Asymmetric Transformations Catalyzed by Enzymes in Organic Solvents

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Received August 7, 1989 (Revised Manuscript Received January 17, 1990)

Introduction

The immense potential of enzymes as catalysts in organic synthesis is well documented.[1-5] Nevertheless, organic chemists have been reluctant to add enzymes to their standard arsenal of synthetic methods. There are several objective, as well as subjective, reasons for this resistance.[6,7] Among the most frequently voiced complaints about enzymes is the perceived necessity to work with them in aqueous solutions, which goes against the everyday rules and customs of organic synthesis. The vast majority of conventional synthetic procedures employ nonaqueous reaction media, and with sound justifications. For example, most compounds of interest to organic chemists are insoluble in water. Also, water often promotes unwanted side reactions and decomposes a number of common organic reagents. Finally, the thermodynamic equilibria of many processes are unfavorable in water, and the latter is far from an ideal milieu for product recovery.

Since synthetic chemists were not about to change their habits, enzyme chemists had to change theirs. Recent research in this direction has resulted in an astonishing realization that many (if not all) enzymes can work in organic solvents containing little or no added water.[8-14] Specifically, when an enzyme powder is placed in an organic solvent, it displays acceptable catalytic activity, especially if certain rules and guidelines are followed:[14] (i) hydrophobic solvents are preferable to hydrophilic ones (although some enzymes remain catalytically active even in the most hydrophilic organic solvents); (ii) some enzymes require exogenous water addition to the dry organic solvent; (iii) enzyme powders are prepared (e.g., freeze-dried) from aqueous solutions at the pH optimal for enzymatic activity; and (iv) since enzymes are insoluble in nearly all organic solvents, the enzyme particles should be sufficiently small and their suspensions should be continuously agitated in order to minimize diffusional limitations.

The realization that enzymes can function in nonaqueous media has eliminated many misgivings about using enzymes as practical catalysts. In addition to expanding the range of reactions feasible via biocatalysis, enzymatic catalysis in organic solvents has revealed some unexpected and beneficial phenomena. For instance, the stability of enzymes in nonaqueous media may be greatly enhanced compared to that in water.[15,16] Substrate specificity of enzymes can be regulated by the solvent.[17,18] Even the enzyme property of particular interest to organic chemists, enantioselectivity, can be controlled by the solvent.[19,20]

The synthetic potential of enzymes in organic solvents has been quickly recognized, and the number of publications on this topic has surged over the last few years. Most of these studies have understandably involved asymmetric conversions, one of the central themes of modern organic synthesis.[21,22] The present Account constitutes an attempt to review and analyze this research area. We will limit our discussion to preparative asymmetric transformations catalyzed by enzymes in organic solvents that either are anhydrous (i.e., contain less than some 0.01% of water) or contain up to a few percent of added water. The term "asymmetric" is used in a broad sense, i.e., encompassing stereoselective and stereospecific,[23] as well as regioselective, reactions.

Asymmetric (Trans)esterifications Catalyzed by Hydrolytic Enzymes

Most of the enzymes used as catalysts of preparative conversions in organic solvents to date have been hydrolases, namely, lipases and proteases. In addition to their catalytic versatility, these enzymes are readily available commercially in sizable quantities and at relatively low cost. For example, one can purchase some 20 lipases isolated from different organisms; even

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(1) Whitesides, G. M.; Wong, C.-H. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 617-638.
(2) Simon, H.; Bader, J.; Gunther, H.; Neumann, S.; Thanos, J. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 539-553.
(3) Jones, J. B. *Tetrahedron* **1986**, *42*, 3351-3403.
(4) Yamada, H.; Shimizu, S. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 622-642.
(5) Wong, C.-H. *Science* **1989**, *244*, 1145-1152.
(6) Klibanov, A. M. *Science* **1983**, *219*, 722-727.
(7) Akiyama, A.; Bednarski, M.; Kim, M.-J.; Simon, E. S.; Waldmann, H.; Whitesides, G. M. *CHEMTECH* **1988**, *18*, 627-634.
(8) Klibanov, A. M. *CHEMTECH* **1986**, *16*, 354-359.
(9) Laane, C.; Tramper, J.; Lilly, M. D., Eds. *Biocatalysis in Organic Media*; Elsevier: Amsterdam, 1986.
(10) Halling, P. J. *Biotechnol. Adv.* **1987**, *5*, 47-84.
(11) Zaks, A.; Russell, A. J. *J. Biotechnol.* **1988**, *8*, 259-270.
(12) Deetz, J. S.; Rozzell, J. D. *Trends Biotechnol.* **1988**, *6*, 15-19.
(13) Dordick, J. S. *Enzyme Microb. Technol.* **1989**, *11*, 194-211.
(14) Klibanov, A. M. *Trends Biochem. Sci.* **1989**, *14*, 141-144.
(15) Zaks, A.; Klibanov, A. M. *Science* **1984**, *224*, 1249-1251.
(16) Aldercreutz, P.; Mattiasson, B. *Biocatalysis* **1987**, *1*, 99-108.
(17) Zaks, A.; Klibanov, A. M. *J. Am. Chem. Soc.* **1986**, *108*, 2767-2768.
(18) Russell, A. J.; Klibanov, A. M. *J. Biol. Chem.* **1988**, *263*, 11624-11626.
(19) Sakurai, T.; Margolin, A. L.; Russell, A. J.; Klibanov, A. M. *J. Am. Chem. Soc.* **1988**, *110*, 7236-7237.
(20) Kitaguchi, H.; Fitzpatrick, P. A.; Huber, J. E.; Klibanov, A. M. *J. Am. Chem. Soc.* **1989**, *111*, 3094-3095.
(21) Eliel, E. L., Otsuka, S., Eds. *Asymmetric Reactions and Processes in Chemistry*; American Chemical Society: Washington, DC, 1982.
(22) Morrison, J. D., Ed. *Asymmetric Synthesis*; Academic Press: New York, 1983-1985.
(23) Eliel, E. L. *Stereochemistry of Carbon Compounds*; McGraw-Hill: New York, 1962; p 436.

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though all of them are called lipases and catalyze the same reactions, their specificities are distinct. One-half kilogram of porcine pancreatic lipase can be obtained from Sigma Chemical Co. for less than $35; although this enzyme is very crude, it has proved to be quite suitable as a practical catalyst. A number of other lipases can be bought in 10- and 100-g amounts for under $100. Therefore organic chemists need not get involved in fermentation, isolation, and purification of enzymes nor must they ruin their laboratories' budgets. Sufficient supplies of various lipases can be acquired from commercial suppliers and then screened for desired specificities.

The ability of lipases and proteases to catalyze the asymmetric hydrolysis of chiral esters in water has been profitably exploited for years.[1,3,4] If one wants to resolve a racemic alcohol or acid, one chemically esterifies it and then finds a hydrolase to cleave this ester with the "right" stereoselectivity (ideally, reacting with only one enantiomer). The general mechanism of such reactions is depicted in eq 1:

$$E + RCOOR' \rightleftharpoons E\cdot RCOOR' \xrightarrow[R'OH]{} RCOOE \xrightarrow{H_2O} E + RCOOH \quad (1)$$

where E is the hydrolytic enzyme, RCOOR′ is the hydrolyzable ester, E·RCOOR′ is the noncovalent enzyme–substrate complex (the Michaelis complex), and RCOOE is the covalent acyl–enzyme intermediate. If the hydrolase reacts with only one enantiomer of the ester RCOOR′, then once a 50% conversion is reached, this enantiomer is completely hydrolyzed to the alcohol and acid, while the unreacted enantiomer remains in the ester form. Even if the enantiodiscrimination is not absolute (i.e., both enantiomers are hydrolyzed by the enzyme, albeit with different rates), a partial resolution is still achieved, with the optical purity dependent on the degree of conversion.[24]

The described approach to the enzymatic resolution of racemates is not without drawbacks, e.g., the necessity to initially convert the racemic target molecule to an ester, the insolubility of most such esters in water, the sensitivity of many compounds to water, and so on. Hence, it was desirable to devise an alternative strategy.

The acyl–enzyme intermediate in eq 1 is formed via the acylation of the active center's nucleophile (usually the strongly activated hydroxyl group of a serine residue) by the acyl moiety of the substrate ester.[25] In water, the acyl–enzyme is subsequently hydrolyzed, thereby regenerating the free enzyme and producing the acid. In principle, other nucleophiles may compete with water for RCOOE, but in aqueous solutions, hydrolysis prevails. On the other hand, if organic solvents are used as the reaction media, then the acyl–enzyme can be exposed to any nucleophile without competition from water, and therefore, hydrolysis can be replaced by a number of alternative (and, in terms of absolute rates, slower) reactions such as transesterification, aminolysis, thiotransesterification, oximolysis, etc.[26] Consequently, novel means for enzymatic resolutions of racemates become feasible, as will be discussed below.

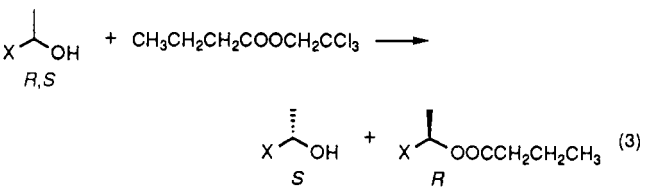
Resolution of Racemic Alcohols. We proposed[27] to use the following scheme (transesterification) for enzymatic resolution of racemic alcohols in nonaqueous media:

$$R_1COOR_2 + (\pm)\text{-R*OH} \xrightarrow{\text{hydrolase}} \text{optically active } (R_1COOR^* + R^*OH) \quad (2)$$

where R_1COOR_2 is an achiral ester and (±)-R*OH is a chiral, racemic alcohol. In this case, water plays no role in the process and can be omitted. In addition, the alcohol does not need to be esterified prior to the enzymatic reaction, thereby saving an extra step. Pig liver carboxyl esterase and yeast (*Candida cylindracea*) lipase were then employed as asymmetric catalysts for the resolution of numerous primary and secondary chiral alcohols.[27]

The transesterification-based enzymatic resolutions of racemic alcohols depicted in eq 2 can be carried out either in biphasic, aqueous–organic (predominantly organic) mixtures[27-29] or in organic solvents.[30] The latter mode appears most preferable because it is so easy to use (a powdered enzyme, free or immobilized, is simply added to a solvent containing the dissolved racemic alcohol and ester; the latter can be also used as the solvent[15]) and has been employed by most investigators.

In the first study of this type,[30] several racemic secondary alcohols were resolved in anhydrous ether or heptane by using crude porcine pancreatic lipase, as exemplified in eq 3:



(3)

where X = hexyl, decyl, tetradecyl, 2-methyl-2-hydroxypropyl, or phenyl. The *S* alcohols and *R* esters, formed enzymatically and separated by conventional means, were found to have enantiomeric excess (ee) in the range 90–100%. The utility of this procedure was illutrated by Theisen and Heathcock[31] in the production of multigram quantities of optically pure *R* and *S* isomers of 1-(1′-naphthyl)ethanol, subsequently employed to prepare useful synthons, such as optically active monoesters of 3-hydroxyglutarate and related compounds.

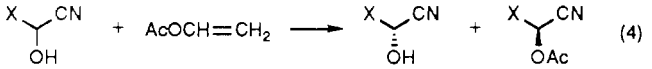
Reaction 3 was used by Veschambre and co-workers[32] for the resolution of racemic sulcatol (6-methylhept-5-en-2-ol) to form the biologically active *S* enantiomer (the aggregation pheromone of an ambrosia beetle). The same synthetic objective was achieved by Stokes and Oehlschlager[33] by using 2,2,2-trifluoroethyl laurate

(24) Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.
(25) Fersht, A. *Enzyme Structure and Mechanism*; Freeman: New York, 1985.
(26) Zaks, A.; Klibanov, A. M. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 3192–3196.
(27) Cambou, B.; Klibanov, A. M. *J. Am. Chem. Soc.* **1984**, *106*, 2687–2692.
(28) Cambou, B.; Klibanov, A. M. *Biotechnol. Bioeng.* **1984**, *26*, 1449–1454.
(29) Chen, C.-S.; Wu, S.-H.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1987**, *109*, 2812–2817.
(30) Kirchner, G.; Scollar, M. P.; Klibanov, A. M. *J. Am. Chem. Soc.* **1985**, *107*, 7072–7076.
(31) Theisen, P. D.; Heathcock, C. H. *J. Org. Chem.* **1988**, *53*, 2374–2378.
(32) Belan, A.; Bolte, J.; Fauve, A.; Gourcy, J. G.; Veschambre, H. *J. Org. Chem.* **1987**, *52*, 256–260.

as the acylating ester. These authors also made the intriguing observation that the enantioselectivity of porcine pancreatic lipase is affected by the water content of the enzyme powder.

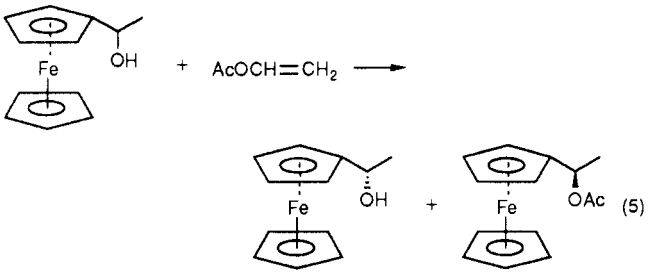
In the studies described above, racemic alcohols were enzymatically acylated with activated esters (trichloroethyl or trifluoroethyl) of carboxylic acids. Such esters are advantageous over nonactivated ones (e.g., methyl) because they are more reactive and also the thermodynamic equilibrium in eq 2 is shifted to the right.[30] The latter task can be accomplished by other means as well. For example, Bianchi et al.[34] asymmetrically acylated racemic alcohols with anhydrides of short-chained carboxylic acids (from acetic to butyric). A number of primary and secondary alcohols were obtained in high optical purity by using *Pseudomonas fluorescens* lipase as a catalyst in anhydrous benzene. Abramowicz and Keese[35] resolved racemic 2-butanol with *C. cylindracea* lipase using diphenyl carbonate as the acylating agent.

Another convenient way to make an enzymatic transesterification essentially irreversible in organic solvents is to employ enol esters as the acylating agent:[36] the leaving groups released from such esters tautomerize to aldehydes or ketones, thus shifting the equilibrium in reaction 2 completely to the right. Wong and coworkers have used this approach for a number of stereoselective transformations.[37-39] For instance, cyanohydrins were efficiently resolved by using *Pseudomonas* lipase as an asymmetric catalyst in anhydrous dichloromethane, as depicted in the following equation:[39]



(4)

where X = $NaphOCH_2$, $PhCH_2$, or $PhCH_2OCH_2$. After the enzymatic resolution, the first compound was chemically converted to *S*-(−)-propranolol, a β-adrenergic blocking agent. The same process has been successfully utilized for the production of many other optically active secondary alcohols.[37,40,41] An indicative example is the enzymatic resolution of 1-ferrocenylethanol:



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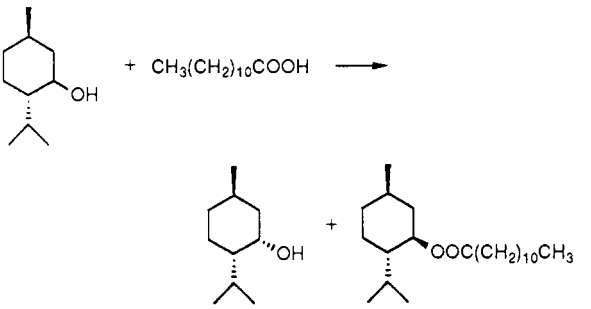
(33) Stokes, T. M.; Oehlschlager, A. C. *Tetrahedron Lett.* **1987**, *28*, 2091–2094.
(34) Bianchi, D.; Cesti, P.; Battistel, E. *J. Org. Chem.* **1988**, *53*, 5531–5534.
(35) Abramowics, D. A.; Keese, C. R. *Biotechnol. Bioeng.* **1989**, *33*, 149–156.
(36) Degueil-Castang, M.; DeJesco, B.; Drouillard, S.; Maillard, B. *Tetrahedron Lett.* **1987**, *28*, 953–954.
(37) Wang, Y.-F.; Lalonde, J. J.; Momogan, M.; Bergbreiter, D. E.; Wong, C.-H. *J. Am. Chem. Soc.* **1988**, *110*, 7200–7205.
(38) Wang, Y.-F.; Wong, C.-H. *J. Org. Chem.* **1988**, *53*, 3129–3130.
(39) Wang, Y.-F.; Chen, S.-T.; Liu, K. K.-C.; Wong, C.-H. *Tetrahedron Lett.* **1989**, *30*, 1917–1920.
(40) Laumen, K.; Breitgoff, D.; Schneider, M. P. *J. Chem. Soc., Chem. Commun.* **1988**, 1459–1461.
(41) Nishio, T.; Kamimura, M.; Murata, M.; Terao, Y.; Achiwa, K. *J. Biochem.* **1989**, *105*, 510–512.

This organometallic compound is decomposed by water, making the conventional methodology (eq 1) impossible; in contrast, enzymatic transesterification in benzene[37] or *tert*-butyl methyl ether[42] results in facile resolutions.

Racemic alcohols can also be resolved via lipase-catalyzed asymmetric acylation with carboxylic acids[43,44] rather than with the esters thereof. Langrand et al.[43] employed this approach for the resolution of numerous alicyclic secondary alcohols, as illustrated for menthol in eq 6, using *C. cylindracea* lipase in heptane. This



(6)

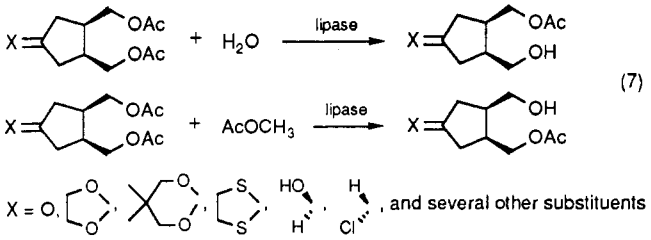
important flavorant and other terpenes have proven to be popular targets for enzymatic resolutions via esterifications in organic solvents.[43-46] In addition, the production of optically active acyclic secondary alcohols[47,48] and primary allenic alcohols[49] has been reported. However, the transesterifications (see also Bevinakatti et al.[50]) seem to be more versatile than direct esterifications in terms of reaction rates, flexibility, and thermodynamic limitations.

Enzymatic resolutions in organic solvents can be extended to polyfunctional compounds: e.g., racemic amino alcohols have been resolved by using porcine pancreatic lipase in ethyl acetate (serving both as a solvent and as an acyl donor) with[51] or without[52] prior protection of the NH_2 group.

Note that enzymatic hydrolysis in water and transesterification in organic solvents yield complementary stereoisomers. Indeed, suppose the *S* enantiomer of a chiral alcohol is much more reactive with a given lipase than its *R* counterpart. In the hydrolysis reaction (eq 1), the *S* alcohol and *R* ester will form, whereas in the transesterification reaction (eq 2), the *S* ester and *R* alcohol will result (assuming the same enantioselectivity in water and organic solvents[30]). This point was elegantly illustrated in the lipase-catalyzed asymmetric acylation of cycloalkanediols.[53,54] For instance, monoesters of *meso*-diols of opposite absolute configura-

(42) Boaz, N. W. *Tetrahedron Lett.* **1989**, *30*, 2061–2064.
(43) Langrand, G.; Secchi, M.; Buono, G.; Baratti, J.; Triantaphylides, C. *Tetrahedron Lett.* **1985**, *26*, 1857–1860.
(44) Langrand, G.; Baratti, J.; Buono, G.; Triantaphylides, C. *Tetrahedron Lett.* **1986**, *27*, 29–32.
(45) Koshiro, S.; Sonomoto, K.; Tanaka, A.; Fukui, S. *J. Biotechnol.* **1985**, *2*, 47–57.
(46) Inagaki, T.; Ueda, H. *Agric. Biol. Chem.* **1987**, *51*, 1345–1348.
(47) Sonnet, P. E. *J. Org. Chem.* **1987**, *52*, 3477–3479.
(48) Okahata, Y.; Fujimoto, Y.; Ijiro, K. *Tetrahedron Lett.* **1988**, *29*, 5133–5134.
(49) Gil, G.; Ferre, E.; Meou, A.; Le Petit, J.; Triantaphylides, C. *Tetrahedron Lett.* **1987**, *28*, 1647–1648.
(50) Bevinakatti, H. S.; Banerji, A. A.; Newadkar, R. V. *J. Org. Chem.* **1989**, *54*, 2453–2455.
(51) Francalanci, F.; Cesti, P.; Gabri, W.; Bianchi, D.; Martinengo, T.; Foa, M. *J. Org. Chem.* **1987**, *52*, 5079–5082.
(52) Gotor, V.; Brieva, R.; Rebolledo, F. *J. Chem. Soc., Chem. Commun.* **1988**, 957–958.
(53) Hemmerle, H.; Gais, H.-J. *Tetrahedron Lett.* **1987**, *28*, 3471–3474.
(54) Ader, U.; Breitgoff, D.; Kelin, P.; Laumen, K. E.; Schneider, M. P. *Tetrahedron Lett.* **1989**, *30*, 1793–1796.

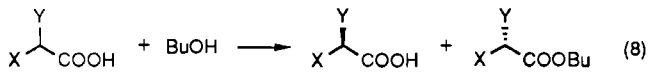
tions were prepared with porcine pancreatic lipase:[53]



Similarly, both enantiomers of 2-substituted 1,3-propanediol monoacetates were prepared by starting from a common prochiral precursor via this process.[55] Other examples of asymmetric enzymatic acylation of prochiral diols in organic solvents have been reported.[37,38]

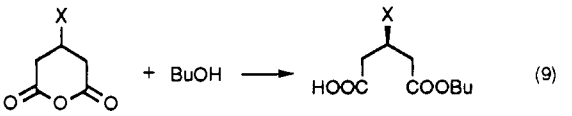
The basic experimental philosophy in all the resolutions described above is to select a solvent that affords the greatest reaction rates and ease of operation and then screen for a hydrolase with a desired enantioselectivity. Recently, we discovered that the latter itself can be greatly affected by the solvent.[19] For instance,[56] the enantioselectivity (the ratio k_{cat}/K_m for *S* and *R* isomers) of subtilisin in the acylation of *sec*-phenethyl alcohol with vinyl butyrate varies from very low in acetonitrile (less than 2) to overwhelming in triethylamine (200). In the short run, this phenomenon adds another variable to enzymatic resolutions: besides the screening of enzymes in a particular solvent, one can conduct a much easier screening of solvents with a given enzyme. In the long run, if understood mechanistically, this phenomenon can lead to the solvent engineering of enzyme enantioselectivity.

Preparation of Optically Active Carboxylic Acids and Their Esters. Asymmetric esterifications and transesterifications catalyzed by lipases in organic solvents can also be used for the resolution of racemic acids.[28,30] For instance, *C. cylindracea* lipase was employed for stereoselective esterifications in hexane:



where X = Br, Cl, Br, Br, Cl, Br, and *p*-ClC_6H_4O and Y = CH_3, CH_3, $CH_3(CH_2)_3$, $CH_3(CH_2)_{13}$, Ph, Ph, and CH_3, respectively. High optical purities were obtained in most cases. This process, applied to the resolution of 2-bromo- and 2-chloropropionic acids (starting materials for the synthesis of phenoxypropionic herbicides), is currently being carried out on a 100-kg scale and is to be scaled up further by Chemie Linz Co. (Austria) under a license from MIT.[57] The same transformations can be conducted, albeit less efficiently, in biphasic aqueous–organic mixtures.[28,29]

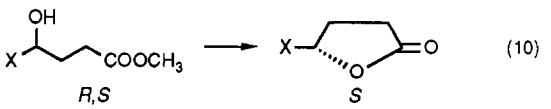
Oda and co-workers[58–60] described an interesting asymmetric ring opening of substituted cyclic anhydrides of dicarboxylic acids catalyzed by *P. fluorescens* lipase in anhydrous organic solvents. For instance, optically active monoesters of 3-substituted glutaric acid were prepared:[58,59]



where X = Me, Et, Pr, *i*-Pr, or Cl. The products of this reaction can be readily converted to optically active δ-valerolactones, which are useful chiral building blocks.[59] In the case of α-substituted cyclic acid anhydrides, the enzyme opens the ring at the less sterically hindered carbonyl group to give monoesters with high regioselectivity.[60]

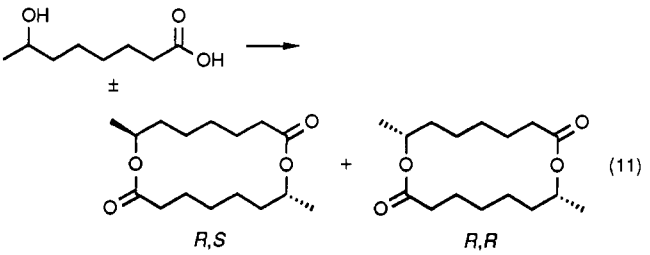
Another intriguing report dealt with lipase-catalyzed stereoselective transesterification of chiral diesters:[61] dimethyl methylsuccinate was subjected to alcoholysis to form the *S* mixed diester (propyl methyl or allyl methyl), with the dimethyl *R* ester remaining (90–95% ee for all esters); also, racemic dimethyl *N*-acetylaspartate and *N*-acetylglutamate were enzymatically reacted with 1-propanol to yield L-propyl methyl and D-dimethyl esters.

Stereoselective Lactonizations and Polycondensations. The groups of Yamada[62] and Gutman[63,64] have described the use of lipases in anhydrous organic solvents for the production of lactones, including chiral ones, from methyl esters of hydroxy acids. For example, optically active γ-methyl- and γ-phenylbutyrolactones were prepared by using porcine pancreatic lipase catalyzed intramolecular transesterification in ether:[63]



where X = CH_3 or Ph.

Sih and co-workers[65–67] have reported the enzymatic synthesis of macrocyclic lactones via intermolecular esterification in organic solvents starting with either hydroxy acids or diols and diacids. The former is illustrated by the following stereoselective lactonization catalyzed by *Pseudomonas* lipase in isooctane:[67]



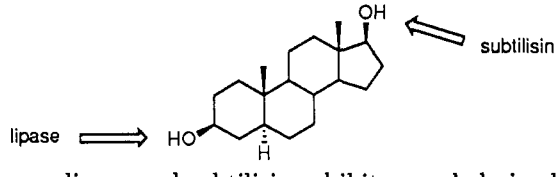
A reaction pathway alternative to lactonization in the foregoing examples is oligomerization, with the ratio between the two processes being dependent on both the

(55) Ramos-Tombo, G. M.; Schar, H.-P.; Fernandez-Busquets, X.; Ghisalba, O. *Tetrahedron Lett.* **1986**, *27*, 5707–5710.
(56) Fitzpatrick, P. A.; Klibanov, A. M. Unpublished results.
(57) Klibanov, A. M.; Kirchner, G. U.S. Patent 4,601,987, July 22, 1986.
(58) Yamamoto, K.; Nishioka, T.; Oda, J.; Yamamoto, Y. *Tetrahedron Lett.* **1988**, *29*, 1717–1720.
(59) Yamamoto, Y.; Yamamoto, K.; Nishioka, T.; Oda, J. *Agric. Biol. Chem.* **1988**, *52*, 3087–3092.
(60) Hiratake, J.; Yamamoto, K.; Yamamoto, Y.; Oda, J. *Tetrahedron Lett.* **1989**, *30*, 1555–1556.
(61) Guibe-Jampel, E.; Rousseau, G. *Tetrahedron Lett.* **1987**, *28*, 3563–3564.
(62) Makita, A.; Nihira, T.; Yamada, Y. *Tetrahedron Lett.* **1987**, *28*, 805–808.
(63) Gutman, A. L.; Zuobi, K.; Boltanski, A. *Tetrahedron Lett.* **1987**, *28*, 3861–3864.
(64) Gutman, A. L.; Oren, D.; Boltanski, A.; Bravdo, T. *Tetrahedron Lett.* **1987**, *28*, 5367–5368. Gutman, A. L.; Bravdo, T. *J. Org. Chem.* **1989**, *54*, 4263–4265.
(65) Guo, Z.-W.; Ngooi, T. K.; Scilimati, A.; Fulling, G.; Sih, C. J. *Tetrahedron Lett.* **1988**, *29*, 5583–5586.
(66) Guo, Z.-W.; Sih, C. J. *J. Am. Chem. Soc.* **1988**, *110*, 1999–2001.
(67) Ngooi, T. K.; Scilimati, A.; Guo, Z.-W.; Sih, C. J. *J. Org. Chem.* **1989**, *54*, 911–914.

reaction conditions and reactant structure.[64,66] Another approach to enzymatic polycondensation is lipase-catalyzed transesterification of diesters with diols in organic solvents. For example, optically active oligoesters were prepared[68] from racemic diesters and achiral diols or vice versa by using various lipases as asymmetric catalysts. This process was refined and further developed by Wallace and Morrow,[69] who achieved high molecular weight optically active polymers, thereby making this procedure attractive for practical stereoselective polycondensations.

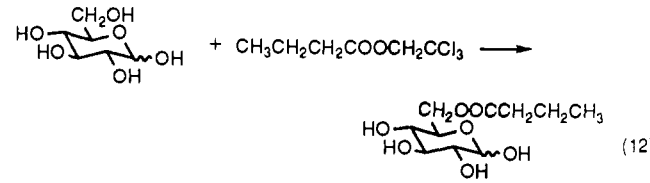
Regioselective Acylation of Glycols, Steroids, and Sugars. Site-selective modification of polyfunctional organic compounds remains a challenging synthetic task. Enzymatic transesterifications in organic solvents have proven to be extremely powerful for such asymmetric transformations. This potential was recognized early on by Cesti et al.,[70] who demonstrated highly regioselective monoacylation of various glycols in ethyl carboxylates (used both as solvents and as acylating agents) catalyzed by porcine pancreatic lipase. For instance, in the reaction of numerous primary–secondary diols with ethyl acetate, almost quantitative conversion was achieved with 98–100% of the product as the primary monoester. Similarly, selective monoacylation of glycals with vinyl esters was reported[71] using *C. cylindracea* lipase as a catalyst. Another hydrolytic enzyme, chlorophyllase, was employed for the monoacylation of symmetrical glycols with chlorophyll a, bacteriochlorophyll a, and pyrobacteriochlorophyll a.[72] Interestingly, when heterobifunctional compounds, such as amino alcohols, are used as nucleophiles in enzymatic transesterifications, the position of modification (OH vs NH_2) can be controlled by both enzyme and ester.[73]

Asymmetric enzymatic acylations of diols can be profitably applied to the classical synthetic problem of regioselective protection of steroids. For instance we found[74] that 5α-androstane-3β,17β-diol (a minimal dihydroxy steroid structure) can be enzymatically acylated with trifluoroethyl butyrate in anhydrous acetone. Strikingly, as shown in the drawing, *Chromobacterium*



viscosum lipase and subtilisin exhibit overwhelming but opposite regioselectivities in this reaction: the former acylates the C-3 hydroxyl group, while the latter prefers the C-17 site. Hence the position of steroid modification can be directed by the choice of the enzyme. In addition, lipase-catalyzed, regioselective acylation of steroids in anhydrous benzene has been demonstrated for many steroid structures, including bile acid derivatives.[75] Yeast lipase can be employed as a catalyst in the regioselective modification of polyhydroxy steroids in a complementary approach:[76] the steroid is first completely acylated by chemical means and then selectively deacylated enzymatically in organic solvents with another alcohol as the nucleophile.

Regioselective acylation of carbohydrates is a particularly arduous task due to the abundance of hydroxyl groups in their molecules.[77] An additional obstacle to the aforementioned enzymatic approach in this case is that unprotected sugars are soluble only in a few, very hydrophilic solvents, such as pyridine and dimethylformamide, which are far from optimal for enzymatic catalysis.[14] Nevertheless, even under these adverse conditions, many lipases and proteases retain useful catalytic properties allowing for the modification of carbohydrates. Therisod and Klibanov[78] established that not only is porcine pancreatic lipase enzymatically active in anhydrous pyridine but, under these conditions, it acylates unprotected monosaccharides at the primary hydroxyl group, as illustrated for glucose in eq 12. This regioselectivity of lipase in the acylation of



(12)

sugars, including numerous furanose and pyranose derivatives, was confirmed by Wong's group.[37,39]

The product of reaction 12 (and analogous sugar monoesters) can be further enzymatically acylated, with the position of the secondary acylation dependent on the enzyme used: e.g., *C. viscosum* and *Aspergillus niger* lipases in tetrahydrofuran acylate exclusively the C-3 OH group, whereas porcine pancreatic lipase under the same conditions acylates predominantly at the C-2 hydroxyl.[80] Similarly, Nicotra et al.[81] found that, upon the enzymatic acylation of the glycolipid 1,4-anhydro-5-*O*-hexadecyl-D-arabinitol with trichloroethyl butyrate in benzene, *Humicula lanuginosa* and *Mucor meheii* lipases modify nearly exclusively at the C-3 hydroxyl, whereas the enzyme from *Rhizopus japonicus* reacts predominantly with the C-2 OH group.

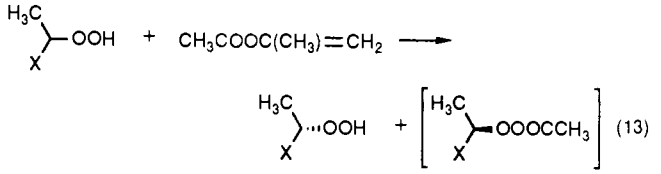
The protease subtilisin is especially useful for the regioselective protection of carbohydrates and related compounds.[82] In both anhydrous dimethylformamide and pyridine, it carries out a one-step acylation of primary hydroxyls in unprotected mono- and oligosaccharides. For instance, the enzyme acylates the C-1′ OH group of sucrose with a 90% regioselectivity (while the other two OH groups of sucrose are more reactive in the chemical acylation).[82] The product of this reaction can be hydrolyzed with the enzyme yeast α-glu-

(68) Margolin, A. L.; Crenne, J.-Y.; Klibanov, A. M. *Tetrahedron Lett.* **1987**, *28*, 1607–1610.
(69) Wallace, J. S.; Morrow, C. J. *J. Polym. Sci., Polym. Chem.* **1989**, *27*, 2553–2567.
(70) Cesti, P.; Zaks, A.; Klibanov, A. M. *Appl. Biochem. Biotechnol.* **1985**, *11*, 401–407.
(71) Holla, E. W. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 220–221.
(72) Michalski, T. J.; Hunt, J. E.; Bradshaw, C.; Wagner, A. M.; Norris, J. R.; Katz, J. J. *J. Am. Chem. Soc.* **1988**, *110*, 5888–5891.
(73) Chinsky, N.; Margolin, A. L.; Klibanov, A. M. *J. Am. Chem. Soc.* **1989**, *111*, 386–388.
(74) Riva, S.; Klibanov, A. M. *J. Am. Chem. Soc.* **1988**, *110*, 3291–3295.
(75) Riva, S.; Bovara, R.; Ottolina, G.; Secundo, F.; Carrea, G. *J. Org. Chem.* **1989**, *54*, 3161–3164.
(76) Njar, V. C. O.; Caspi, E. *Tetrahedron Lett.* **1987**, *28*, 6549–6552.
(77) Haines, A. H. *Adv. Carbohydr. Chem. Biochem.* **1976**, *33*, 11–109.
(78) Therisod, M.; Klibanov, A. M. *J. Am. Chem. Soc.* **1986**, *108*, 5638–5640.
(79) Hennen, W. J.; Sweers, H. M.; Wang, Y.-F.; Wong, C.-H. *J. Org. Chem.* **1988**, *53*, 4939–4945.
(80) Therisod, M.; Klibanov, A. M. *J. Am. Chem. Soc.* **1987**, *109*, 3977–3981.
(81) Nicotra, F.; Riva, S.; Secundo, F.; Zucchelli, L. *Tetrahedron Lett.* **1989**, *30*, 1703–1704.
(82) Riva, S.; Chopineau, J.; Kieboom, A. P. G.; Klibanov, A. M. *J. Am. Chem. Soc.* **1988**, *110*, 584–589.

cosidase to yield 1-*O*-fructose esters, which are not easily attainable by chemical methods.[83] Subtilisin could also butyrylate sugar moieties attached to bulky aromatic and heterocyclic rings and regioselectively conjugate sugars and amino acid derivatives.[82]

Other Asymmetric Nucleophilic Substitutions Catalyzed by Hydrolases

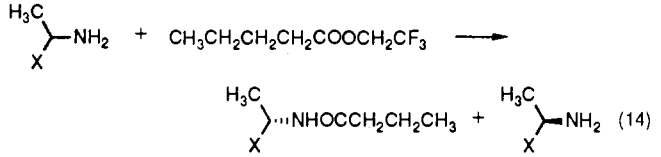
Other nucleophiles can be employed instead of the alcohol R*OH in eq 2, thereby leading to the resolution of various classes of racemic compounds. This notion is well illustrated by a study of Oda and co-workers[84] on the lipase-catalyzed resolution of racemic hydroperoxides. Using *P. fluorescens* lipase as an asymmetric catalyst in cyclohexane or isopropyl ether, the following regioselective conversion was carried out:



(13)

where X = phenyl, naphthyl, or *n*-propyl. In all instances, optically active (*S*)-hydroperoxides were prepared (which, if desired, could be chemically reduced to the corresponding chiral alcohols); the acylated hydroperoxides, shown in brackets, are unstable and decompose to a ketone and acetic acid.

Another indicative example is the subtilisin-catalyzed resolution of racemic amines in anhydrous 3-methyl-3-pentanol:[20]



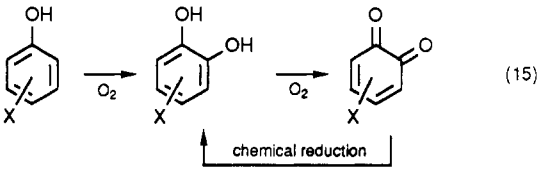
(14)

where X = phenyl, 1-naphthyl, 1-phenylethyl, hexyl, indolylmethyl, isobutyl, cyclohexyl, or 1-(1,2,3,4-tetrahydro)naphthyl. (*S*)-Amides and (*R*)-amines were prepared, in most cases, with good enantiomeric excess. Another noteworthy feature of this resolution is that it was optimized with respect to the reaction medium: of the many solvents tested, 3-methyl-3-pentanol afforded the highest enantiodiscrimination, while some solvents showed none. Gotor et al.[85] examined the opposite situation to that depicted in eq 14: a racemic ester was reacted with an achiral amine to result in optically active amides as catalyzed by *C. cylindracea* lipase.

The same approach can presumably be used for the enzymatic resolution of other nucleophilic compounds, e.g., thiols, oximes, and perhaps even carbanions (to asymmetrically form a carbon–carbon bond).

Asymmetric and Regioselective Conversions Catalyzed by Non-Hydrolases

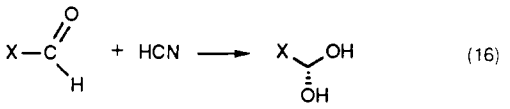
To date, there are only a few examples of the use of nonhydrolytic enzymes as regioselective or stereoselective catalysts in organic media. Kazandjian and Klibanov[86] employed polyphenol oxidase for the hydroxylation of phenols:



(15)

where X = H, CH_3, CH_3O, $HOOCCH_2CH_2$, Cl, Br, I, $HOCH_2CH_2$, or $PhCONHCH_2$. The enzyme catalyzes both the hydroxylation and the subsequent dehydrogenation. In water, the *o*-quinones formed are unstable and rapidly polymerize, thus making the process synthetically useless. In organic solvents (e.g., in chloroform), the *o*-quinones are much more stable and consequently were reduced with ascorbic acid to the precursor catechols, thereby affording a net *ortho* hydroxylation of substituted phenols. Enzymatic reaction 15 was also conducted in supercritical carbon dioxide and fluoroform.[87]

Effenberger et al.[88] reported the enantioselective addition of hydrogen cyanide to various aldehydes, catalyzed by the enzyme mandelonitrile lyase, to give optically active *R* cyanohydrins:



(16)

where X = Ph, 3-$PhOC_6H_4$, furyl, nicotinyl, CH_3CH=CH, benzyl, $CH_3SCH_2CH_2$, $CH_3CH_2CH_2$, or $(CH_3)_3C$. In water, only moderate optical purities were attained because of a chemical side reaction leading to racemic cyanohydrins. In ethyl acetate, the nonenzymatic reaction was negligible, and consequently, greater than 95% ee's were obtained in most cases.

Cofactor-requiring enzymes can also be employed as asymmetric catalysts in nonaqueous media, even if the cofactors are insoluble in such a reaction milieu. This can be accomplished by co-recovering the enzyme and its cofactor from the same aqueous solution and suspending the resultant complex in an organic solvent (containing enough water for cofactor mobility). Grunwald et al.[89] illustrated this approach using horse liver alcohol dehydrogenase which was responsible for its own cofactor regeneration. The racemic aldehyde and ketone were enzymatically reduced to optically active alcohols, while the concomitant oxidation of ethanol regenerated NADH. In a complementary process, alcohol dehydrogenase oxidized racemic secondary alcohols to optically active ketones, and reduced isobutyraldehyde to regenerate NAD^+. In all cases, high optical purities (greater than 95% ee) and cofactor turnover numbers (10^5–10^6) were obtained.[89]

Although most of the enzymes used for asymmetric transformations in organic solvents thus far have been hydrolases (due to their ready availability, low cost, and simplicity of reactions), it is likely that other classes of enzymes will catch up. In particular, oxidases seem

(83) Carrea, G.; Riva, S.; Secundo, F.; Danieli, B. *J. Chem. Soc., Perkin Trans. 1* **1989**, 1057–1061.
(84) Baba, N.; Mimura, M.; Hiratake, J.; Uchida, K.; Oda, J. *Agric. Biol. Chem.* **1988**, *52*, 2685–2687.
(85) Gotor, V.; Brieva, R.; Rebolledo, F. *Tetrahedron Lett.* **1988**, *29*, 6973–6974.
(86) Kazandjian, R. Z.; Klibanov, A. M. *J. Am. Chem. Soc.* **1985**, *107*, 5448–5450.
(87) Hammond, D. A.; Karel, M.; Klibanov, A. M.; Krukonis, V. J. *Appl. Biochem. Biotechnol.* **1985**, *11*, 393–400.
(88) Effenberger, F.; Ziegler, T.; Forster, S. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 458–460.
(89) Grunwald, J.; Wirz, B.; Scollar, M. P.; Klibanov, A. M. *J. Am. Chem. Soc.* **1986**, *108*, 6732–6734.

suitable for such exploitation, e.g., for selective oxidation of hydrocarbons.

Concluding Remarks

It should be encouraging, for organic chemists and chemical engineers alike, to know that enzymes can work not only in water but also in organic solvents. The still-existing prejudice against enzymes as practical catalysts will be lessened further by the forthcoming greater availability of a wider variety of enzymes at a lower cost, brought about by modern biotechnology. Moreover, protein engineering, particularly redesigning enzymes by site-directed mutagenesis,[90] has an exciting potential of altering the enzymatic properties at will, e.g., broadening substrate specificity,[91] as well as enhancing enzyme action in organic media.[92]

The ever intensifying search for novel biologically active compounds inevitably results in increasingly complex molecules, often containing stereocenters and existing as distinct structural isomers. Usually, one of the enantiomers or isomers has a higher biological activity than others. Consequently, there is mounting pressure on the manufacturers of pharmaceuticals and agricultural chemicals to produce only the desired stereoisomer, which would enhance the compound's benefit-to-harm ratio. As a result, the need for simple and scalable asymmetric conversions will grow. Hopefully, this Account demonstrates that enzymatic catalysis in organic solvents provides a useful methodology toward that aim.

I am grateful to David Volkin and Paul Burke for their help in the preparation of this manuscript and to the National Institutes of Health for financial support (Grant GM39794).

(90) Fersht, A. R.; et al. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 467–473. Knowles, J. R. *Science* **1987**, *236*, 1252–1258.
(91) Bone, R.; Silen, J. L.; Agard, D. A. *Nature* **1989**, *339*, 191–195.
(92) Arnold, F. H. *Protein Eng.* **1988**, *2*, 21–25.

Encoding and Decoding Hydrogen-Bond Patterns of Organic Compounds

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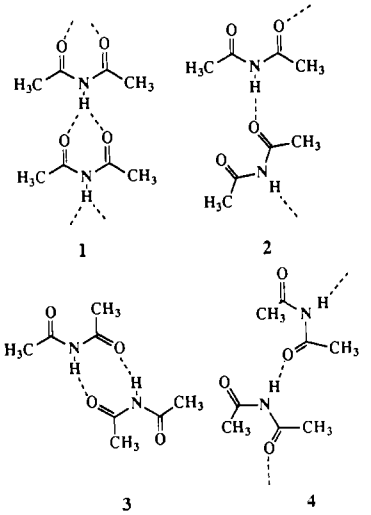
Received June 20, 1989 (Revised Manuscript Received January 31, 1990)

Are hydrogen-bond patterns predictable? Benzoic acids form cyclic dimers. Salicylic acid has an intramolecular hydrogen bond. These hydrogen-bond patterns seem obvious because they satisfy the chemical criteria of pairing a somewhat acidic hydrogen with an electronegative atom, and because spectroscopic and crystallographic studies have confirmed our chemical intuition about how and when hydrogen bonds should form in these and in similar frequently studied structures.[1] But what about other, less studied, yet simple compounds like diacetamide? Diacetamide has two proton acceptors, one proton donor, and three possible conformers each of which could form two- or three-centered hydrogen-bonded chains or dimers. Four of the hydrogen-bond possibilities are shown. Patterns 1 and 3 are found in the two polymorphs of diacetamide.[2]

Besides these patterns, diacetamide also cocrystallizes with many other hydrogen-bonding molecules.[3] The inherent complexities in making such predictions are apparent in the intricate hydrogen-bond pattern observed for diacetamide and acetamide,[4] shown below. It certainly is not obvious that the cyclic pattern shown



1 2 3 4

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(1) The idea that some hydrogen-bond patterns are obvious and can be used to prepare aggregate patterns with predictable structures has been demonstrated by several different groups. (a) Ducharme, Y.; Wuest, J. D. *J. Org. Chem.* **1988**, *53*, 5789–5791. (b) Chang, S. K.; Hamilton, A. D. *J. Am. Chem. Soc.* **1988**, *110*, 1318–1319. (c) Askew, B.; Ballester, P.; Buhr, C.; Jeong, K. S.; Jones, S.; Parris, K.; Williams, K.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1989**, *111*, 1082–1090, (d) Stadler, R. *Prog. Colloid Polym. Sci.* **1987**, *75*, 140–145.
(2) (a) Kuroda, Y.; Taira, Z.; Uno, T.; Osaki, K. *Cryst. Struct. Commun.* **1975**, *4*, 321–324. (b) Kuroda, Y.; Taira, Z.; Uno, T.; Osaki, K. *Ibid.* **1975**, *4*, 325–328.
(3) Etter, M. C.; Reutzel, S., unpublished results.

0001-4842/90/0123-0120$02.50/0