrate concentrations in which the concentration of enzyme limits the rate (V_{max} conditions). If substrate concentrations are too low, a certain fraction of enzyme active sites are vacant at equilibrium; if substrate concentrations are too high, the enzyme is deactivated. The Michaelis constant K_m is thus invaluable in judging the *minimum* concentration of substrate which must be maintained in solution to achieve efficient use of enzyme: V_{max} is useful in indicating the most rapid rate of production of product which can be achieved for a given quantity of enzyme under ideal circumstances. Both K_m and V_{max} are measured under assay conditions.

In practice, values of K_m are often roughly the same under assay conditions and conditions used in synthesis, although higher apparent values of K_m may be observed in synthesis using immobilized enzymes if mass transport limitations are important; V_{max} under assay conditions is often much larger than the maximum rate of operation observed for the enzyme under synthetic conditions, because inhibition of enzymatic activity is common.

The discrepancy between the catalytic activity of an enzyme obtainable under ideal (assay or V_{max}) conditions and the lower value often observed under synthetic conditions is a major source of frustration to inexperienced users of enzymatic catalysis for synthetic applications. This discrepancy may have several origins: nonspecific deactivation of the enzyme by high or inappropriate concentrations of substrates, products, buffers, or ions; loss of activity on immobilization due to chemical modification or mass transport limitations; or reversible kinetic inhibition by product. The importance of product inhibition is only now beginning to be appreciated. Specific examples are given later, and a complete kinetic discussion is unnecessarily complex for synthetic applications.[20] It is, however, important to recognize that there are several important classes of inhibition (Fig. 2). The first—competitive inhibition—reflects competitive binding of substrate and product at the enzyme active site. This type of inhibition influences only K_m; its effects can, in principle, be overcome by increasing the concentration of substrate. The second—noncompetitive inhibition—leaves K_m unchanged and decreases V_{max}. This type of inhibition *cannot* be overcome by increasing substrate concentration: the only generally effective strategy is to decrease the concentration of product (e.g., by removing it continuously during reaction or by limiting the total quantity of substrate processed).[21] Yet other types of inhibition—mixed inhibition and uncompetitive inhibition—influence V_{max} and K_m simultaneously.

The important data to extract from kinetic analysis of an enzyme in order to optimize its use in synthesis are two: first, the values of K_m and V_{max} are critical in choosing values of the concentrations of substrate and enzyme; second, the product inhibition must be recognized, if present, and approximate values of K_i determined in order to choose the correct strategy—increasing substrate concentration and/or decreasing product concentration—to limit its unfavorable influence on rates of reaction.

5. Solved Problems; Current Activities; Future Problems and Opportunities

Because application of enzymes as catalysts in organic synthesis is a relatively new field, a substantial number of technical problems require solution before these applications become routine. Table 2 summarizes the status of several generic problems in this area. Those problems classified as "solved" do not necessarily have *optimal* solutions at present. The solutions now available are, however, sufficient that these problems no longer limit progress in the field. For example, many methods are now available for immobilization of enzymes.[34–39] The best strategy for immobilization of any particular enzyme is not necessarily clear, without substantial trial and error. Particularly for process applications involving long-term stability and good performance in fixed-bed reactors, improved immobilization technology would be very useful. Nonetheless, for laboratory and pilot-plant scale, existing technology is probably such that more difficult problems will be found elsewhere.

Several of the topics listed in Table 2 are discussed more fully below, but a few of the items listed in the table deserve brief comment here:

— The general thrust of *current* research in this area of applied enzymology is to establish the real practicality of

Table 2. Status of certain generic problems in enzymatic catalysis.

Solved	Current Research	Immediate Future	More Distant Fuuture
Immobilization	Development of new laboratory-scale uses of enzymes, especially simple enzymes (eaterases, amidases, isomerases)	Uses of recombinant DNA for large-scale production of enzymes	Development of new types of enzymatic activities using genetic engineering
Cofactor Regeneration AMP or ADP→ATP NAD(P)H ⇌ NAD(P)	Use with unnatural substrates; definition of the generality of synthetic applicability of readily available enzymes	Site-specific mutagenesis for changes in properties (K_m, K_i, selectivity, stability)	
Deactivation Thiol Autoxidation Protease Action	Use with organic cosolvents; one- and two-phase systems	Exploration of enzymes from thermophilic and halophilic organisms for synthetic utility	
	Overcoming product inhibition	Evaluation of complex multienzyme systems in synthesis; comparison with whole-cell systems; "artificial metabolism"	
	Cofactor regeneration Acetyl CoA; PAPS [a]	Applications in synthesis of fine and specialty chemicals	
	Scale-up in pharmaceutical synthesis	Chemical modification of enzymatic activities: "semisynthetic enzymes"	

[a] PAPS: 3′-phosphoadenosine-5′-phosphosulfate.

enzymes as catalysts in organic synthesis. How broadly applicable are they? Can they be used with substrates very different from their natural substrates? To what extent can their activity be changed by adding organic cosolvents? How should they be used: immobilized, in membrane reactors, or in free solution? In general, in what circumstances do they provide methods superior to existing or easily developed classical techniques for the solution of problems in organic synthesis?

— The *immediate future* will see the application of the techniques of site-specific mutagenesis and recombinant DNA technology to the large-scale production of uncommon enzymes (if warranted by the development of important processes requiring large quantities of these enzymes), the discovery of new enzymatic activities from new sources, and modest modification of enzymatic properties (for example, changes in Michaelis-Menten constants, substrate selectivities, or thermal stabilities). The first useful examples of site-specific mutagenesis are now appearing (for example, generation of T4 lysozyme containing an engineered disulfide bond to improve stability[40a] and of tyrosyl-tRNA synthetase altered to lower K_m values[40b]). The immediate future will also see the exploration of complex multienzyme systems for synthesis. In principle, the complexity of these systems may approach that of major metabolic paths (and, since these systems may not exist in nature, the phrase "artificial metabolism" seems appropriate for them). A major question in this area concerns the degree of complexity required before cell-free enzymology becomes inferior to whole-cell synthesis. The chemical modification of existing enzymes and proteins to change activity, stability, or selectivity—that is, the generation of "semisynthetic enzymes"—also holds promise.[41]

— The *distant future* holds out the potential of using highly developed structure-reactivity reactions for protein chemistry to "design" new enzymes to catalyze new reactions, and of using molecular genetics to produce these new enzymes. The prospect of being able to design and produce new catalysts is an alluring one. Its realization is, however, very far away, and even the basic principles on which the design of a new protein catalyst might be built are still being explored at the level of basic research.

With these generalities in hand concerning the use of enzymes as catalysts in organic synthesis, we turn to a discussion of specific classes of enzymes, and illustrations of their use in synthesis. In this discussion, we will consider specific enzymes, rather than generalities concerning classes of enzymes. The discussion will proceed in terms of the present ease of use of these enzymes, although not necessarily in order of the ultimate usefulness of the enzymes.

6. Specific Applications

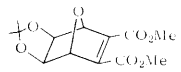
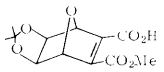
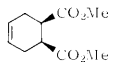
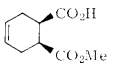
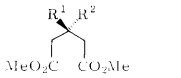
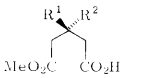
6.1. Enzymes Not Requiring Cofactors

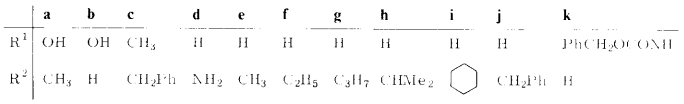
Certain of these enzymes are among the most readily available, least expensive, and most stable. They have seen the most widespread use by synthetic organic chemists, and probably comprise the group which will first become a standard part of the synthetic organic repertoire (especially for the preparation of chiral synthons).

6.1.1. Esterases: Pig Liver Esterase and Lipases

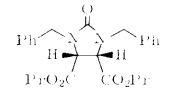
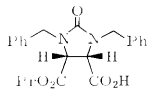
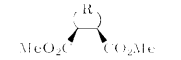
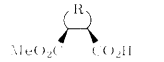
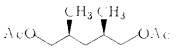
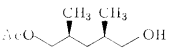
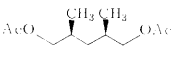
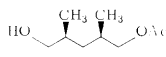
A large number of examples exist illustrating the use of these classes of enzymes for kinetic resolutions.[42–68] The hydrolysis of *meso*-diesters has proved a particularly attractive strategy for obtaining chiral synthons, and both examples and theoretical analysis of this strategy are well-developed.[42–53] Table 3 gives representative examples.

Table 3. Enantioselective hydrolysis of *meso* substrates catalyzed by pig liver esterase and hog pancreatic lipase (last example).

Substrate	Product	*ee* [%]	Ref.
		77	[43]
		96	[51, 52]
		100	[42, 47, 49]



	a	b	c	d	e	f	g	h	i	j	k
R^1	OH	OH	CH_3	H	H	H	H	H	H	H	$PhCH_2OCONH$
R^2	CH_3	H	CH_2Ph	NH_2	CH_3	C_2H_5	C_3H_7	$CHMe_2$	⬡	CH_2Ph	H

		87 [46]
		90 [50]
		95 [53]
		90 [53]

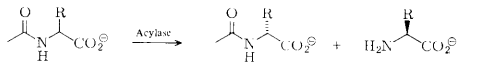
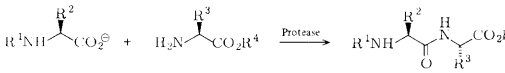
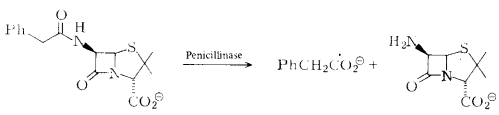
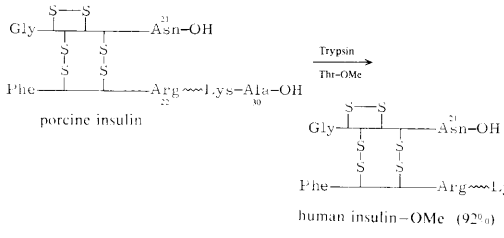
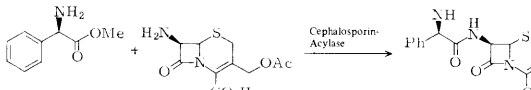
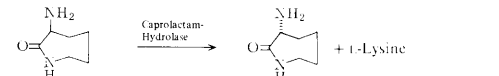
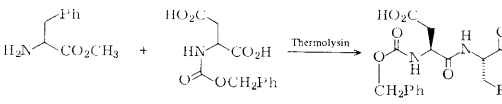
Several recent studies with these enzymes deserve further comment, since they point to certain generally useful features and problems. The lipases are rapidly developing into an extremely useful class of enzymes, especially for chiral synthesis. They have the characteristics of low cost, high stability, and high tolerance for variation in substrate structure.[53–57] They have the additional attractive feature that they do not require water-soluble substrates: They operate best at water-organic interfaces, and recent work suggests that they retain a high degree of activity in strongly dehydrating and organic systems.[60, 69, 70] When operating with *meso* substrates, they can generate chiral products in high yield. The utility of lipases as catalysts for the preparation of chiral substances has been demonstrated in a number of types of systems. In addition to kinetic resolution by hydrolysis, transesterification is a useful procedure.[60, 61]

A recent study has compared the utility of lipase-catalyzed hydrolysis as a route to enantiomerically enriched epoxy alcohols[21] with transition-metal-catalyzed epoxidation.[17] The enzymatic hydrolysis suffers from the disadvantage that it is a kinetic resolution: that is, it relies on the selective hydrolysis of one enantiomer in the presence of the second to achieve separation, and thus, in principle, yields a maximum of 50% of product based on racemic starting material. Even given this disadvantage, however, it is in certain instances a more practical route to large quantities of an optically active epoxy alcohol than the transition-metal-catalyzed system. The enzymatic and transition-metal catalysts now available show very different substrate selectivities, and are thus, in a broad sense, complementary rather than competitive.

6.1.2. *Amidases*

A second broadly applicable class of hydrolytic enzymes is the amidases. The most important applications for these enzymes to date have been in kinetic resolutions of amino acids[35, 72–80] (typically by hydrolysis of *N*-acylamino acids)[35, 72] and in formation of amide bonds[12, 81–83] in polypeptides and proteins (Table 4).[11, 84–101]

Table 4. Illustrations of uses of amidases.

Reaction	Ref.
	[35, 72]
	[83, 101]
	[102–104]
	[94]
	[102]
	[105]
	[23]

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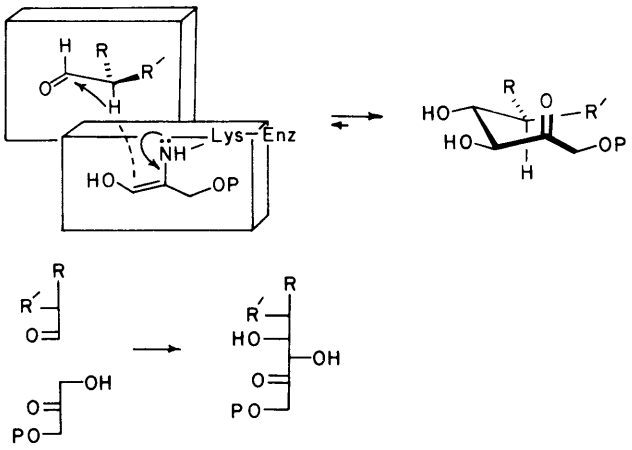
A substantial body of work has been devoted to the development of methods for formation of amide bonds between unprotected amino acids and amino acid esters using amidases. The several successful demonstrations of this type of enzyme-catalyzed dehydration establish that this type of process will be important in specific circumstances.[83] It is also clear, however, that the development of the specific reaction conditions required to effect a given dehydration demand patience and skill, and will be worthwhile only in instances in which substantial quantities of the product are desired. The most spectacular example of this technology to date is certainly the semisynthesis of human insulin from porcine insulin by Novo.[94] Future applications will rest in routine kinetic resolutions of amines, in the preparation of di- or oligopeptides,[23] in reactions incorporating organic media or reversed micelles,[101] and in the post-translational modification of proteins[106, 107] obtained either by isolation from natural sources or by recombinant DNA methodology.

6.1.3. *Aldolases*

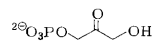
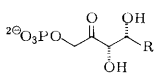
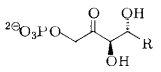
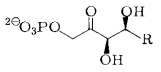
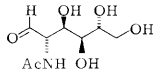
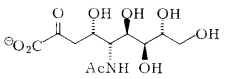
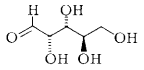
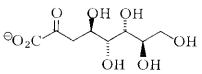
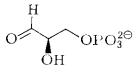
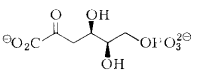
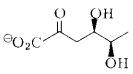
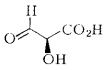
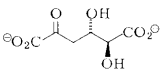
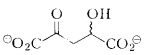
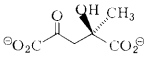
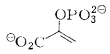
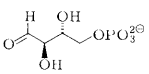
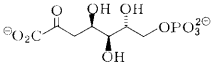
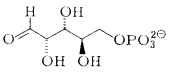
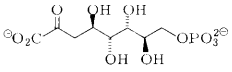
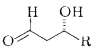
Rabbit muscle aldolase is a readily available enzyme that catalyzes the condensation of dihydroxyacetone phosphate (and a few close analogues) with aldehydes (Scheme 1).[108] This enzyme accepts a number of aldehydes as substrates, and has proved useful in the preparation of a number of rare, unnatural, and isotopically labeled sugars.[109, 110] The preparation of 6-deoxyfructose-1-phosphate (a precursor to the flavoring agent Furaneol) provides one example.[109]



Scheme 1. Stereochemistry of carbon-carbon bond formation catalyzed by rabbit muscle aldolase. R is widely variable. R′ must be H or OH. Dihydroxyacetone phosphate is only slightly variable. $P = PO_3^{2\ominus}$.

Although rabbit muscle aldolase is the only enzyme in this class which has been explored for synthetic application, a large number of relatively readily available enzymes display the ability to catalyze aldol and reverse aldol reactions (Table 5).[111–121] Given the current interest in synthetic organic chemistry in directed aldol reactions,[122–126] and in the synthesis of carbohydrates,[127–130] we expect that the aldolases will be one class of enzymes which will experience substantial development as synthetic catalysts over the next years.

Table 5. Enzymes that catalyze aldol reactions.

Donor	Acceptor	Enzyme [a]	Product	Ref.
		FDP-Aldolase		[108–111]
		Fuc-1-P-Aldolase		[112]
		Rha-1-P-Aldolase		[113]
		Sialinsäure-Aldolase		[114]
		KDO-Aldolase		[115]
		KDPG-Aldolase		[116]
		KDF-Aldolase		[116]
		KDG-Aldolase		[116]
		KHG-Aldolase		[117]
		MHKG-Aldolase		[116]
		KDHA-Aldolase		[118]
		KDO-8-P-Synthase		[119]
		Desoxyribose-Aldolase		[120, 121]

[a] Abbreviations: FDP, D-fructose-1,6-diphosphate; Fuc-1-P, L-fuculose-1-phosphate; Rha-1-P, L-rhamnulose-1-phosphate; KDO, 3-deoxy-D-mannooctulosonate; KDPG, 2-keto-3-deoxy-6-phosphogluconate; KDF, 2-keto-3-deoxy-D-fuconate; KDG, 2-keto-3-deoxy-D-glucarate; KHG, 2-keto-4-hydroxyglutarate; MHKG, 4-methyl-4-hydroxy-2-ketoglutarate; KDHA, 2-keto-3-deoxyheptonate.

6.1.4. Isomerases and Lyases

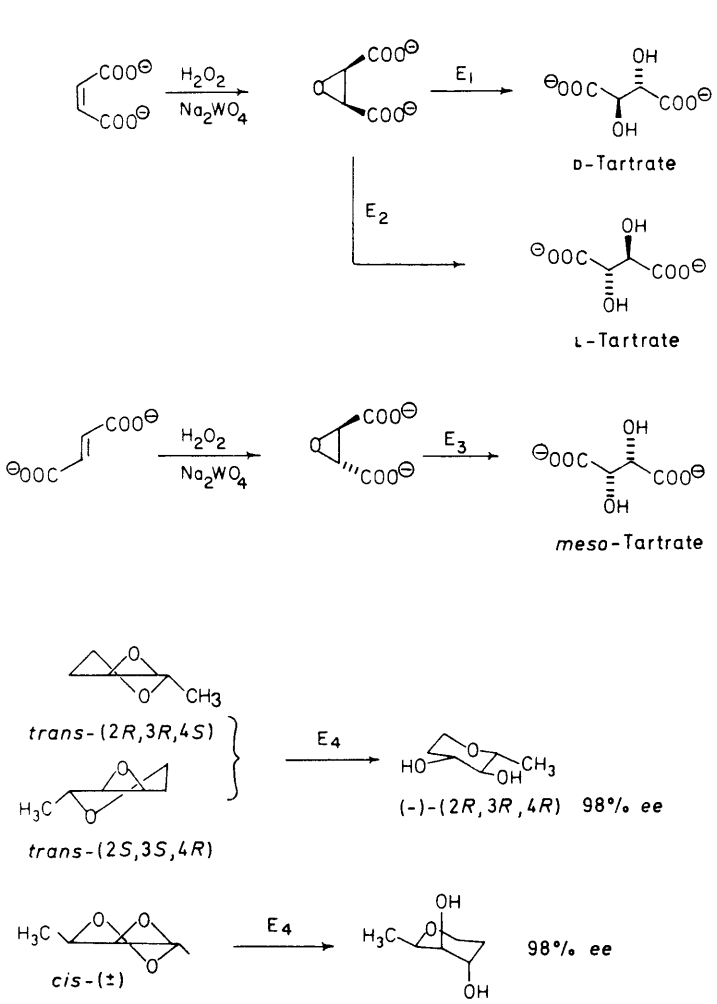
Several important commercial applications of enzymes rest on these types of enzymes. For example, glycosidases are used in large quantity in conversion of cornstarch to glucose,[131–134] and glucose isomerase catalyzes the equilibration of glucose and fructose.[135–141] Aspartic acid is prepared by addition of ammonia to fumaric acid in a reaction catalyzed by aspartase,[142] and malic acid by hydration of fumaric acid catalyzed by fumarase.[143] These transformations, by themselves, are not of major interest to synthetic chemists. The enzymes responsible for them are, however, readily available, and the range of substrates that they will accept has not been explored in detail.

6.1.5. Other Enzymes

Cyanohydrolases from several sources catalyze the enantioselective condensation of HCN with aldehydes.[144] *S*-Adenosylhomocysteine hydrolase catalyzes the synthesis of *S*-adenosylhomocysteine (or analogues) from homocysteine and adenosine (or analogues).[145–149] NADase catalyzes exchange of the nicotinamide residue of NAD with nicotinamide analogues. This enzymatic process provides a useful route to analogues of NAD.[150–152] Different phospholipases are available for selective hydrolysis of phospholipids. Phospholipases A_1 and A_2, for example, catalyze the hydrolysis of the ester groups at *sn*-1 and *sn*-2 positions of the glycerol moiety, respectively.[153–155] Phos-

pholipase D catalyzes transphosphatidylation using phosphatidylcholine and other amino alcohols as substrates.[153-155] These three enzymes have been used to prepare different types of phospholipids.[156] Glycosidases (for example, β-galactosidases) are useful in preparing glycosides and oligosaccharides.[157, 158] 1-Deoxy-1-fluorosucrose—a compound which is stable to hydrolysis by invertase and which is recognized by sucrose carrier protein—has been prepared from UDP-glucose and 1-deoxy-1-fluoro-D-fructose in a reaction catalyzed by sucrose synthetase.[159]

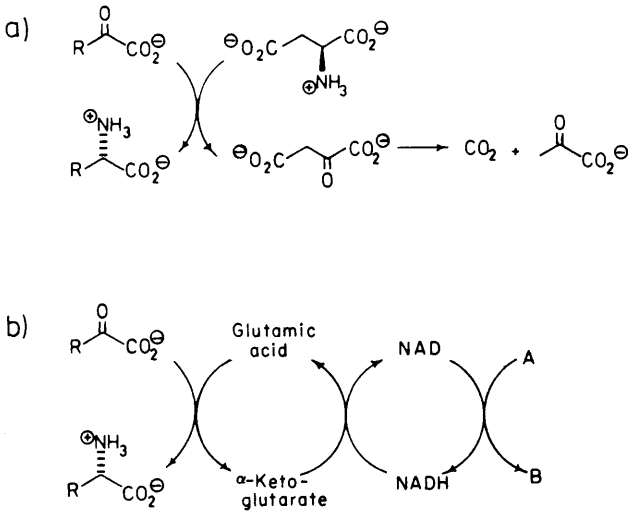
Enzymes catalyzing addition of ammonia or hydrolytic removal of amino groups also have potential application. L-Phenylalanine has been synthesized by addition of isotopically labeled ammonia to cinnamic acid catalyzed by phenylalanine ammonia lyase.[4] Large-scale production of L-citruline from L-arginine provides a representative example of hydrolytic deamination.[35] Regiospecific epoxide opening catalyzed by epoxide hydrases may have potential in the preparation of polyhydroxy compounds.[160] Scheme 2 illustrates the use of this class of enzymes in the synthesis of D-,[161] L-,[162] and *meso*-tartaric acid,[163] and hydroxypyran derivatives.[164, 165] Another potentially useful group of hydrolytic enzymes is the nitrile hydratases.[166] One such enzyme catalyzes the hydrolysis of acrylonitrile to acrylamide.[167]



Scheme 2. Reactions involving epoxide hydrolases. Abbreviations: E_1, D-tartrate epoxidase; E_2, L-tartrate epoxidase; E_3, *meso*-tartrate epoxidase; E_4, epoxide hydrolase from rabbit liver microsomes.

6.2. Enzymes Not Requiring Added Cofactors, Especially Enzymes Containing Flavins, Pyridoxal Phosphate, Porphyrins, and Coordinated Metals

In these cases, cofactors are either covalently attached to or tightly non-covalently bound to the enzymes. They are regenerated automatically during the catalytic cycle. The pyridoxal phosphate-dependent transaminases catalyze the synthesis of amino acids via transfer of the amino group from an amino acid to an α-keto acid. In most cases L-glutamate is the amine donor and is converted into α-ketoglutarate. A useful strategy for the synthesis of α-amino acids is based on transaminase-catalyzed amine transfer between an α-keto acid and L-glutamate and coupled in situ regeneration of the glutamate by reductive amination of α-ketoglutarate in a reaction catalyzed by glutamic dehydrogenase. Another group of transamination reactions using L-aspartic acid as an amine donor has the possible advantage for synthetic application that the keto acid produced (oxaloacetic acid) decarboxylates spontaneously in situ to pyruvate; this decarboxylation drives the transamination reactions[168] (Scheme 3). These methods are particularly useful for the preparation of compounds containing isotopes of short half-life, such as ^{11}C (20 min) or ^{13}N (10 min), in high purity.[169, 170]



Scheme 3. Procedures for enzyme-catalyzed transamination involving an α-keto acid and a) aspartic acid or b) glutamic acid as the amine donor. Compound A is a hydride donor (for examples, see Table 7) used for in situ regeneration of NADH from NAD.

The pyridoxal phosphate-dependent enzymes tyrosinase and tryptophanase catalyze the synthesis of L-tyrosine and L-tryptophan, respectively, by condensation of three small molecules: phenol (indole for tryptophanase), pyruvate, and ammonia. By replacing the aromatic components with other phenolic analogues, new amino acids can be prepared. L-DOPA, for example, can be prepared by replacing phenol with catechol. A similar approach has been used to prepare tryptophan analogues.[171, 172] An efficient synthesis of porphobilinogen from δ-aminolevulinate using catalysis by the pyridoxal phosphate-containing enzyme δ-aminolevulinate dehydratase has been demonstrated.[173, 174] The flavoenzyme glucose oxidase is widely

used in the food industry as an antioxidant.[175] Under anaerobic conditions, glucose oxidase also catalyzes the transfer of electrons from glucose to acceptors such as benzoquinone.[176] In addition to determination of the optical purity of amino acids,[177] L- and D-amino acid oxidases have been used to produce α-keto acids.[35]

The $Cu^{2\oplus}$-containing enzyme galactose oxidase catalyzes the oxidation of D-galactose to D-galactaldehyde; this enzyme also catalyzes the stereospecific oxidation of glycerol and 3-halo-1,2-propanediols to L-glyceraldehyde and L-3-haloglyceraldehydes.[178] Investigations of the substrate specificity of this enzyme using a number of polyols has permitted the development of a model of the enzyme active site which rationalizes the stereospecific transformation of polyhydroxy compounds to aldehydes.[179] Several unusual L-sugars have been prepared using this method.[179] Another potentially useful $Cu^{2\oplus}$-containing enzyme, dopamine β-monooxygenase, catalyzes the stereospecific hydroxylation of the *pro-R* benzylic hydrogen of dopamine to give norepinephrine. It also catalyzes the stereoselective oxidation of heteroatoms such as S and Se, and of certain olefins.[180]

The $Fe^{2\oplus}$-containing peroxidases are able to catalyze the addition of the elements of HOX to olefin substrates starting with H_2O_2 and $X^{\ominus}$;[181-184] they also catalyze oxidation of primary alcohols and heteroatoms.[185-187] Mixtures of organic dihalides are generated by reaction between olefins, H_2O_2, and a mixture of inorganic halides; the products include compounds containing C–F bonds.[188] Horseradish peroxidase also catalyzes the selective hydroxylation of aromatic compounds at 0°C in the presence of dihydroxyfumarate, and several pharmaceuticals including L-DOPA have been prepared using this enzyme on a millimolar scale.[189] Peroxidase appears to generate highly reactive and unselective species such as singlet oxygen and hydroxy radicals during its catalytic reactions.[190, 191] Whether these enzyme-based hydroxylation processes are competitive with those using fermentation[192] or organometallic catalysis[14] remains to be demonstrated. In recent, interesting work horseradish peroxidase has been modified with 2-chloro-4,6-bis[ω-methoxypoly(oxyethylene)]-*s*-triazine.[193] The modified enzyme is soluble and still active in benzene. This technique allows manipulation of apparently homogeneous solutions of the enzyme in an organic solvent. The same immobilization procedure has been used to prepare hydrophobic lipase for use in synthesis in organic media.[62]

Lipoxygenase has been used to prepare a hydroperoxy derivative for use in the synthesis of prostaglandins.[194, 195] The $Fe^{2\oplus}$-containing enzyme isopenicillin cyclase has been isolated and used to prepare several analogues of isopenicillin N.[196-199] Such enzymatic reactions may be useful in generating new β-lactam antibiotics.

Another class of iron and copper monooxidases that incorporate molecular oxygen into organic substances also has been explored with interesting results, but so far without real practical utility. These enzymes convert saturated hydrocarbons to alcohols, olefins to epoxides, and heteroatoms such as N and S to oxides. They also cleave aromatic rings and steroid derivatives and dealkylate *N*- or *O*-alkyl groups.[180] Of the presently known oxygen-incorporating enzymes, bacterial methane monooxygenase[200-202] (catalyzing the hydroxylation of saturated hydrocarbons), ω-hydroxylase (catalyzing the selective epoxidation of olefins),[203-206] hepatic hydroxylase (catalyzing the oxidation of heteroatoms for preparation of drug metabolites),[207, 208] and steroid hydroxylases[209, 210] are in principle attractive for synthetic development. Practical use of these enzymes is limited by severe technical problems: the instability and low specific activity of the enzymes; the requirement for multiple interacting enzymes to couple NAD(P)H oxidation to reduction of O_2; the inefficient coupling of NAD(P)H oxidation to oxygen activation (with production of hydrogen peroxide and superoxide as the undesirable competing side reactions); the requirement for NAD(P)H regeneration. Certain of these problems will not be easily solved, and although these monooxygenases are potentially attractive catalysts, procedures based on fermentation and other whole-cell processes[211] are presently more practical. The problems associated with these systems are discussed in more detail below. Other oxidative enzymes—particularly cyclohexanone monooxygenase[212] (which catalyzes enzymatic Baeyer-Villager oxidation) and lipoxygenase[213]—have seen some applications in synthesis, and deserve development.

6.3. Enzymes Requiring Added Cofactors

6.3.1. Enzymes Requiring Nucleoside Triphosphates, Especially ATP

A substantial fraction of the enzymes involved in complex biosynthesis (as opposed to biodegradation) require as cofactor a nucleoside triphosphate (especially ATP) or a nicotinamide cofactor (NAD(P)H). These enzymes have not been widely used in practical-scale organic synthesis, in large part because of the cost of the cofactors when used as stoichiometric reagents. ATP costs approximately US $ 800/mol. It is, however, a relatively stable substance: in solution, the most important degradation reaction is usually the hydrolysis of the ribose triphosphate group to a di- or monophosphate (probably by traces of ATPases present in solution).[214] Thus, an efficient strategy for regeneration of ATP from ADP and AMP renders enzymatic systems requiring these cofactors practical for use. The problem of ATP regeneration is now effectively solved.[215-221] The general strategy is to use a readily obtained phosphate donor, and to regenerate ATP from ADP or AMP in situ enzymatically.[222-224] Table 6 summarizes the most

Table 6. Procedures for ATP regeneration [a].

Procedure	$\Delta G^{\circ\prime}_{hyd}$ [kcal/mol]	Ref.
$CH_3COPO_3^{2\ominus}$ + ADP $\xrightarrow{AcK}$ ATP + $CH_3CO^{\ominus}$	−10.1	[218]
$=\!\!<^{OPO_3^{2\ominus}}_{CO_2^{\ominus}}$ + ADP $\xrightarrow{PK}$ ATP + $CH_3CCO_2^{\ominus}$	−12.8	[220]
$CH_3OCOPO_3^{2\ominus}$ + ADP $\xrightarrow{AcK}$ ATP + CH_3OH + CO_2	−12.4	[221]

[a] Abbreviations: AcK, acetate kinase; PK, pyruvate kinase.

useful procedures now available for ATP regeneration. Procedures based on different phosphate donors (e.g., carbamoyl phosphate), fermentation, and other techniques are not presently practical.[225]

The most generally useful of the procedures using high-energy phosphate donors is based on acetyl phosphate. This material is readily obtained,[218,221] and two acetate kinases are commercially available. Although most of the published work has involved *E. coli* acetate kinase, the *Bacillus stearothermophilus* enzyme may be superior: the *E. coli* enzyme contains an SH group close to the active site, and is sensitive to autoxidation; the *B. stearothermophilus* enzyme is thiol-free.[219] Acetyl phosphate has two disadvantages: it is only a moderately strong phosphorylating agent (as judged by its free energy of hydrolysis, $\Delta G^{\circ\prime}$ at pH 7, see Table 6) and it is relatively hydrolytically unstable in aqueous solution. The hydrolytic instability both destroys acetyl phosphate and produces phosphate ion in solution; this phosphate complexes with and sometimes precipitates the $Mg^{2\oplus}$ required for kinase activity, and may render slow reactions difficult to control.

Regeneration systems based on phosphoenol pyruvate (PEP) are more stable: PEP is resistant to hydrolysis, and is also a very strong phosphorylating agent. Its synthesis is, however, more complex than that for acetyl phosphate.[220] A newly developed reagent, methoxycarbonyl phosphate, is as easily prepared as acetyl phosphate and roughly as strong a phosphorylating agent as PEP; it has, however, the disadvantage of even more severe hydrolytic instability than acetyl phosphate.[221] A number of other systems for regeneration of ATP have also been suggested,[35,225] but do not seem to be practical.

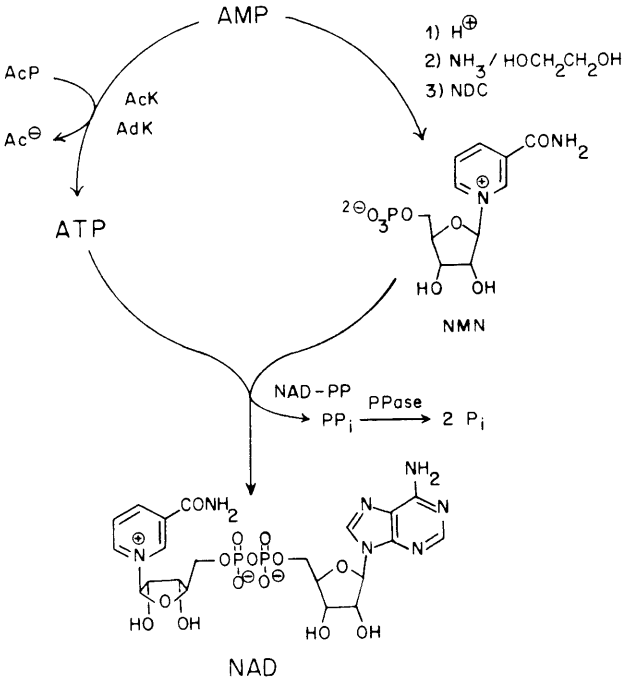
For certain large-scale applications, even given a good regeneration system for in situ reconversion of AD(M)P to ATP, the initial cost of the ATP may be significant. AMP can be purchased inexpensively in kilogram quantities and converted to ATP either in situ or in a separate step.[226] Alternatively, an efficient preparation of crude ATP from RNA has been developed.[226-228] These syntheses should render the cost of the adenosine mononucleotide moieties required for this type of synthesis unimportant on a process scale.

The other nucleoside triphosphates (GTP, UTP, CTP) and the deoxynucleoside triphosphates (dATP, dGTP, dUTP, TTP) are less important in most plausible organic synthetic applications than is ATP. Fortunately, acetate kinase and pyruvate kinase will accept all of these species as cofactors,[218,229] and thus the acetyl phosphate (methoxycarbonyl phosphate)/acetate kinase and PEP/pyruvate kinase systems will regenerate all of these nucleoside and deoxynucleoside triphosphates from the corresponding diphosphates.

Most of the simple applications of ATP regeneration systems have been to the preparation of sugar phosphates using kinases. Glucose-6-phosphate is a useful reagent for reduced nicotinamide cofactor regeneration;[230] dihydroxyacetone phosphate is a reagent for use in aldol reactions catalyzed by rabbit muscle aldolase;[108,109] *sn*-glycerol-3-phosphate[233] has the correct absolute configuration to serve as the basis for the synthesis of phospholipids; arginine phosphate[221,231] and creatine phosphate[224] illustrate the synthesis of materials which themselves have high phosphate-donor potentials, and are therefore thermodynamically difficult to prepare. These kinases are also useful in the preparation of modified nucleoside triphosphates (e.g., ATP-γ-S[232] and ATP-α-S[233]).

Of these simple kinase-based systems, the most broadly applicable in synthesis is, at present, that using glycerol kinase. This enzyme accepts a number of three- and four-carbon substrates, and yields enantiomerically enriched products.[234] It can be used in kinetic resolutions.

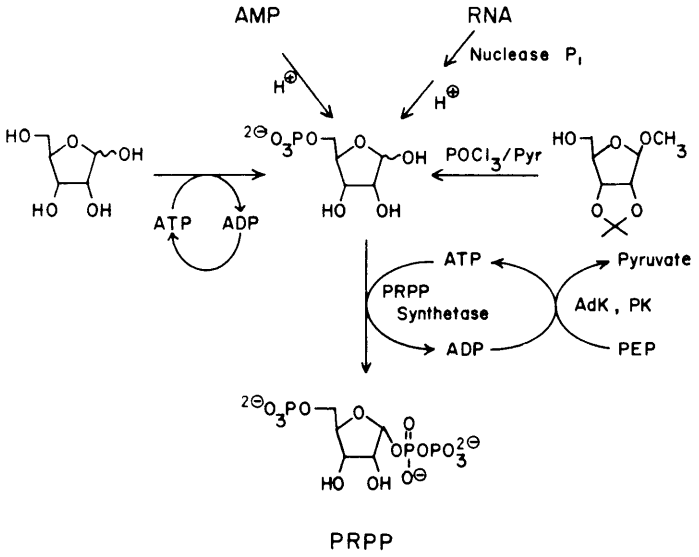
More complicated processes requiring regeneration of ATP are illustrated by syntheses of NAD(P) and phosphoribosyl pyrophosphate (PRPP). The synthesis of NAD(P) (Scheme 4)[235,236] illustrates a practical procedure which successfully combines classical organic and enzymatic synthetic methods. The reaction sequence avoids the use of protecting groups, chromatographic separations, and complicated purifications. This preparation still does not compete with isolation as a source of NAD,[237-239] but it does provide sufficient chemical flexibility to permit synthesis of a number of isotopically and structurally modified analogues of NAD(P).



Scheme 4. Synthesis of NAD from AMP. Abbreviations: AcP, acetyl phosphate; AcK, acetate kinase; AdK, adenylate kinase; NAD-PP, NAD pyrophosphorylase; PPase, pyrophosphorylase; PP_i, pyrophosphate; NMN, nicotinamide mononucleotide; NDC, *N*-(2,4-dinitrophenyl)-3-carbamoylpyridinium chloride.

The synthesis of 5-phospho-D-ribosyl-1-pyrophosphate (PRPP)—a key intermediate in the biosynthesis of nucleotides—is another example which involves the use of an ATP regeneration system in a large-scale preparation of a substance difficult to synthesize using classical techniques.[240] This procedure should open new routes to nucleotides and other analogues.

One feature of the syntheses summarized in Schemes 4 and 5 deserves special comment: the use of enzymatic selectivity to circumvent the normal requirement for purifi-



Scheme 5. Synthesis of 5-phospho-D-ribosyl-1-pyrophosphate (PRPP). Abbreviations: Pyr, pyridine; PK, pyruvate kinase; PEP, phosphoenolpyruvate.

cation of intermediates. In classical multistep synthesis, it is important to purify intermediates, because impurities present in them will react in subsequent steps and, ultimately, generate mixtures of products which can be purified only with difficulty. In enzyme-catalyzed synthesis, requirements for purification of an intermediate may be less strict, because the enzyme will only accept its substrate. Processing of a crude reaction mixture through an enzyme-catalyzed step thus constitutes a form of purification. In Scheme 4, for example, the conversion of AMP to NAD was carried through with essentially no purification of intermediates at any point; the final crude NAD was accepted as a substrate by enzymes. Since purification of these types of molecules is often a major nuisance, the ability to use crude reaction mixtures directly in enzymatic synthesis may be a major practical advantage of an enzyme-based strategy.

6.3.2. Enzymes Requiring Reduced Nicotinamide Cofactors (NADH or NADPH)

The problem of cofactor regeneration involving the nicotinamide cofactors is more complex than that for the nucleoside triphosphates: the nicotinamide cofactors are more expensive than ATP (the least expensive is NAD: ca. US$ 1000/mol), and are, more importantly, intrinsically unstable in solution. The reduced nicotinamide cofactors are destroyed by several processes, the most important of which is a Lewis acid-catalyzed protonation on the dihydronicotinamide ring, followed by addition of water to the resulting iminium salt and further irreversible degradation.[230, 241] Thus, even given the existence of a good regeneration system, it is still important to keep the rate of the enzymatic reaction high, since the ratio of productive turnover of the nicotinamide cofactor by enzyme-catalyzed reaction to nonproductive hydrolytic destruction depends upon this rate. A very large amount of work has been devoted to developing and testing various types of systems for NAD(P)H reduction;[36, 230, 242–255] examples are summarized in Table 7. Of these, three now seem useful for synthetic applications.

Table 7. Systems for in situ regeneration of NAD(P)H from NAD(P) [a].

Regeneration Reaction	Ref.
$HCOO^{\ominus} + NAD \xrightarrow{FDH} CO_2 + NADH$	[36, 244]
$G\text{-}6\text{-}P + NAD(P) \xrightarrow{G\text{-}6\text{-}PDH} 6\text{-}PG + NAD(P)H$	[230]
$Glucose + NAD(P) \xrightarrow{GDH} Gluconate + NAD(P)H$	[255]
$H_2 + NAD(P) \xrightarrow[MV,\ LipDH]{H_2ase} H^{\oplus} + NAD(P)H$	[247]
$C_2H_5OH + NAD(P) \xrightarrow{ADH/AldDH} CH_3CO_2^{\ominus} + NAD(P)H$	[251]
$CH_3OH + NAD \xrightarrow{ADH/AldDH/FDH} CO_2 + NADH$	[251]
$H_2 + NAD \xrightarrow[MV]{Proteus\ mirabilis} H^{\oplus} + NADH$	[252]

[a] Abbreviations: FDH, formate dehydrogenase; G-6-P, glucose-6-phosphate; G-6-PDH, glucose-6-phosphate dehydrogenase (*L. mesenteroides*); 6-PG, 6-phosphogluconate; GDH, glucose dehydrogenase (*B. cereus*); H_2ase, hydrogenase; MV, methyl viologen; LipDH, lipoamide dehydrogenase; ADH, alcohol dehydrogenase; AldDH, aldehyde dehydrogenase.

Formate/Formate Dehydrogenase has two advantages as a regeneration scheme: formate is a strong reducing agent, and the product of reaction, CO_2, causes no complications in workup. Formate dehydrogenase (from yeast), although commercially available, is still relatively expensive; a good preparation has been reported by *Kula* et al.[36]

Glucose/Glucose Dehydrogenase is also an attractive system.[255] The enzyme (from *B. cereus*) is commercially available, thermally stable, and applicable to both NAD and NADP. The major disadvantages of the system are the expense of the enzyme and the requirement to separate product from glucose and gluconic acid.

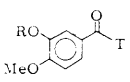
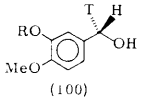
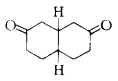
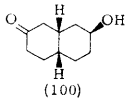
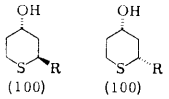
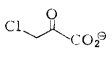
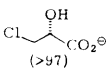
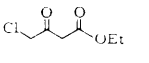
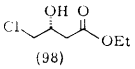
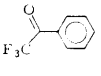
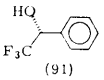
Glucose-6-phosphate/Glucose-6-phosphate Dehydrogenase has the advantage that the enzyme is very inexpensive and stable, and that the enzyme system accepts both NAD and NADP as substrates.[230] The glucose-6-phosphate must, however, be prepared (by hexokinase-catalyzed phosphorylation of glucose with in situ ATP regeneration) and the product must be separated from the 6-phosphogluconic acid produced as product.

A scheme starting with methanol and using a coupled enzyme system of methanol dehydrogenase, formaldehyde dehydrogenase, and formate dehydrogenase probably represents the most economical system available from the vantage of the ultimate hydride donor.[251] The system is not convenient to use for small-scale work, because it requires three enzymes. For large-scale work, however, it might well be worth the effort to develop a single microbial source for the three enzymes which could be used in a whole-cell package. The remaining systems for reduced nicotinamide cofactor regeneration are presently primarily of academic interest, although electrochemical procedures continue to attract attention.[252] In practice, schemes that use inexpensive reducing agents (H_2, electrons) emphasize the wrong feature: the expense in the reaction lies in the enzyme and the cofactor, not in the ultimate reducing

agent. These systems should not be discounted: with appropriate development, they may ultimately prove useful. They are not, however, the systems which are presently the most useful in organic synthesis.

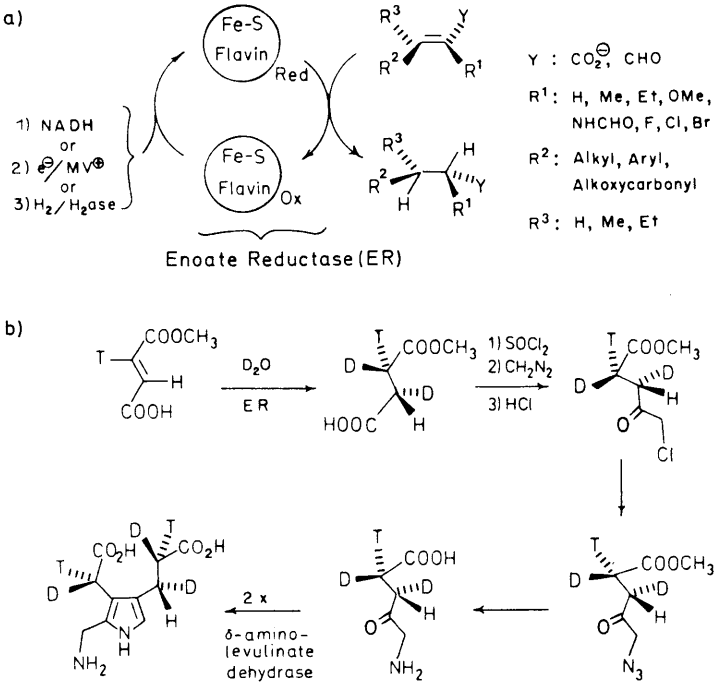
Synthetic application of the enzymes requiring reduced nicotinamide cofactors has so far been restricted to research applications. The most highly developed process is that described by *Kula* et al. for the production of amino acids.[36] Other applications have involved asymmetric reductions of carbonyl compounds, and production of isotopically labeled species (Table 8).[256-259] (A system based on deuterated formate and formate dehydrogenase provides the best system presently available for the introduction of deuterium through a nicotinamide-cofactor-catalyzed process.)[259] It is, however, important to emphasize that the problem of cofactor regeneration is only one of several that must be solved in utilization of the NAD(P)H-requiring oxidoreductases. At least as important are the problems of enzyme cost, enzyme stability, and product inhibition. This last problem is particularly difficult to solve in a general way, and is important because it places constraints on the ultimate concentrations of products attainable and on the rates of reaction.[260]

Table 8. Alcohol dehydrogenase-catalyzed reductions of carbonyl compounds using NAD(P)H as the hydride donor.

Substrate	Enzyme [a] (cofactor)	Product (*ee* [%])	Ref.
	HLADH (NADH)	 (100)	[256]
	HLADH (NADH)	 (100)	[261]
	HLADH (NADH)	 (100) (100)	[261]
	HLADH (NADH)	 (>97)	[259]
	L-LDH (NADH)	 (>97)	[262]
	HLADH (NADH)	 (98)	[255]
	TADH (NADPH)	 (91)	[255]

[a] Abbreviations: HLADH, horse liver alcohol dehydrogenase; L-LDH, L-lactic dehydrogenase; TADH, alcohol dehydrogenase from *Thermoanaerobium brockii*.

Another reducing enzyme that shows promising synthetic utility is the enoate reductase from the anaerobe *Clostridium kluyvery*.[252,263] The enzyme reduces different α,β-unsaturated carboxylate anions stereospecifically to the saturated carboxylate anions using NADH as a cofactor (Scheme 6a). A preliminary study regarding the reaction mechanism indicated that the reducing equivalent was transferred from NADH through the enzyme-associated iron-sulfur-flavin cluster to the substrate, and proton exchange between the reduced cluster and water was observed. The iron-sulfur cluster can be reduced with reduced methyl viologen produced electrochemically, and large-scale organic synthesis using a reactor suitable for electro-enzymatic reductions has been demonstrated.[264] Regeneration of NADH using H_2 and an anaerobic microorganism that contains hydrogenase has been developed and the regeneration system has been coupled with the *Clostridium* reduction of enoates.[252,265] The enoate reductase has also been used to prepare isotopically labeled chiral δ-aminolevulinic acid (Scheme 6b).[4] Similar asymmetric reduction of the CC double bond of α,β-unsaturated carbonyl compounds catalyzed by fermenting yeast has also been reported, but the enzyme responsible has not been isolated.[266] The reaction proceeds with formal *trans* addition of hydrogen across the double bond, with a *pro-R* hydrogen being introduced at the position α to the electrophilic substituent. It is unlikely that the yeast enzyme contains an oxygen sensitive iron-sulfur cluster because fermenting yeast is aerobic.



Scheme 6. Asymmetric reduction of carbon-carbon double bonds catalyzed by enoate reductase (ER). a) General reaction scheme; b) synthesis of isotopically labeled, chiral δ-aminolevulinic acid.

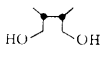
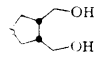
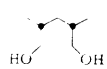
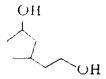
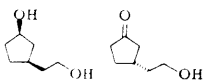
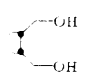
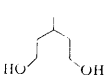
6.3.3. Enzymes Requiring Oxidized Nicotinamide Cofactors (NAD or NADP)

The problem of regeneration of *oxidized* nicotinamide cofactors is still not completely solved. The same constraints apply in this system as to those involving the reduced nicotinamide cofactors, but, in addition, the problem of product inhibition (especially kinetically mixed and uncompetitive inhibition[20]) is often severe.[260,267] The oxidized nicotinamide cofactors are also hydrolytically un-

stable in solution, and are subject to destruction in several types of enzyme-catalyzed processes. The most widely used procedure for in situ regeneration of NAD from NADH is that developed by *Jones* et al.[268] This system is based on the (non-enzyme-catalyzed) reoxidation of NADH by oxidized flavin; the reduced flavin is in turn oxidized by molecular oxygen. The disadvantage of this system is that large quantities of flavin are required to achieve useful rates, and that the separation of product from flavin is inconvenient. A number of other systems have been proposed for this regeneration.[2,242,243,269,270] Probably the most general involves oxidation of NADH to NAD coupled with enzyme-catalyzed reduction of α-ketoglutarate and ammonia to glutamic acid.[267,269] This system has the advantage that it avoids the exposure of the enzymatic system to dioxygen, with the concomitant risk of oxidation of enzymes or substrates. It has the disadvantage that glutamic acid must be separated from the product at the conclusion of the reaction. An enzyme-catalyzed system based on diaphorase and methylene blue is also useful for reoxidation of NADH to NAD when dioxygen (the ultimate oxidizing agent) can be tolerated by the system.[243,267,270,271] The flavin mononucleotide (FMN) reductase (E.C. 1.6.8.1) from photobacterium species catalyzes the reaction of NAD(P)H with dioxygen and FMN;[271] this enzyme has satisfactory specific activity (ca. 100 U/mg) and markedly improves the efficiency of the regeneration system developed by *Jones* et al.

The most important body of work devoted to synthetic applications of enzymes requiring oxidized nicotinamide cofactors has been that of *Jones* and coworkers, and of other groups,[272-277] concerning applications of horse liver alcohol dehydrogenase. Examples of these transformations are given in Table 9. These reactions, although highly practical on the scale of several grams, have not been carried to large-scale work. Several problems have hindered this stage of development. First, until recently, the available regeneration systems have not been truly practical for large-scale work. Second, horse liver alcohol dehydrogenase is particularly subject to product inhibition. Many of the most successful examples of *Jones* et al. are based on the oxidation of *meso*-diols to optically active lactones. These systems seem to be relatively free of product inhibition. Attempts to achieve kinetic resolutions of simple alcohols by oxidation of one enantiomer have, in general, been relatively unsuccessful on large scale. The active site of alcohol dehydrogenase appears to be hydrophobic, and to bind the product ketone more strongly than the reactant alcohol.[274,278,279] This specific system has been analyzed in some detail. There appears to be no general strategy to deal with product inhibition, other than to remove the product as it is formed by extraction or by some other technique.[21,30] When extraction is feasible (using, for example, a two-phase water-organic system to remove the ketone selectively as it is produced), the problem can be circumvented. In other circumstances, it is more troublesome.

Table 9. HLADH-Catalyzed oxidation of diols using NAD as a cofactor.

Substrate	Product	*ee* [%]	Ref.
		100	[272]
		100	[272]
		100	[272]
		100	[274]
		100	[275]
		96	[276]

6.3.4. *Enzymes Requiring S-Adenosyl Methionine, Acetyl-CoA, PAPS, or Other Cofactors*

S-Adenosylmethionine (SAM) is an important one-carbon donor in many enzyme-catalyzed reactions.[280-282] A study of the enzymatic synthesis of SAM from ATP and methionine catalyzed by SAM synthetase from yeast concluded that the practicality of the reaction hinged upon the availability of SAM synthetase.[282] The preparation of substantial quantities of homogeneous SAM synthetase from a genetically engineered strain of *E. coli* has been described.[283] This enzyme is, however, seriously inhibited by product. The inhibition constant (K_i) of SAM vs. ATP (a competitive inhibitor) or methionine (a noncompetitive inhibitor) is ca. 0.01 mM.[282] The corresponding K_i values for the enzyme from yeast are ca. 6 mM.[284] If the *E. coli* enzyme could be modified using site-specific mutagenesis and recombinant DNA technology to increase the K_i values significantly, this procedure for the preparation of SAM could form the basis for a practical method for preparation of quantities of SAM for *stoichiometric* use in enzyme-catalyzed synthesis. No strategy for in situ regeneration of SAM is now evident. In a transmethylation using SAM as methyl donor, the product of SAM is *S*-adenosylhomocysteine (SAH). At present, there is no enzyme known which catalyzes the selective methylation of SAH to SAM. Although chemical procedures are available for the synthesis of SAM from SAH,[285] they produce racemic products and enzymes only accept the (−)-isomer.[280,286] In an alternative route to (±)-SAM, which does not require ATP as a starting material, L-homocysteine and adenosine are converted into SAH chemically[287] or enzymatically,[145-149] and SAH is methylated chemically. If resolution of SAM could be accomplished conveniently, this procedure might be valuable.

Little work has been devoted so far to synthetic applications of reactions requiring acetyl-CoA and its analogues as cofactors. Although CoASH is a broadly important cofactor in biochemistry, from a strategic viewpoint it is unclear that reactions involving it will be as important in enzyme-catalyzed synthesis as are reactions involving other

cofactors. Acetyl-CoA is central in reactions leading to fatty acids, polyketides, and derived species.[288] It is precisely these classes of compounds which classical organic synthesis is most successful in preparing. Whether enzymatic procedures offer advantages relative to chemical or fermentation routes to these compounds remains to be established.

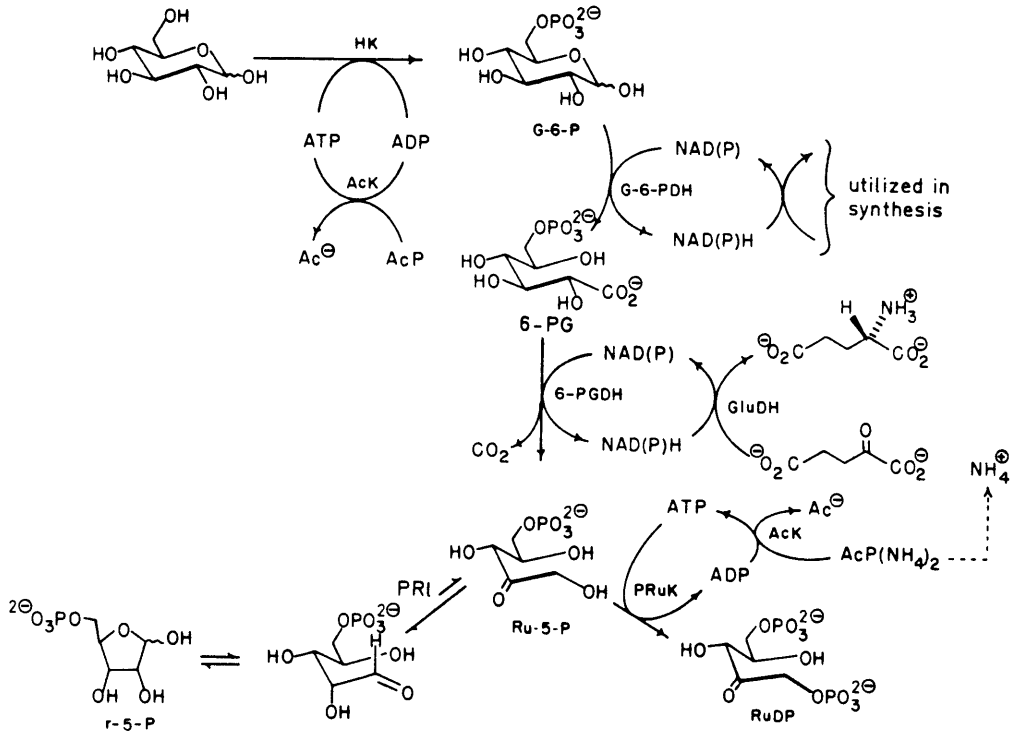
3'-Phosphoadenosine-5'-phosphosulfate (PAPS) is a sulfate donor, universally used in biochemistry for sulfation of polysaccharides and for activation of sulfate. It is regenerated in vivo by a complex set of reactions involving the enzymes ATP-sulfurylase and adenylsulfate kinase.[289, 290] Although sulfation of polysaccharides is an interesting and potentially important target reaction for enzyme-catalyzed synthesis (for example, in the modification of heparin[291] or the synthesis of chondroitin sulfate[292]), no straightforward method now exists to achieve either the synthesis or regeneration of PAPS.

7. Syntheses Involving Multienzyme Systems

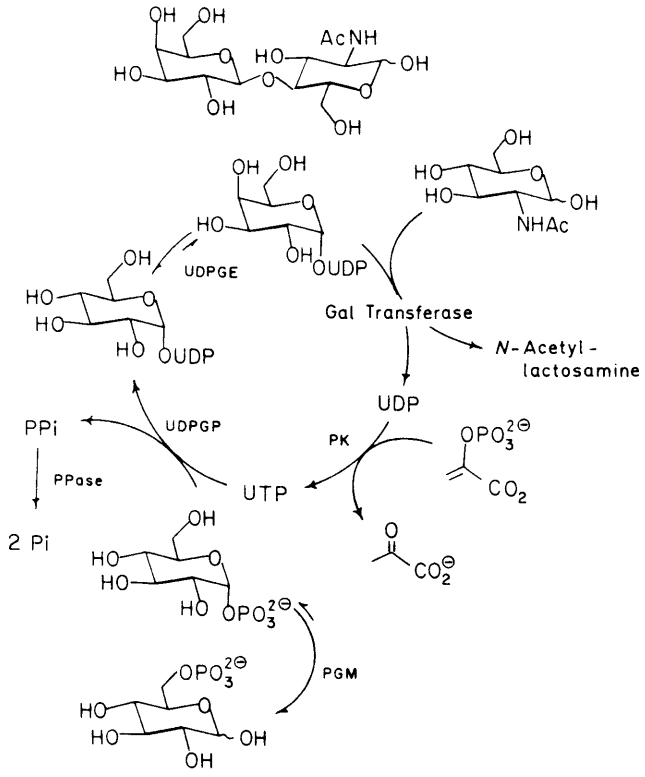
A potentially important characteristic of enzymes as catalysts in organic synthesis is that, because most enzymes operate with reasonable efficiency at values of pH between 6.5 and 8.5 and at temperatures around room temperature, it is often possible to assemble complex systems containing multiple enzymes cooperating in sequence or in parallel to achieve multi-step processing of substrates. The synthesis of ribulose-1,5-diphosphate (RuDP), an important substrate in plant biochemistry (Scheme 7),[217] provides an example of a synthesis for which enzymatic catalysis provides the best solution. This synthesis, despite its apparent complexity, is actually quite straightforward, and several hundered grams of RuDP has been prepared in the course of the several reactions devoted to developing it. The point of technical interest in this synthesis is the final conversion of 6-phosphogluconate to RuDP. The kinase that converts ribulose-5-phosphate to RuDP is oxygen sensitive. Thus, the in situ regeneration of NAD from NADH required in conversion of 6-phosphogluconate to ribulose-5-phosphate cannot be carried out using an oxygen-based regeneration system. This regeneration is, however, conveniently carried out anaerobically using the α-ketoglutarate/glutamate dehydrogenase system. This part of the synthesis requires the cooperative action of four enzymes: 6-phosphogluconate dehydrogenase and 5-phosphoribulokinase for the principal conversions, and glutamate dehydrogenase and acetate kinase for in situ regeneration of cofactors.

The conversion of glucose-6-phosphate and *N*-acetylglucosamine to lactosamine provides a more complex example.[293] Lactosamine is a core disaccharide, common in glycoproteins. This synthesis (Scheme 8) illustrates the cooperative interaction of six enzymes, and establishes the utility (howbeit on a small scale) of the Leloir pathway enzymes for oligosaccharide synthesis. This type of procedure has subsequently been extended to trisaccharides.[294]

The conversion of glucose to ethanol provides a final example of a coupled multienzyme system (Scheme 9; in this scheme, several enzymes required to regulate the concen-



Scheme 7. Enzymatic syntheses of RuDP. Abbreviations: G-6-P, glucose-6-phosphate; 6-PG, 6-phosphogluconate; Ru-5-P, ribulose-5-phosphate; AcP, acetyl phosphate; Ac, acetate; 6-PGDH, 6-phosphogluconate dehydrogenase; GluDH, glutamic acid dehydrogenase; PRuK, 5-phosphoribulokinase; PRI, phosphoriboisomerase; AcK, acetate kinase; HK, hexokinase; r-5-P, ribose-5-phosphate.



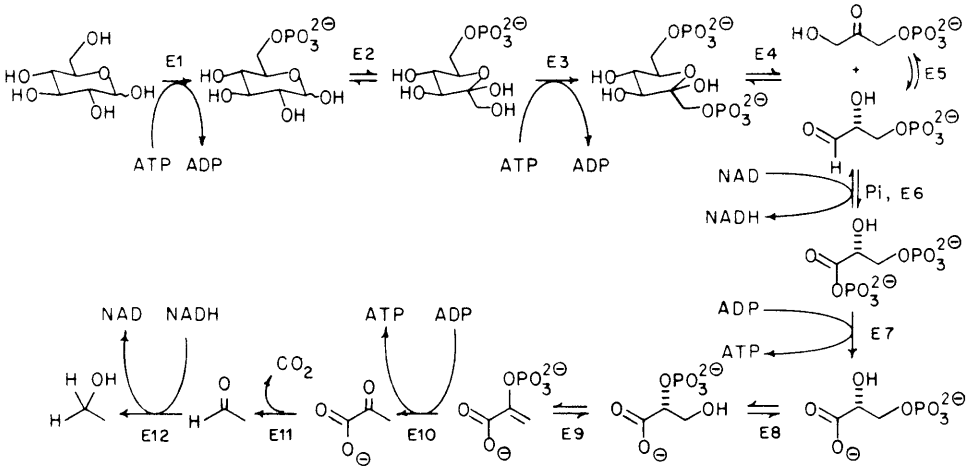
Scheme 8. Synthesis of *N*-acetyllactosamine. Abbreviations: Gal transferase, galactosyl transferase; PK, pyruvate kinase; PGM, phosphoglucomutase; UDPGP, UDP-glucose pyrophosphorylase; UDPGE, UDP-glucose epimerase; PPase, inorganic pyrophosphatase.

tration of ATP are omitted for simplicity). This system was not developed for synthetic purposes—there are simpler ways of obtaining ethanol—but rather to study the mechanism of ethanol toxicity in yeast and to examine the kinetic characteristics of a regulated multienzyme system.[295] Nonetheless, it establishes that it is practical to construct processes requiring sequential operation of many cooperating enzymes.

8. More Difficult Enzymatic Systems: Oxygenases and Systems for Syntheses of Macromolecules

The practicality of using monooxygenase systems has been the subject of speculation in applied enzymology for some years. Certain enzyme systems, operating in vivo, catalyze hydroxylation, epoxidation, and related oxidation reactions with selectivities that cannot be matched using classical synthetic technology. These in-vivo systems are widely important in the oxidative functionalization of steroids and other hydrocarbons (using fungal systems), and interesting isolated examples having emerged from other areas of fermentation chemistry.[211,296-299] An important recent example is the conversion of benzene to *cis*-3,5-cyclohexadiene-1,2-diol by fermentation.[300] Many of these transformations are accomplished by cytochrome P450 or non-heme iron-sulfur enzymes.[301,302] The cytochrome P-450's have been widely studied mechanistically, because of their importance in steroid metabolism and carcinogen production; the non-heme iron-sulfur proteins are less well understood.

From a practical point of view, microbial systems based on non-heme iron-sulfur and copper-sulfur enzymes are the most interesting.[200-202,303] Although these systems have been extensively examined, current indications suggest that they will not be useful for enzymatic transformations in vitro in the immediate future. They suffer from several disadvantages. First, in vitro, operating lifetimes of the enzymes are short. Many of the cytochrome P450 enzymes are membrane-bound or membrane-associated, and it is conceivable (although not yet practical) that appropriate use of surfactants or model membranes might provide valuable stabilization. The in-vivo lifetimes of the simpler microbial monooxygenases have not been carefully examined, but they also seem to be short. Perhaps more importantly, these enzymes require accessory enzyme systems as a source of reducing equivalents, ultimately used to convert dioxygen to a more reactive species at the



Scheme 9. Enzymatic pathway from glucose to ethanol. Enzymes: E1, hexokinase; E2, phosphoglucomutase; E3, phosphofructokinase; E4, aldolase; E5, triosephosphate isomerase; E6, 3-phosphoglycerate dehydrogenase; E7, 3-phosphoglycerate kinase; E8, phosphoglycerate mutase; E9, enolase; E10, pyruvate kinase; E11, pyruvate decarboxylase; E12, alcohol dehydrogenase.

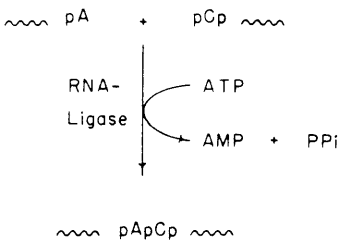
hydrogen peroxide oxidation level. These accessory enzymes typically require NADH or NADPH as the ultimate reducing agent. Thus, the final system contains a number of sensitive enzymatic components. Further, the efficiency of the coupling of oxidation of NADH to reduction of dioxygen has, so far, been poor in vitro: the majority of NAD(P)H is "wasted" in production of hydrogen peroxide or superoxide, both themselves species capable of reacting with and inactivating enzymes. It may be that the problems associated with these potentially valuable monooxygenases will ultimately be solved, but for the immediate future, these systems do not represent practical methods for oxidative functionalization of hydrocarbons.

A second class of enzymes that is attracting interest is that responsible for the synthesis of polysaccharides, oligosaccharides, and proteins.[304-306] Each of these systems represents a different set of problems. Cell-surface oligosaccharides are of substantial interest as, inter alia, disease-associated antigens and histocompatibility markers, and as reagents of possible interest in drug delivery.[304,307] In most systems, they are assembled by enzymes utilizing the Leloir pathway and localized in the Golgi apparatus.[308] These membrane-bound enzymes are usually obtainable only in small quantities. It is unresolved whether it will be more practical to base synthetic schemes on isolated enzymes or to rely on isolation from natural sources or screening for high-producing mutants for fermentation synthesis. The enzymatic systems involved in synthesis of many polysaccharides (especially those of microbial origin) are, in principle, more straightforward. Since polysaccharides cannot presently be prepared by any classical chemical technique, and because these substances have properties valuable in modification of the rheological properties of aqueous solutions and in pharmacological applications,[304-306] enzyme systems that synthesize polysaccharides may prove valuable. Again, however, no practical example yet demonstrates the utility of such systems.

Cell-free protein synthesis might provide a technique complementary to DNA methodology for certain applications.[309,310] At present, however, the complexity of the systems of enzymes required to accomplish this type of process is such that it is impractical on scales larger than micrograms.

The central scientific discoveries that have focused interest on biologically based syntheses are those leading to recombinant DNA (rDNA) technology. This technique depends on a number of enzymes that make it possible to implant desired genes into a foreign organism to produce desired proteins.[311,312] Of the enzymes used in rDNA technology, DNA ligase and restriction endonucleases are two of the most important. DNA molecules can be cleaved specifically with specific restriction endonucleases and joined with another restriction-enzyme-modified DNA fragment in a reaction catalyzed by DNA ligase. These chemical transformations cannot be accomplished by classical synthetic techniques, and the enzymatic methods might be useful in practical-scale nucleic acid chemistry. The enzymes used in rDNA research are, however, expensive. Technical problems concerning stabilization and large-scale production of enzymes need to be solved if this type of enzymology is ever to be used for preparative chemical synthesis (large-scale work is unnecessary for use in genetic engineering, because microbial growth provides large amplification). We note also that since the condensation reactions involved in forming phosphodiester links require either NAD (in reactions catalyzed by NAD ligase from bacteria, and generating AMP and NMN) or ATP (catalyzed by T_4 DNA ligase from phage-infected bacteria or from eukaryotes, and generating AMP and pyrophosphate),[310] cofactor regeneration may be required for large-scale production.

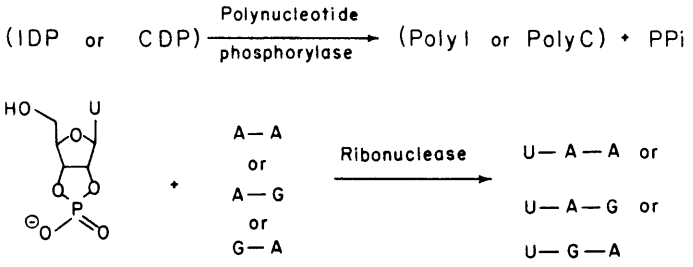
Another ligase that has been used increasingly is RNA ligase.[311,313-315] The enzyme T_4 RNA ligase catalyzes the synthesis of single-stranded oligonucleotides of different lengths from a 3′-terminal hydroxyl acceptor and a 5′-terminal phosphate donor oligoribonucleotide through the formation of a 3′→5′ phosphodiester bond, with hydrolysis of ATP to AMP and pyrophosphate. The 5′-terminal phosphate donor can be nucleotides as small as monomer units and the 3′-terminal hydroxyl acceptor can be a dimer, trimer, or oligomer.[311] The enzyme also accepts single-stranded DNA as a substrate (Scheme 10). This enzyme



Scheme 10. A representative scheme for the RNA-ligase-catalyzed formation of a 3′→5′ phosphodiester bond between oligonucleotides containing terminal A and pC.

has been used to couple short, chemically synthesized oligonucleotides to longer oligomers.[313] It also has been used for modification of nucleic acids by introducing radioactive probes and by replacing nucleotide residues.[311-316] Since applied biology based on rRNA technology may be important for the future, and since chemical methods for the synthesis of RNA are not as well developed as those for DNA, RNA ligase could play an important role in this area.

Two other examples of polynucleotide synthesis deserving mention are the polynucleotide phosphorylase-catalyzed synthesis of polyI:C[317] (an inducer of interferon) and the ribonuclease-catalyzed synthesis of trinucleotide codons[318] (Scheme 11). These two enzymes have a broad



Scheme 11. Synthesis of polynucleotides catalyzed by polynucleotide phosphorylase, and of trinucleotides catalyzed by riconuclease.

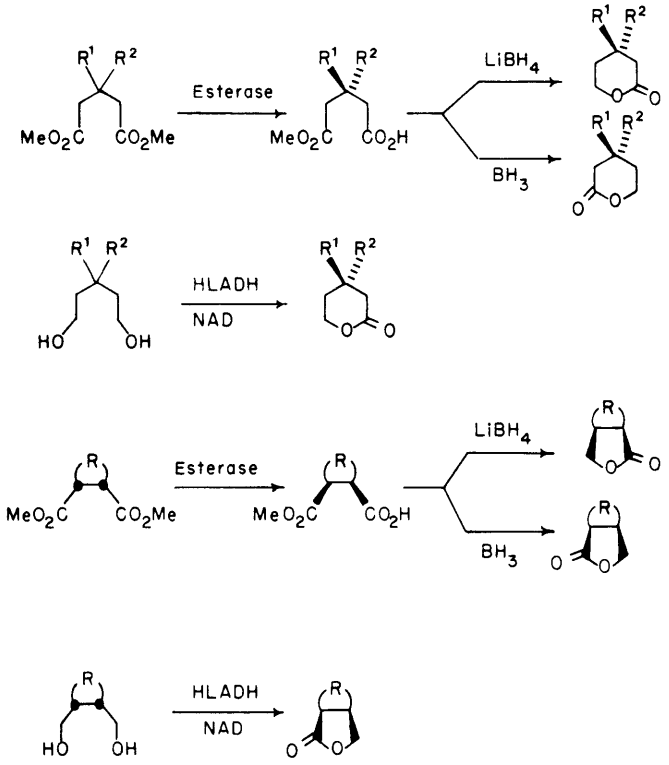
range of specificity; they can thus be useful for the synthesis of other polynucleotides.

9. Summary and Outlook

Enzymes are catalysts that are useful in a variety of synthetic problems: synthesis of chiral fragments; transformations of sugars, nucleotides, and related species; synthesis of compounds important in metabolism and analogues of these metabolites (amino acids, sugar phosphates, etc.); transformations of peptides and proteins; transformations of compounds present in foods and other transformations in which classical chemical methodology is constrained by regulation. What are the technical problems that must be solved to consolidate and extend these uses? What are the barriers that a synthetic organic chemist must overcome in order to use enzyme-catalyzed synthesis?

The two major barriers to the use of enzymes are probably educational. The first is that of learning the basic techniques of enzymology and biochemistry, especially the specialized techniques of analysis and isolation of enzymes. Neither is difficult, but both require skills that are not familiar to most classically trained synthetic chemists. Standard biochemistry textbooks are helpful in learning these techniques; *Bergmeyer*'s series provides an indispensible reference for enzymatic assays.[319] A short apprenticeship in a biochemistry laboratory is exceedingly helpful. A second, more serious problem (at least in considering the feasibility of including enzymology in a proposed synthetic sequence) is finding out whether an enzyme is known (or, better yet, commercially available) that can plausibly carry out the transformation of interest. This problem is a difficult one to solve. There is presently no reference that describes enzymology from the vantage of a synthetic chemist, that outlines the types of synthetically useful transformations which might be expected from a given class of enzymes, or that details the enzymes capable of conducting a particular type of transformation. Most enzymes have not, in fact, been examined for their utility in synthesis, and the nomenclature of enzymology is not helpful in establishing activity for new transformations. This article lists several of the enzymatic systems that have been explored in sufficient detail to be helpful in organic synthesis; the series *Methods in Enzymology* and *Bergmeyer*'s series give sample procedures involving enzymes (usually on an analytical scale) that are helpful in suggesting new applications. In the absence of specific solutions from these sources, the synthetic chemist can only consult his biochemical colleagues, and test enzymes known to operate on substrates analogous to those of interest in the proposed process.

If an enzyme can be found that effects the desired transformation, what determines the practicality of a process based on this enzyme? Clearly, technical features—the availability of the enzyme, its specificity, specific activity, and stability, its cofactor requirements—are of primary importance in evaluating the practicality of a specific enzyme. Of greater interest to most organic synthetic chemists is a broader question of strategy. Enzymes should be regarded simply as one more type of catalyst, and the best synthetic procedures will be those that make the most efficient and best integrated use of all of the available techniques—classical organic synthesis, homogeneous and heterogeneous catalysis by metals, catalysis by enzymes, fermentation, electrochemistry, conventional resolution, and other methods. In certain instances—for example, the use of DNA ligase and restriction endonuclease to close and open nicks in DNA—enzymes have no competition from other techniques. In production of chiral synthons for use in synthesis of pharmaceuticals, the range of options open to the synthetic chemist is much larger. The optimal strategies for using enzymes are still evolving. For example, is it more efficient to use horse liver alcohol dehydrogenase to oxidize a *meso*-diol to a lactone (taking into account the problems of cofactor regeneration and product inhibition) or to use an esterase in combination with a chemical oxidation (Scheme 12)? For resolution of a new epoxy alcohol, is it better to use asymmetric epoxidation[17] or an esterase? For production of large quantities of short oligopeptides, is catalysis by selected amidases starting from minimally protected amino acids in dehydrating media preferred, or are conventional chemical coupling procedures superior? None of these questions presently have categorical answers. The general point is, however, that the range of plausible applications of enzymes in organic synthesis is much larger than might be supposed by the range of their present use. Enough experimental background is now available that many of the generic problems involved in applying enzymes as catalysts have been solved.



Scheme 12. Alternative routes to optically active lactones.

Certain problems in synthesis will unquestionably benefit from use of enzymatic catalysis. Synthetic chemists capable of using this class of catalysts will have a clear advan-

tage in their ability to tackle the new generation of problems in synthesis appearing at the border between chemistry and biology. Those unwilling to use these and other biologically derived synthetic techniques may find themselves excluded from some of the most exciting problems in molecular science.

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