Section VI - Topics in Chemistry and Drug Design

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Chapter 26. Enzymic Methods in Organic Synthesis

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Introduction - Enzyme-based synthetic chemistry has continued to grow
rapidly in recent years.[1-6] Technical problems, which have inhibited the
widespread use of enzymes as catalysts, have been much reduced by the
introduction of new procedures for enzyme immobilization and stabiliza-
tion and for in situ cofactor recycling. A widespread interest in asym-
metric synthesis has focussed attention on the demonstrated utility of
enzymic catalysis in producing chiral fragments. In fact, the major
hindrance to the widespread use of enzymic catalysis is the residual
unfamiliarity of many classically trained synthetic chemists in the
techniques of enzyme isolation, manipulation, and assay. This last
barrier is disappearing as biochemistry and enzymology become an ac-
cepted part of the education of an organic chemist.

This review emphasizes procedures which use partially or highly
purified enzymes to catalyze organic reactions potentially useful in
medicinal chemistry. Biochemical procedures using microbiological
transformations or cell culture are not discussed.

General Techniques - More than two thousand enzymes are known,[7] and
several hundred can be obtained commercially. Many other enzymic activ-
ities are available through straightforward isolations or small-scale
fermentations. Increasing attention is being paid to large-scale pro-
duction of enzymes;[8] Kula has developed efficient methods based on
liquid-liquid extractions.[9-11] Given sufficient demand, many enzymes
can be produced in quantity by recombinant DNA techniques.

We note that highly purified enzyme preparations are not always
necessary in synthetic applications since contaminating enzymes may have
no effect on the reactants and products present in the reaction mix-
ture. If an enzyme to be used in synthesis has intrinsically low spe-
cific activity (units of catalytic activity per weight of protein),
crude preparations can cause practical problems by requiring large
volumes of immobilization medium and correspondingly large reactor
volumes. The use of microbial cells in enzyme-catalyzed synthesis
represents a limiting case in purification; since no purification is
involved, their manipulation is straightforward. The activity of these
preparations may be low. In favorable cases, however, they represent

the simplest basis for enzyme-catalyzed reactions, and several success-ful industrial processes have been developed using immobilized whole cells.[12]

Enzyme Immobilization - Enzymes used in synthetic applications are commonly immobilized in or on insoluble materials because immobilization enhances their stability and allows their recovery and reuse. While bench-scale experiments are most conveniently carried out as batch processes, industrial processes often depend on long-lived immobilized enzymes in continuous processes.

Many immobilization methods have been developed.[12-14] Only a few of these techniques deserve explicit comment. Glutaraldehyde is the most commonly used bifunctional reagent in immobilization,[12] forming covalent linkages of still incompletely defined nature between amino groups. It is used to bind enzymes to solid supports or cross-link enzymes adsorbed on a support, and to cross-link enzymes with carrier proteins or with themselves to form insoluble aggregates. Immobiliza-tion procedures for industrial applications based on functionalized ceramics cross-linked with enzyme via glutaraldehyde have been devel-oped.[14,15] Wood et al. have developed an immobilization procedure using polyurethane-based membranes.[16] Whitesides et al. have developed an immobilization method based on polyacrylamide-co-N-acryloxysuccinimide cross-linked with triethylene tetramine in the presence of enzyme.[17] This method is particularly useful with the relatively delicate enzymes useful in organic synthesis. Kula et al. have used membrane reactors containing soluble enzymes.[18] The enzymes are not immobilized, but reactor performance has been good.

Enzyme Stabilization - Enzyme stabilization is a concern before, during, and after immobilization. Immobilization often greatly increases the stability of enzymes.[19-22] Reasons for this stabilization are not clearly understood. Enzyme deactivation during the immobilization pro-cess is often troublesome. Addition of substrates or inhibitors of the enzyme during immobilization helps to occupy and protect the active site, and increases yields on immobilization.[17] A number of strategies are useful in maintaining activity in soluble and immobilized enzymes during use.[23-26] Thiol reagents (dithiothreitol, β-mercaptoethanol, 1,3-dithiopropan-2-ol) maintain the reduced state of catalytically essential thiols in the enzyme. Chelating agents inhibit metal ion catalyzed oxidations of enzymes.[27] The stability of soluble enzymes can be enhanced by the addition of polyols, salts, and certain polymers, and by chemical modification.[23,28-32] Thermally inactivated immobilized enzymes have been reactivated by thiol reagents and reversible heat treatment.[33]

Cofactor Regeneration - About 70% of enzymes use nucleoside triphos-phates, nicotinamide derivatives [NAD(P)(H)], or CoA as cofactors. These enzymes include many of those of greatest interest in the syn-thesis of fine chemicals. Since these cofactors are too expensive to be used stoichiometrically, it has been necessary to develop recycling systems for them. The problem of recycling the nucleoside triphosphates

is essentially solved at the level of laboratory-scale synthesis,[34-38] and that of recycling NAD derivatives is well-advanced toward solution.[39-46] None of these schemes has been tested on a production scale, although they work satisfactorily for syntheses of several moles of products. Recycling of ATP from ADP or AMP rests on the development of practical syntheses of the phosphate donors acetyl phosphate[37] and phosphoenolpyruvate[35] to be used with the enzymes acetate kinase and pyruvate kinase, respectively. Acetate kinase is also applicable to recycling of GTP, UTP, CTP and the corresponding 2'-deoxy-nucleoside triphosphates.[1,34] Regeneration of these species from nucleoside monophosphates is not truly practical since adenylate kinase is specific for AMP, but relatively few reactions generate nucleoside monophosphate. CoA recycling has not been explored. Recycling of S-adenosyl-L-methionine, a cofactor in enzyme-catalyzed transmethylations, is currently difficult.[47] The conversion of reduced nicotinamide cofactors [NAD(P)H] to the oxidized form [NAD(P)], developed by Jones et al., is the most widely used procedure for this transformation, but suffers from the need for large amounts of flavin and from slow reaction rates.[39] Oxidative regeneration based on the conversion of α-ketoglutarate to glutamic acid works well and does not require the presence of oxygen.[36] The best system for reductive regeneration of NADH from NAD is based on formate dehydrogenase.[18,46,48] This procedure works very well, although the enzyme does not accept NADP as a substrate and is relatively expensive. The most practical procedure for regeneration of NADPH uses glucose-6-phosphate dehydrogenase.[42]

Equilibrium Manipulation - Enzymes are catalysts and therefore serve only to accelerate attainment of equilibrium. In many instances the equilibrium constant for a given reaction in water does not adequately favor the desired product or water acts as an undesired reactant. Sometimes product can be favored using excess starting material, or by reacting the product in an irreversible manner.[49] Occasionally precipitation of product will drive an unfavorable reaction, but precipitation can foul immobilized enzymes. Kinetic control of a reaction can sometimes be used to maximize yields.[49] In cases where less polar materials are being manipulated, water miscible organic cosolvents have been used, but this technique can reduce enzyme activity.[50-53] Careful selection of cosolvent can, however, maintain enzyme activity and dramatically shift equilibrium.[54] A more general approach for working with less polar compounds is the use of a two-phase system incorporating a water-immiscible organic solvent. This approach has been discussed extensively by Martinek and coworkers.[55-59]

Enzyme Mediated Processes

Simple Hydrolases and Isomerases - These are enzymes requiring no added cofactors, and which catalyze hydrolyses, isomerizations, some condensations, and related reactions. Such enzymes are among the simplest to use and are the most widely used in industry (Table 1). A valuable introduction to processes using these enzymes has been published.[12]

Table 1. Selected Industrial Applications of Enzymes.

			ref.
penicillin-G	⟶	6-aminopenicillanic acid	12,60,61
fumaric acid	⟶	L-aspartic acid	12,62
fumaric acid	⟶	L-malic acid	12,63
starch	⟶	glucose	30,64
glucose	⟶	fructose	30,65,66
N-acetyl-D,L-amino acids	⟶	L-amino acids	12
L-arginine	⟶	L-citrulline	12

Amidases - Acylase is used in continuous production of L-amino acids.[12] It has been used to resolve D,L-phenylalanine[67] and α-formyl-ε-acyl-D,L-lysine.[68] Penicillins have been synthesized from 6-aminopenicillanic acid with penicillinase.[69]

Esterases - Esterases from several sources have been used in stereoselective and regioselective hydrolysis of esters in the preparation of chiral esters, acids, and alcohols. Sih and coworkers have developed a valuable theoretical treatment for quantitative analysis of such biochemical kinetic resolutions relating the extent of conversion (c) of racemic substrate, the optical purity (ee) of the starting material and products, and the enantiomeric selectivity of the enzyme.[70] Recycling of enantiomerically enriched substrate can greatly increase ee. Racemic threo ester 1 was hydrolyzed with pig liver esterase (PLE) to (-)-acid 2 (64% ee). Reesterification and incubation to 80% c resulted in 2 with >90% ee. Erythro ester 3 was treated with <u>Gliocladium roseum</u> to hydrolyze the 2S,3S isomer. At 70% c the remaining ester had 95% ee.


(±) 1, R = CH$_3$
(-) 2, R = H

(+) 3
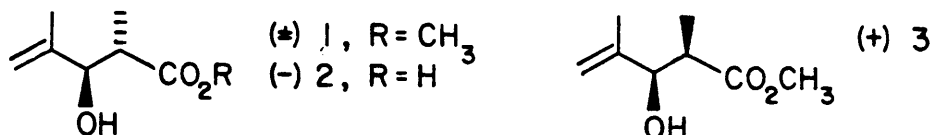
PLE was used in the enantiotopically selective hydrolysis of diethyl-β-methylglutarate 4 to the half-ester 5. Reduction, derivatization and resolution raised the ee of 6 from 69% to 91%.[70]

4, R=Et; 5, R=H   6(69% ee)   6 (91% ee)
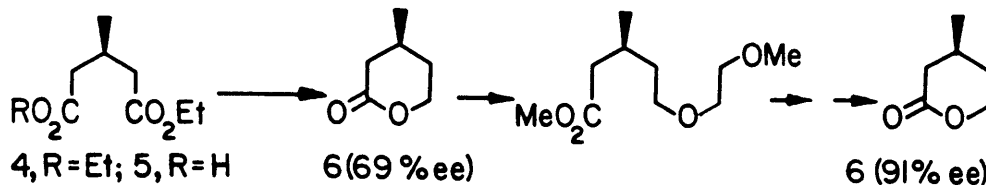
Substituent effects have been studied in PLE-catalyzed hydrolysis of a range of symmetrical dicarboxylic acids and a model has been developed to determine absolute configuration in the monoester products.[71]

Esterases have successfully resolved compounds 7-11. Imidazolone 7 was converted to (+)-biotin.[72] Dimethyl-2,4-dimethylglutarates 8 and 9 were resolved with PLE and G. roseum.[73] Dimethyl-β-aminoglutarate 10 was used as starting material for syntheses of R- and S-4-[(methoxycarbonyl)methyl]-2-azetidinones,[74] useful nuclei for carbapenem β-lactam antibiotic synthesis. R-Esters of 3-hydroxy-3-methylalkanoic acids 11

were prepared for testing as inhibitors of 3-hydroxy-3-methylglutarylCoA reductase and in compactin analogue syntheses.[75] Schneider and coworkers have used PLE-catalyzed hydrolyses in asymmetric syntheses of (1R, 3R)-chrysanthemic 12, permethrinic 13 and caronic acid 14 derivatives,[76] and in syntheses of disubstituted monoalkyl malonates[77] and 1,2-cycloalkane dicarboxylate monomethyl esters.[78]
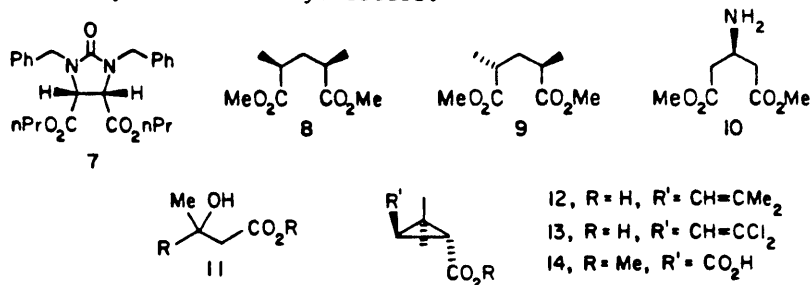
Cambou and Klibanov[79] have used transesterifications catalyzed by PLE and yeast lipase to resolve a variety of alcohols with great effectiveness. Their novel approach employed a biphasic system of aqueous enzyme solution absorbed in a porous solid phase placed in a mixture of "matrix ester" (methyl propionate or tributyrin) and racemic alcohol (Scheme I).

Scheme I.
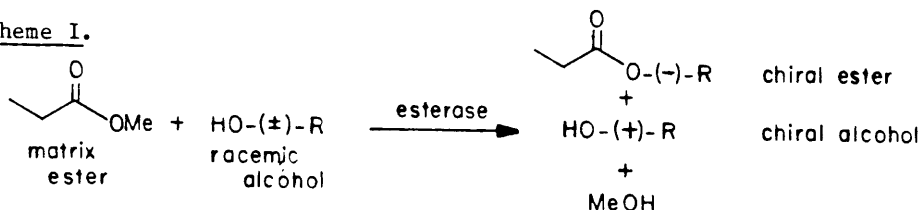
The low water content of this system simplified subsequent purification and effectively suppressed ester hydrolysis. Lipases have also been applied to resolutions of chloroglycerol derivatives,[80,81] 3-acetylthiocycloheptene,[82] and 2-chloromethyl-1-propyl propionate.[83] Cholinesterases have been used to resolve D,L-carnitine.[84]

Proteases - Proteases have been applied to several synthetic purposes the most important of which are peptide bond synthesis and protein semisynthesis. Recent extensive reviews cover this area.[85,86] Proteases have been used in ester synthesis[87] and resolution.[88] Semisynthesis of human insulin has been achieved by enzymic removal and replacement of one amino acid in porcine insulin.[89,90] All peptide bonds in the N-terminal octapeptide of dynorphin [H-Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-OH] have been formed using proteases.[91] A precursor of aspartame has been made by thermolysin-catalyzed condensation of benzyloxycarbonyl-L-aspartic acid and L-phenylalanine methyl ester.[92]

Aldolases - Aldolases catalyze reversible aldol condensations of sugars.[93] A well-studied enzyme is fructose-1,6-diphosphate aldolase from rabbit muscle. This enzyme exhibits a high specificity for dihydroxyacetone phosphate as the nucleophile, but tolerates a range of aldehydes as electrophiles (Scheme II).[94] This broad specificity allows synthesis of sugars such as 6-deoxyfructose[95] and isotopically labeled glucose

derivatives.[94] Other aldolases exist with different substrate specific-
ities for possible application to preparative sugar synthesis.

Scheme II.
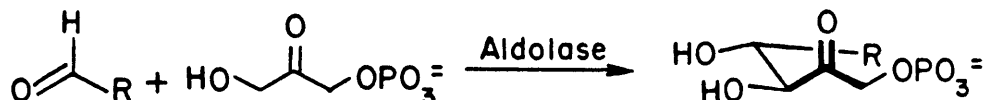
Other - Farnesylpyrophosphate synthetase has been used in asymmetric
synthesis of isoprenoids.[96] Potato acid phosphatase has been applied to
mild hydrolysis of polyprenyl pyrophosphates.[97] Sulfatase-catalyzed
hydrolysis of β-napthol sulfate has been used to separate α- and
β-napthols.[98] NAD[99] and flavin adenine dinucleotide[100] have been made
by enzymic coupling reactions.

## Cofactor Requiring Enzymes

Phosphorylation - Enzymic phosphorylation with coupled in situ ATP
regeneration has been used to prepare glucose-6-phosphate,[101] sn-gly-
cerol-3-phosphate,[102] creatine-phosphate,[103] and 5-phosphoribosyl-1-
pyrophosphate.[104]

Chiral Redox Chemistry - Nicotinamide cofactor dependent oxidoreductases
have been applied in chiral synthesis. Enantioselectivity is often only
moderate, but in certain cases excellent results have been obtained.
Horse liver alcohol dehydrogenase (HLADH) is the most widely explored
enzyme for these purposes with most of the work in this area having been
done by Jones and coworkers.[105] HLADH has been studied sufficiently
thoroughly to allow modeling of the active site.[106] Cis-3-(2-hydroxy-
ethyl)cyclopentanol 15 was oxidized by HLADH to (+)3S-(2-hydroxyethyl)
cyclopentanone 16 with 97% ee; the remaining diol had 70% ee.[107]
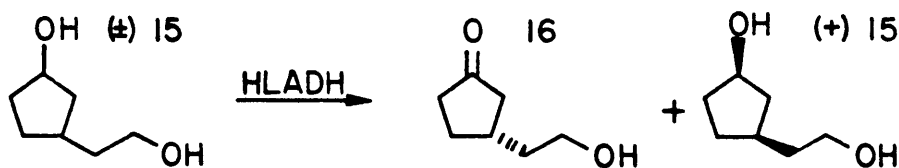
2-Substituted tetrahydropyran-4-ones were reduced to (-)trans alcohols
with 100% ee.[108] Remaining (+)ketones had 51-86% ee. HLADH-catalyzed
oxidation of monocyclic mesodiols 17 proceeds to bicyclic lactones 18 in
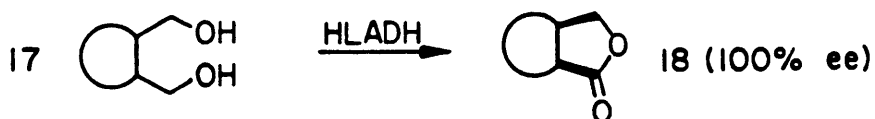
high yield with complete stereotopic selectivity.[109] Essentially no
selectivity is seen in oxidation of the trans diols. Cis-decalin-2,7-
dione 19 is reduced specifically to (-)(7S,9S,10R)-7-hydroxy-cis-dec-
alin-2-one 20[110,111] which can be converted to (+)4R-twistanone 21 in
51% yield from the dione. D- and L-lactic dehydrogenases reduce
chloropyruvic acid 22 to D- and L-chlorolactic acids 23; these can be
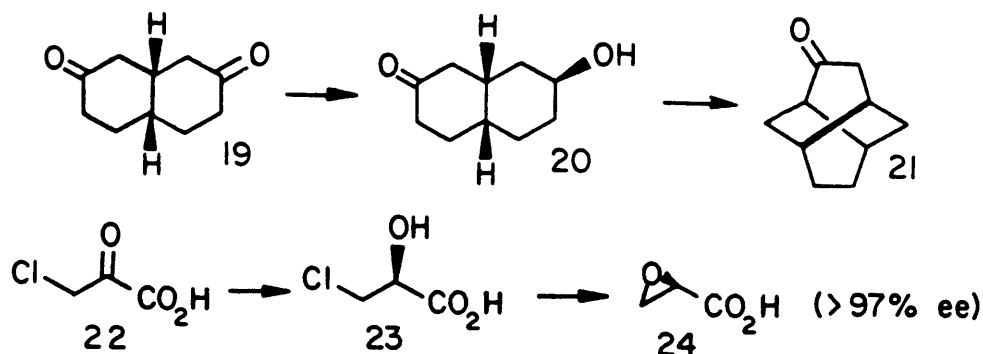
converted to epoxyacrylic acids 24.[112] Progesterone has been reduced to 20-β-hydroxy-pregn-4-ene-3-one with 20-β-hydroxysteroid dehydrogenase using reversed micelles in organic solvent and H_2 as ultimate reductant.[113] Microbial reductions have been used in intermediate steps in syntheses of natural brefeldin-A,[114] (+)compactin[115] and L-carnitine.[116] L-leucine dehydrogenase has been used in the reductive amination of α-ketoisocaproate to L-leucine[18] in a membrane reactor. NAD was covalently modified by attachment of polyethylene glycol to make it unable to cross the membrane.

Multi-Enzyme Cofactor Requiring Processes — A more complex level of applied enzymology is reached in the use of multi-enzyme schemes to synthesize complex molecules. Examples of such syntheses include ribulose-1,5-diphosphate,[36] important in the study of ribulose-diphosphate carboxylase; lactosamine,[117] from the first use of the Leloir pathway enzymes in synthesis; and S-adenosyl-L-methionine.[47]

Oxidations — Enzymes which functionalize inactivated carbon are often difficult to obtain and handle. Many examples exist of oxidations using microbial fermentations,[118] e.g. for steroids, and recently in olefin oxidation.[119] Such preparative transformations have not been achieved with purified enzymes and are unlikely to be amenable to large-scale in vitro approaches because of instability and complexity of the enzyme systems. Klibanov and coworkers, however, have developed systems with horseradish peroxidase[120,121] and xanthine oxidase[122] for oxidation of aromatic alcohols and amines, for use in syntheses and waste water treatment. Hydroxyphenyl compounds can be oxidized to dihydroxy derivatives. L-DOPA has been made from L-tyrosine in this manner.[123] Cyclohexanone was oxidized to ϵ-caprolactone with a bacterial oxygenase.[124]

The Future — Enzymic synthetic methods will see increased use in research and industry. Numerous examples exist of preparations of useful quantities of chiral compounds for use in synthesis, and the use of hydrolytic enzymes for simple synthesis. More importantly, enzymes will allow the facile synthesis of complex molecules important in biological research. Immunology, neurobiology, endocrinology, molecular genetics, membrane biology, and plant and insect biology are areas becoming more molecular in scope. Research in such fields will increasingly depend on biologically active compounds not readily accessible by more conventional chemistry. Molecules that are water soluble, or highly functional-

ized such as carbohydrates, nucleic acids, lipids, and proteins may prove available by enzymic synthetic methods. Enzymology will be useful in modifications of poly- and oligosaccharides and proteins. Enzymes will also see growth in applications in medical diagnostics and treatment, and in food chemistry.[125]

Recombinant DNA and RNA methods rely on enzymes, and as these methods are developed, the opportunity for enzyme engineering of synthetic catalysts will grow.[126,127] Enzyme-based synthetic methods will be an important part of future organic synthesis, especially in synthesis of new pharmaceutical products.

References

1.   G.M. Whitesides and C.-H. Wong, Aldrichimica Acta, 16, 27 (1983).
2.   J.B. Jones in "Asymmetric Synthesis," J.D. Morrison, Ed., Academic Press, New York, N.Y., 1983.
3.   C.J. Suckling and H.C.S. Wood, Chem. Brit., 15, 243 (1979).
4.   J.B. Jones, C.J. Sih and D. Perlman, Eds., "Applications of Biochemical Systems in Organic Chemistry," Tech. Chem., 10, Wiley, New York, N.Y., 1976.
5.   J.B. Jones, Method. Enzymol., 44, 831 (1976).
6.   C.J. Sih, E. Abushanab and J.B. Jones, Annu. Repts. Med. Chem., 12, 298 (1977).
7.   International Union of Biochemistry. Nomenclature Committee. "Enzyme Nomenclature, 1978," Academic Press, New York, N.Y. (1979).
8.   B.S. Hartley, T. Atkinson and M.D. Lilly, Eds., Phil. Trans. R. Soc. Lond., B 300, 237 (1983).
9.   M.R. Kula, K.H. Kroner and H. Hustedt. Adv. Biochem. Eng., 24, 73 (1982).
10. P.-A. Albertsson, Adv. Protein Chem., 24, 309 (1970).
11. P.-A. Albertsson, "Partition of Cell Particles and Macromolecules," 2nd ed., Almquist and Wiksell, Stockholm, Wiley-Interscience, N.Y., 1971.
12. I. Chibata, "Immobilized Enzymes--Research and Development." Halsted Press, New York, N.Y., 1978.
13. K. Mosbach, Ed., "Immobilized Enzymes," Method. Enzymol., 44 (1976).
14. A.M. Klibanov, Science, 219, 722 (1983).
15. R.P. Rohrbach, U.S. 4,268,419 (1979).
16. L.L. Wood, F.J. Hartdegen, P.A. Hahn, U.S. 4,312,946 (1982).
17. A. Pollak, H. Blumenfeld, M. Wax, R.L. Baughn and G.M. Whitesides, J. Am. Chem. Soc., 102, 6324 (1980).
18. C. Wandrey, A.F. Buckmann and M.R. Kula, Biotechnol. Bioeng., 23, 2789 (1981).
19. A.M. Klibanov, Anal. Biochem., 93, 1 (1979).
20. K. Martinek, A.M. Klibanov, V.S. Goldmacher and I.V. Berezin, Biochim. Biophys. Acta, 485, 1 (1977).
21. K. Martinek, A.M. Klibanov, V.S. Goldmacher, A.V. Tchernysheva, V.V. Mozhaev, I.V. Berezin and B.O. Glotov, Biochim. Biophys. Acta, 485, 13 (1977).
22. A.M. Klibanov, N.O. Kaplan and M.D. Kamen, Arch. Biochem. Biophys., 199, 545 (1980).
23. R.D. Schmid, Adv. Biochem. Eng., 12, 41 (1979).
24. A.M. Klibanov, Biochem. Soc. Trans., 11, 19 (1983).
25. A.M. Klibanov, N.O. Kaplan and M.D. Kamen, Enzyme Eng., 5, 135 (1980).
26. A.M. Klibanov, N.O. Kaplan and M.D. Kamen, Proc. Nat. Acad. Sci. U.S.A., 75, 3640 (1978).
27. A.M. Klibanov, N.O. Kaplan and M.D. Kamen, Biochim. Biophys. Acta, 547, 411 (1979).
28. J.B. Jones and D.R. Dodds, Can. J. Chem., 57, 2533 (1979).
29. S. Branner-Jørgensen, Biochem. Soc. Trans., 11, 20 (1983).
30. C. Bucke, Biochem. Soc. Trans., 11, 13 (1983).
31. K. Gekko and S.N. Timasheff, Biochem., 20, 4667; 4677 (1981).
32. T. Arakawa and S.N. Timasheff, Biochem., 21, 6536; 6545 (1982).
33. A.M. Klibanov and V.V. Mozhaev, Biochem. Biophys. Res. Comm., 83, 1012 (1978).
34. C.-H. Wong, S.L. Haynie and G.M. Whitesides, J. Am. Chem. Soc., 105, 115 (1983).
35. B.L. Hirschbein, F.P. Mazenod and G.M. Whitesides, J. Org. Chem., 47, 3765 (1982).
36. C.-H. Wong, A. Pollak, S.D. McCurry, J.M. Sue, J.R. Knowles and G.M. Whitesides, Method. Enzymol., 89, 108 (1982).
37. D. Crans and G.M. Whitesides, J. Org. Chem., 48, 3130 (1983).
38. R.L. Baugh, O. Adalsteinsson, and G.M. Whitesides, J. Am Chem. Soc., 100, 304 (1978).

39. J.B. Jones and K.E. Taylor, Can. J. Chem., 55, 2969 (1976).
40. C.-H. Wong and G.M. Whitesides, J. Am. Chem. Soc., 104, 3542 (1982).
41. C.-H. Wong and G.M. Whitesides, J. Org. Chem., 47, 2816 (1982).
42. C.-H. Wong and G.M. Whitesides, J. Am. Chem. Soc., 103, 4890 (1981).
43. C. Burstein, H. Ounissi, M.D. Legoy, G. Gellf and D. Thomas, Appl. Biochem.
     Biotechnol., 6, 329 (1981).
44. E. Chave, E. Adamowicz and C. Burstein, Appl. Biochem. Biotechnol., 7, 431 (1982).
45. C.-H. Wong and G.M. Whitesides, J. Am. Chem. Soc., 105, 5012 (1983).
46. Z. Shaked and G.M. Whitesides, J. Am. Chem. Soc., 102, 7104 (1980).
47. A. Gross, S. Geresh and G.M. Whitesides, Appl. Biochem. Biotechnol., 8, 415 (1982).
48. H. Schutte, M.R. Kula and H. Sahm, Method. Enzymol., 89, 527 (1982).
49. K. Martinek and A.N. Semenov, J. Appl. Biochem., 3, 93 (1981).
50. L.G. Butler, Enzyme Microb. Technol., 1, 253 (1979).
51. J.B. Jones and H.M. Schwartz, Can. J. Chem., 60, 335 (1982).
52. J.B. Jones and D.H. Pliura, Can. J. Chem., 59, 2921 (1981).
53. J.B. Jones and M.M. Mehes, Can. J. Chem., 57, 2245 (1979).
54. G.A. Homandberg, J.A. Mattis and M. Laskowski, Jr., Biochem., 17, 5220 (1978).
55. K. Martinek, A.N. Semenov and I.V. Berezin, Biochim. Biophys. Acta, 658, 76 (1981).
56. K. Martinek and A.N. Semenov, Biochim. Biophys. Acta, 658, 90 (1981).
57. K. Martinek, A.N. Semenov and I.V. Berezin, Dokl. Akad. Nauk. SSSR, 253, 358 (1980).
58. K. Martinek, A.N. Semenov and I.V. Berezin, Dokl. Akad. Nauk. SSSR, 252, 394 (1980).
59. A.M. Klibanov, G.P. Samokhin, K. Martinek and I.V. Berezin, Biotechnol. Bioeng., 19,
     1351 (1977).
60. B.J. Abbott, Adv. Appl. Microbiol., 20, 203 (1976).
61. E. Lagerlof, L. Nathorst-Westfelt, B. Ekstrom and B. Sjoberg, Method. Enzymol., 44,
     759 (1976).
62. I. Chibata, T. Tosa and T. Sato, Method. Enzymol., 44, 739 (1976).
63. I. Chibata, T. Tosa and I. Takata, Trends. Biotechnol., 1, 9 (1983).
64. M.K. Weibel, W.H. McMullen and C.A. Starace, Dev. Ind. Microbiol., 19, 103 (1977).
65. N.B. Havewala and W.H. Pitcher, Jr., Enzyme Eng., 2, 315 (1974).
66. N.H. Mermelstein, Food Technol., 29, 20 (1975).
67. T. Kitahara and S. Asai, Agric. Biol. Chem., 47, 991 (1983).
68. Y. Minematsu, Y. Shimohigashi, M. Waki and N. Izumiya, Bull. Chem. Soc. Jap., 51,
     1899 (1978).
69. A.N. Semenov, K. Martinek, V. Yu.-K. Shvyadas, A.L. Margolin and I.V. Berezin, Dokl.
     Akad. Nauk. SSSR, 258, 1124 (1981).
70. C.-S. Chen, Y. Fujimoto, G. Girdaukas and C.J. Sih, J. Am. Chem. Soc., 104, 7294
     (1982).
71. P. Mohr, N. Waespe-Sarcevic, C. Tamm, K. Gawronska and J.K. Gawronska, Helv. Chim.
     Acta, 66, 2501 (1983).
72. S. Iriuchijima, K. Hasegawa and G. Tsuchihashi, Agric. Biol. Chem., 46, 1907 (1982).
73. C.-S. Chen, Y. Fujimoto, C.J. Sih, J. Am. Chem. Soc., 103, 3580 (1981).
74. M. Ohno, S. Kobayashi, T. Iimori, Y.-F. Wang and T. Izawa, J. Am. Chem. Soc., 103,
     2405 (1981).
75. W.K. Wilson, S.B. Baca, Y.J. Barber, T.J. Scallen and C.J. Morrow, J. Org. Chem.,
     48, 3960 (1983).
76. M. Schneider, N. Engel, and H. Boensmann, Angew. Chem. Int. Ed. Engl., 23, 64
     (1984).
77. M. Schneider, N. Engel, and H. Boensmann, Angew. Chem. Int. Ed. Engl., 23, 66
     (1984).
78. M. Schneider, N. Engel, P. Honicke, G. Heineman and H. Gorisch, Angew. Chem. Int.
     Ed. Engl., 23, 67 (1984).
79. B. Cambou and A.M. Klibanov, J. Am. Chem. Soc., 106, in press (1984).
80. S. Iriuchijima and N. Kojima, Agric. Biol. Chem., 46, 1153 (1982).
81. S. Iriuchijima, A. Keiyu and N. Kojima, Agric. Biol. Chem., 46, 1593 (1982).
82. S. Iriuchijima and N. Kojima, J.C.S. Chem. Comm., 185 (1981).
83. J. Lavayre, J. Verrier and J. Baratti, Biotechnol. Bioeng., 24 2175 (1982).
84. E.P. Dropsy and A.M. Klibanov, Biotechnol. Bioeng. 26, in press (1984).
85. J.S. Fruton, Adv. Enzymol., 53, 239 (1982).
86. I.M. Chaiken, A. Komoriya, M. Ohno and F. Widmer, Appl. Biochem. Biotechnol. 7, 385
     (1982).
87. D. Tarquis, P. Monsan and G. Durand, Bull. Soc. Chim. Fr. II., 76 (1980).
88. Y. Nishida, H. Ohrui and H. Meguro, Agric. Biol. Chem., 47, 2123 (1983).
89. K. Morihara, T. Oka, H. Tsuzuki, Y. Tochino and T. Kanaya, Biochem. Biophys. Res.
     Comm., 92, 396 (1980).
90. K. Inouye, K. Watanabe, K. Morihara, Y. Tochino, T. Kanaya, J. Emura and S. Sakaki-
     bara, J. Am. Chem. Soc., 101, 751 (1979).
91. W. Kullman, J. Org. Chem., 47, 5300 (1982).
92. K. Oyama, S. Nishimura, Y. Nonaka, K. Kihara and T. Hashimoto, J. Org. Chem., 46,
     5241 (1981).

93. P.D. Boyer, Ed., "The Enzymes," 7, Chapters 6-9 (1972).
94. C.-H. Wong and G.M. Whitesides, J. Org. Chem., 48, 3199 (1983).
95. C.-H. Wong, F.P. Mazenod and G.M. Whitesides, J. Org. Chem. 48, 3493 (1983).
96. T. Koyama, A. Saito, K. Ogura and S. Seto, J. Am. Chem. Soc., 102, 3614 (1980).
97. H. Fujii, T. Koyama, K. Ogura, Biochem. Biophys. Acta, 712, 716 (1982).
98. G. Pelsy, A.M. Klibanov, Biotechnol. Bioeng., 25, 919 (1983).
99. D.R. Walt, M.A. Findeis, V.M. Rios-Mercadillo, J. Augé and G.M. Whitesides, J. Am. Chem. Soc., 106, 234 (1984).
100. S. Shimizu, K. Yamane, Y. Tani and H. Yamada, Appl. Biochem. Biotechnol., 8, 237 (1983).
101. A. Pollak, R.L. Baughn and G.M. Whitesides, J. Am. Chem. Soc., 99, 2366 (1977).
102. V.M. Rios-Mercadillo and G.M. Whitesides, J. Am. Chem. Soc., 101, 5828 (1979).
103. Y.-S. Shih and G.M. Whitesides, J. Org. Chem., 42, 4165 (1977).
104. A. Gross, O. Abril, J.M. Lewis, S. Geresh and G.M. Whitesides, J. Am. Chem. Soc., 105, 7428 (1983).
105. T. Takemura and J.B. Jones, J. Org. Chem., 48, 791 (1983), and previous papers.
106. J.B. Jones and I.J. Jakovac, Can. J. Chem., 60, 19 (1982).
107. A.J. Irwin and J.B. Jones, J. Am. Chem. Soc., 99, 1625 (1977).
108. J.A. Haslegrave and J.B. Jones, J. Am. Chem. Soc., 104, 4666 (1982).
109. I.J. Jacovac, H.B. Goodbrand, K.P. Lok and J.B. Jones, J. Am. Chem. Soc., 104, 4659 (1982).
110. D.R. Dodds and J.B. Jones, J.C.S. Chem. Comm., 1080 (1982).
111. M. Nakazaki, H. Chikamatsu and M. Taniguchi, Chem. Lett., 11, 1761 (1982).
112. B.L. Hirschbein and G.M. Whitesides, J. Am. Chem. Soc., 104, 4458 (1982).
113. R. Hilhorst, C. Laane and C. Veeger, FEBS Lett., 159, 225 (1983).
114. C. LeDrian and A.E. Greene, J. Am. Chem. Soc., 104, 5473 (1982).
115. C.T. Hsu, N.Y. Wang, L.H. Latimer and C.J. Sih, J. Am. Chem. Soc., 105, 593 (1983).
116. B. Zhou, A.S. Gopalan, F. Vanmiddlesworth, W.-R. Shieh and C.J. Sih, J. Am. Chem. Soc., 105, 5925 (1983).
117. C.-H. Wong, S.L. Haynie and G.M. Whitesides, J. Org. Chem., 47, 5416 (1982).
118. K. Kieslich, "Microbial Transformations of Non-Steroid Cyclic Compounds," John Wiley & Sons, New York, N.Y., 1976.
119. H. Ohta and H. Tetsukawa, J.C.S. Chem. Comm., 849 (1978).
120. A.M. Klibanov, B.N. Alberti, E.D. Morris and L.M. Felshin, J. Appl. Biochem., 2, 414 (1980).
121. A.M. Klibanov and E.D. Morris, Enzyme Microb. Technol., 3, 119 (1981).
122. G. Pelsy and A.M. Klibanov, Biochim. Biophys. Acta, 742, 352 (1983).
123. A.M. Klibanov, Z. Berman and B.N. Alberti, J. Am. Chem. Soc., 103, 6263 (1981).
124. J.M. Schwab, J. Am. Chem. Soc., 103, 1876 (1981).
125. H. Samejima, K. Kimura and Y. Ado, Biochemie, 62, 299 (1980).
126. W.H. Rastetter, Appl. Biochem. Biotechnol., 8, 423 (1983).
127. T.H. Maugh, II, Science, 223, 154 (1984).