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Asymmetric Synthesis of Organometallic Reagents Using Enzymatic Methods

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ABSTRACT

The use of enzymatic catalysis in the synthesis and resolution of organometallic complexes is reviewed and discussed. Examples show the potential of biological catalysts for oxidation, reduction, hydrolysis, and esterification of both transition metal and main group organometallic substrates. Chirality in organometallic complexes caused by the presence of chiral carbon centers in substituent groups, tetrahedral or pseudotetrahedral metal centers, and planes of asymmetry can all be recognized by whole cell or isolated enzyme catalysts. Biocatalysts that achieve high levels of kinetic resolution are described. Other catalysts that produce high levels of asymmetric induction in reactions of a prochiral substrate are also described.

Index Entries: Catalysis; enzymatic lipase dehydrogenase; enzyme resolution; organometallic synthesis resolution.

INTRODUCTION

Chiral organometallic compounds are widely useful in asymmetric catalysis and in asymmetric organic synthesis. These compounds often act either as homogeneous catalysts, electrophilic synthons, or nucleophilic synthons in the synthesis of chiral nonracemic organic molecules (*1,2*). Often, the enantiomeric or diastereomeric purity of the product obtained in these syntheses can reach 100%. In the case of homogeneous

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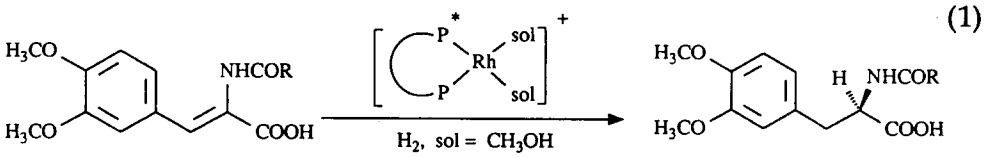
catalysts, this efficiency for asymmetric induction exceeds that of traditional heterogeneous catalysts and can be competitive with that resulting from enzyme-catalyzed processes. However, synthesis of these chiral organometallic compounds and of the associated ligands that sometimes provide the asymmetric environments needed for this chemistry has generally relied on classical procedures. While organometallic compounds are unnatural substrates for asymmetric synthesis and kinetic resolution using biological reagent, results from a variety of laboratories over the past few years have demonstrated that biological catalysts are useful with these substrates. Here we review some of these applications.

Chiral organometallic compounds used in asymmetric catalysis most often contain chiral phosphine ligands. For example, the chiral rhodium catalyst used in the first industrial process for the asymmetric synthesis of L-dihydroxyphenylalanine (L-DOPA) (Equation 1), a drug used to treat Parkinson's disease, derived its chirality from the presence of chiral phosphines such as those shown in Schemes 1–4 (*4*). Subsequent work by many groups has extended this concept beyond asymmetric hydrogenation and has led to many catalysts capable of expressing high levels of asymmetric induction (*1,2*). Thus, asymmetric catalysis with chiral transition metal catalysts is now an achievable goal with most transition metal catalyzed reactions.



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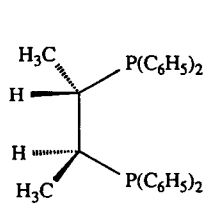

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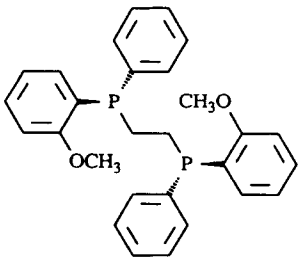

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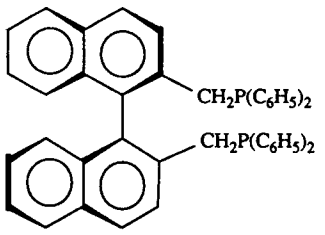

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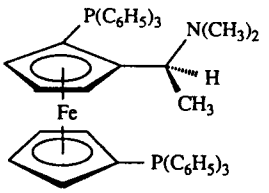

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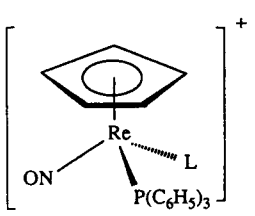
Schemes 1–4



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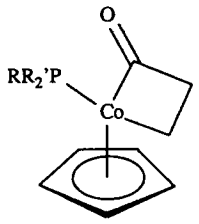

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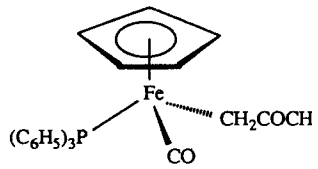

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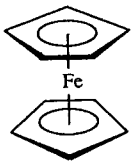
Schemes 5–7

There are also transition metal catalysts and reagents where the source of chirality is a pseudotetrahedral metal center. The chiral rhenium compound indicated in Scheme 5 acts as a chiral Lewis acid center (*5*). The acylcobalt catalyst in Scheme 6 has been prepared as an enantiomercially pure material and has been shown to produce chiral cyclobutanones in high optical yield (*6*). The chiral iron complex prepared by Davies is a very efficient reagent in asymmetric synthesis of chiral enolates (Scheme 7). Similar chiral organometallic reagents can be used in asymmetric synthesis of β-hydroxy carbonyl derivatives via stereoselective aldol condensations. Such reagents are also useful in the synthesis of esters and β-lactams via asymmetric conjugate addition alkylations (*7,8*).

The importance of chiral organometallic complexes such as those in Schemes 4–7 has led to resurgent interest in their asymmetric synthesis and in generally applicable methods for resolution of chiral organometallic reagents (*9*). Biotechnological approaches to this problem are a recent innovation. These approaches for syntheses of these chiral organometallic derivatives, which rely on either whole cells or enzyme isolates, are discussed in more detail below. Much of the work in synthesis of optically active organometallic compounds can be illustrated by the synthesis of ferrocenyl derivatives. Such ferrocene derivatives derive their chirality from the presence of a chiral side chain on the cyclopentadiene moiety or from the presence of a chiral plane arising from disubstitution on one of the cyclopentadiene rings.

The ferrocene nucleus itself can be a target of biological action (Scheme 8). In the presence of horseradish peroxidase and hydrogen peroxide, ferrocene was converted to the ferricinium ion in 4 h (*10*). In rats, ingested



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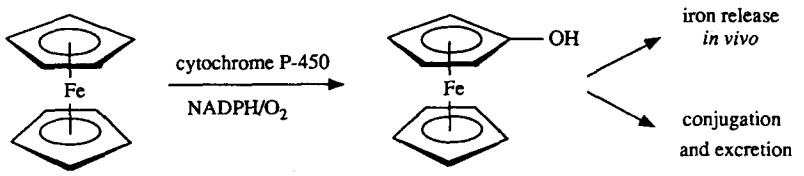
Scheme 8



(2)

ferrocene was metabolized and excreted in urine as the glucuronide of hydroxyferrocene. The hydroxylation process is believed to be mediated by cytochrome P-450 in the presence of NADPH and molecular oxygen in the liver. Hydroxyferrocene that escapes conjugation with glucuronic acid or sulfate may be used as an iron source for the synthesis of hemoglobin (Equation 2). Interest in ferrocene and its derivatives includes their use as hematinic agents, photoaffinity labels, and radiopharmaceutical diagnostic agents incorporating the ^{59}Fe nucleus (*11*).

Ferrocenyl analogs of phenylalanine have also been used in studies on the activity of some peptides where the effect of the aromatic ring is important. [Fer^4,Leu^5]-Enkephalin as well as ferrocene-containing bradykinin and substance P peptides have been synthesized to make use of the ferrocene nucleus as a steric probe in the study of peptide-receptor interactions. In contrast to other aromatic systems that are usually planar, the ferrocene aromatic system has a unique, elongated cylindrical shape (*12,13*).

Much of the work on the stereochemistry of ferrocene was done by Ugi in the early 1970s (*14,15*). Ugi's work established that most nucleophilic substitution reactions on the α-carbon of chiral nonracemic ferrocenes proceed with complete retention of configuration. Retentive S_N1 nucleophilic substitution implies that asymmetric synthesis of just one ferrocenyl compound would provide a route for the synthesis of other ferrocenyl compounds of similar stereochemistry. In Ugi's chemistry, racemic ferrocenylethylamine was resolved via formation of a salt with *R*-(+)-tartaric acid. Both antipodes of the amine were obtained in high yield. The *S* enantiomer produced a salt that crystallized out of methanol, whereas the tartrate of the R enantiomer was recovered from the mother liquor and recrystallized from aqueous acetone (*14*).

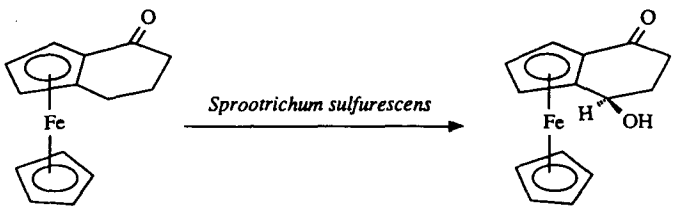
This classical method of optical resolution through the synthesis and separation of diastereomeric derivatives has become the standard approach for asymmetric synthesis of ferrocenyl compounds. However, although resolution is a well-established method by which one can obtain pure enantiomers of chiral ferrocenes and is the preferred method for synthesis of other organometallic compounds, modern methods of asymmetric synthesis including enzymatic catalysis have been shown to be synthetically useful, too (*9*).

Enzymes have long been used by the pharmaceutical and food industries for the production of valuable commodities. The organic chemist, however, failed to recognize immediately the potential value of enzyme-

based technologies for asymmetric synthesis. This may have been the result of preconceived ideas about fragility, instability, and narrow substrate specificities, as well as plain unfamiliarity with the behavior and handling of enzymes. Within the last decade, however, there has been a burgeoning interest in the use of enzymes and other biocatalysts for organic synthesis (*16–19*). This can be attributed to increased interest in bioorganic chemistry in general. In part, this heightened interest is also due to the lowered costs and ready availability of these biochemical catalysts due to the rapid developments in bioorganic chemistry and biotechnology. Current environmental concerns—and the realization that despite high substrate specificities, enzymes can also act on unnatural substrates and retain their activities in organic solvents as well as in water—have further contributed to the upsurge of interest in this biocatalysts.

In organic chemistry, enzymatic methods have been most successfully applied to the synthesis of chiral synthons either via asymmetric induction on a prochiral substrate or kinetic resolution of a racemic mixture. The reactions occur under very mild conditions of temperature and pH in the case of aqueous reactions. They can also be run using the common hydrophobic organic solvents or in a biphasic mixture. These reactions can be manipulated to attain very high chemical and optical yields. Among the reactions that have been studied extensively, oxidation-reductions, group tansfers, hydrolyses, transacylations, and additions to sp^2-hybridized carbon centers are espeically suitable for these enzymatic methods. Except for the synthesis of unusual sugars, C–C bond formation using enzymes is not as facile as with traditional metal catalysts. Because of the high chemo-, regio-, and stereoselectivity, there is no need for the use of protecting groups. There is even the potential for coupling several reactions in one pot, a feature that is less common in traditional chemical catalysis.

There are some drawbacks to the use of enzymatic methods. Although use of organic solvents has now become routine, especially for lipases, enzymes require some amount of associated water to retain their activity (*19*). This presents only mild hindrance to the use of enzymes with organometallics. Specifically excluded are reactive organometallics, which would react with water or other heteroatom XH groups (X = S, N, or O). In some cases, the need to use organic solvents can pose additional problems, since hydrophilic solvents tend to strip away water from the enzyme and inactivate it. There are also kinetic problems, such as, diffusional limitations and inhibition by both substrate and products. All these problems are encountered in application of enzymatic methods in organic synthesis and are being addressed. These limitations do not preclude applications of enzymes to asymmetric synthesis of organometallics. Ongoing development of flow reactors, improved immobilization techniques, use of reverse micelles, or chemical modification of the enzyme are likely to minimize these problems in the future when they are encountered. Finally, in



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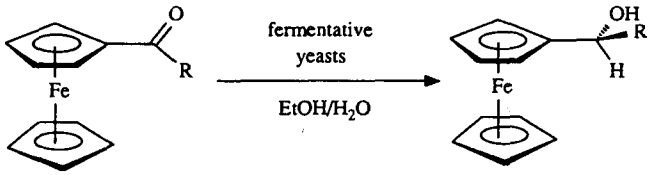


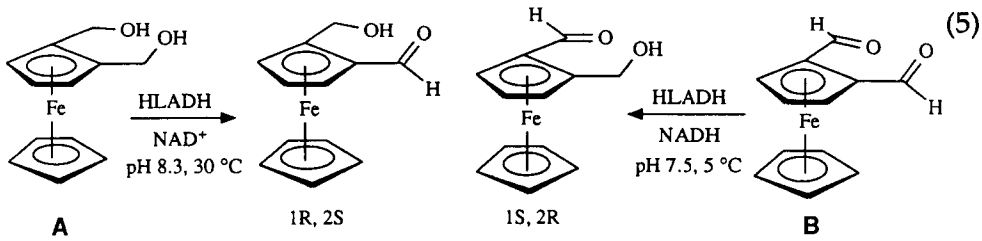
(4)

cases where an expensive cofactor is necessary, economical cofactor regeneration schemes have been developed to make the use of cofactor-requiring enzymes feasible.

Although the use of enzymes in asymmetric syntheses has become increasingly important in the last decade or so, there has been less work done on their application to the synthesis of chiral organometallics (*16–19*). Few organometallic compounds have been tested as substrates for enzyme action. This is a bit curious because in biological systems, metal-containing compounds do exist and unnatural compounds like that in Scheme 8 are associated with enzyme action. Lack of studies concerning organometallics may just reflect the research interests of researchers in this field. Most of the literature on the enzymatic modification of organometallic compounds has dealt with two approaches: Stereoselective oxidation-reduction reactions with either isolated enzymes or whole cells is a common approach. The other approach used has relied on hydrolysis or formation of carboxylic acid derivatives using various esterases or amidases.

Microbial oxidation is perhaps the earliest example of the use of biocatalysts in asymmetric synthesis of organometallic reagents (*17*). In this first example (Equation 3), the mold *Sporotrichum sulfurescens* was used to hydroxylate the known chiral 1,2-(α-oxotetramethylene)ferrocene to produce a hydroxylated product that was optically active. However, the stereochemistry of the product was not clearly determined. Moreover, whether the optical activity in the product resulted from a resolution of what was apparently racemic starting material, or if the overoxidation of one diastereomeric product ketoalcohol to a diketone was slower than that of the other diasteromer, was not established.

Reduction of ferrocenylcarbonyl compounds by fermentative yeasts has been studied beginning as early as 1976. In a typical example of this chemistry shown in Equation 4, ferrocene-carboxyaldehyde was reduced quantitatively in 2 h to ferrocenylethanol at 33°C. Propionyl-, isobutyryl-, benzoyl-, 1,1'-diacetylferrocenyl ketones, [3]-ferrocenophane-1-one, and ferrocenyltrifluoromethyl ketone were also tested as substrates. Only



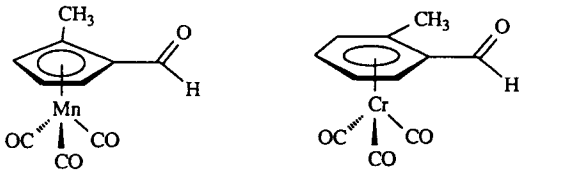
(5)

ferrocenyltrifluoromethyl ketone and [3]-ferrocenophane-1-one gave measurable yields after 60 h of incubation. (-)-Trifluoromethyl-1-ferrocenylethanol was obtained in 50% yield, but the optical yield was not reported. [3]-Ferrocenophan-1-ol was obtained in only 10% yield after chromatography, with an optical rotation that was 84% of the literature value for the (-)-antipode (*22*).

The reduction of an ethanolic solution of ferrocenecarboxyaldehyde-*d* to ferrocenyldeuteromethanol proceeded with 60% conversion along with 8% conversion to ferrocenyldeuteromethyl ethyl ether. The enantiomeric excess was not reported, but the reaction was assumed to be stereospecific for the production of the (+) isomer by comparison with the optical rotation of 1-*d*-benzyl alcohol (*23*).

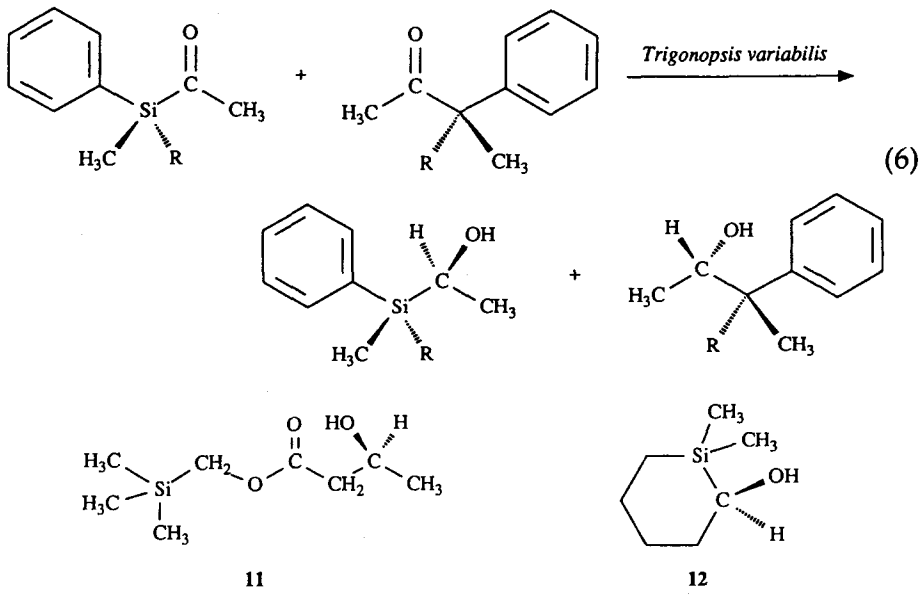
Oxidation of hydroxy groups on the side chain of a ferrocene with a dehydrogenase has also proven to be feasible. For example, the meso diol in Equation 5A was oxidized with horse liver alcohol dehydrogenase (HLADH) in the presence of an NAD^+ recycling system using glutamate dehydrogenase at 30°C at pH 8.3 for 9 h to yield 81% of the hydroxyaldehyde in 86% enantiomeric purity (*24*). A similar reduction of the meso dialdehyde in Equation 5B using HLADH, NADH, and ethanol at 5°C and pH 7.5 for 57 h produced an 80% yield of the enantiomer of the hydroxyaldehyde product in 94% *ee*. Since these reactions generate chirality by virtue of starting with achiral starting materials, the yields are greater than could be expected in a kinetic resolution. The separate generation of both possible enantiomers by use of starting materials in different oxidation states with either an enzymatic oxidation or a reduction also makes this example noteworthy.

Liver alcohol dehydrogenase oxidoreduction appears to be a generally useful biocatalytic route for kinetic resolution through reduction of chiral aldehyde derivatives of metallocenes. As the examples below indicate, high *ee* values can be obtained for a range of products (*25*). In these cases,



9, ~ 100 % ee (by ^1H NMR) **10**, ~ 100 % ee (by ^1H NMR)

Schemes 9–10



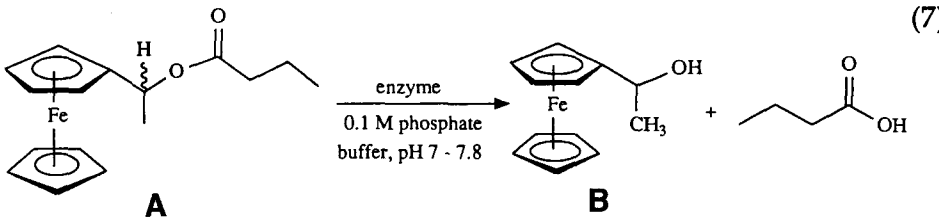
Schemes 11 and 12

the enantiomers of the aldehydes shown could be isolated by chromatography directly from the reaction mixtures. The aldehydes (-)-9 and (-)-10 shown were isolated after oxidation of the hydroxymethyl products with MnO_2 and DMSO-Ac_2O, respectively. Analysis of these products by ^1H NMR spectroscopy with chiral shift reagents showed a single enantiomer (> 95% *ee*)

Baker's yeast reductions on transition metal complexed ketones have also been reported and are successful. For example, in the case of the methyl ketone $^6\eta$-$Cr(CO)_3C_6H_5COCH_3$, the product alcohol was produced in > 99% *ee* and in quantitative yield (*26*). Similar baker's yeast kinetic resolutions of planar chiral chromocene organometallics and ferrocenophanes have been reported (*27*).

Oxidation-reduction chemistry is also feasible with non-hydrolytically labile organosilicon compounds. The enantioselective reduction of silylated alkyl acetoacetates has been conducted on a preparative scale with good-excellent chemical yields and high stereoselectivities using growing cells of microorganisms like *Kloeckera corticis* and *Trigonopsis variabilis* (*29–31*). Additional studies have screened 30 strains of microorganisms for similar reductions to determine the optimum conditions and optimum biocatalyst for achieving high chemical and high optical yields in this chemistry (*32*). Finally, while the chiral center in compounds like 13 and 14 is an asymmetric carbon center, studies using growing cells of *Trigonopsis variabilis* have shown that asymmetric reduction of silylated ketones containing chiral silicon centers is also feasible (Equation 6) (*33*).

Hydrolytic enzymes, e.g., lipases and amidases, are among those that have been most successfully used for the synthesis of chiral molecules. These enzymes are usually cheap, versatile, and do not require expensive cofactors for activity. Lipases, in particular, show enhanced



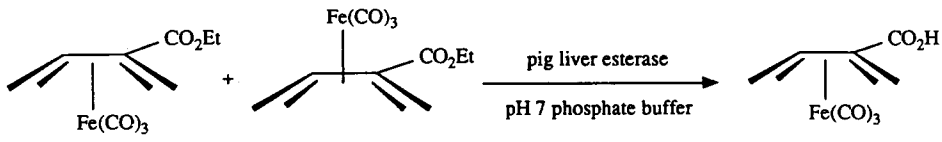
(7)

stability in organic solvents (*34*). While most enzymes become deactivated rapidly at high temperature, porcine pancreatic lipase (PPL) has a life span of tens of h in anhydrous organic media and a catalytic activity that is five times higher at 100°C than it is at 20°C for the transesterification of tributyrin with *n*-heptanol (*35*). Although their normal physiological function is hydrolytic, lipases have been found to catalyze transacylation reactions in the absence of water, as in the case just mentioned. Because of the enantioselectivity of these reactions, the maximum conversion is 100% for meso diester substrates but only 50% if a racemic substrate is used. This 50% limit on yield that is typical of kinetic resolutions can be a drawback unless both product and remaining substrate can be obtained in high optical purity and are equally useful, if the desired enantiomer is so valuable but difficult to obtain by any other means or if there is a means of converting the less reactive substrate to the more reactive antipode.

An advantage of kinetic resolution is that one can obtain both antipodes of the product. This meets an important requirement for the synthesis of chiral metal catalysts or chiral ligands for transition metal catalysts, since in these cases, it is desirable that both of the antipodes of the catalyst or ligand be available (*36*). In this sense, enzymatic resolution may be regarded as a useful tool for the synthesis of chiral ferrocenyl ligands because the product and the unreacted starting material, if obtained in high optical yields, may be reacted further via stereospecific reactions to yield both antipodes of the ligand.

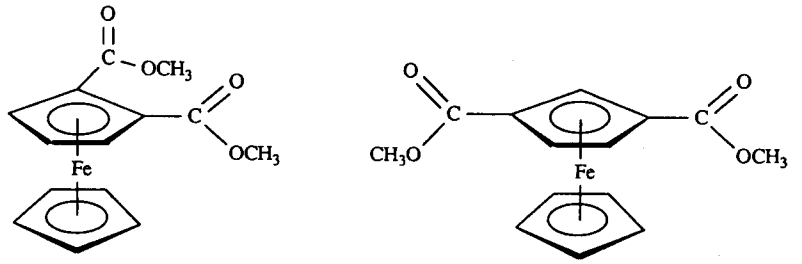
Our group has addressed the problem of enzymatic resolution of ferrocenyl substrates via transacylations using various hydrolytic enzymes for the synthesis of optically active ferrocene compounds (*37*). Our goal was to synthesize a chiral ferrocenyl alcohol, 1-ferrocenylethanol, and its esters in high optical yield using hydrolytic enzymes. These compounds were chosen as model compounds to determine the feasibility of applying enzymatic methods to the asymmetric synthesis of chiral transition metal organometallics because of their utility, unusual structure and properties, and ease of preparation.

Since the natural biological function of these enzymes is to catalyze hydrolysis, we initially screened enzymes for hydrolytic activity on a model substrate shown in Equation 7A. Two lipases, PPL and *Candida cylindracea* lipase, and two proteases, α-chymotrypsin and papain, were tested. None of these enzymes was able to effect enantioselective hydrolysis, although the hydrolysis rates were relatively fast. Control reactions showed that 20% DMSO–water, the medium used for optimum protease



(8)

17, 85% ee, 40% yield



(9)

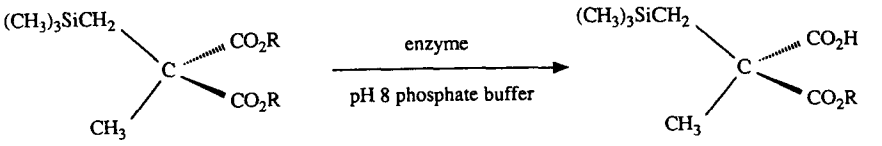
A B

activity, accelerated the nonenzymatic hydrolysis of 7A. In pH 7.8 phosphate buffer (0.1 M), the medium used for the lipases, nonenzymatic hydrolysis of 7A was somewhat slower and was complete in 14 d in the absence of the enzyme. Thus, although it was difficult to gauge the effect of protease on reaction rate, the results indicate that lipases did significantly increase the rate of hydrolysis. The reactions, however, were not enantioselective. We also ran a hydrolysis reaction using 0.033 mol/L of the ester to 1.2 g/L of α-chymotrypsin in methylene chloride treated with 8% water by wt, since Dastoli reported that α-chymotrypsin was able to effect the hydrolysis of an ester in methylene chloride containing 0.25% water (*38*). However, we observed no significant reaction in 2 wk.

Our group was unsuccessful in its attempts to prepare chiral organometallic complexes by hydrolysis of esters; however, others have reported significant successes. For example, Crout has described the enzymatic resolution of an iron tricarbonyl diene complex using pig liver esterase (Equation 8) (*39*). In this example, a 40% yield (based on the total amount of starting meterial) was obtained after 30 h. The enantioselectivity was verified by ^{1}H NMR analysis of an amide salt whose structure was in turn verified by X-ray crystallography.

More extensive studies of asymmetric syntheses by enzymatic hydrolyses have been performed by Yamazaki's group (*40*). Among the esters hydrolyzed using pig liver esterase were the compounds indicated in Equations 9A and 9B. As might have been expected, the achiral starting material with substituents in adjacent positions (i.e., Equation 9A) was a more suitable substrate for achieving high stereoselectivity forming a chiral product in 88% yield with 74% *ee* in contrast to the observed 70% yield and 16% *ee* for the compound in 9B.

Enantioselective enzymatic hydrolysis of trimethylsilyl-substituted malonic acid diesters has also been reported (*41*). In these examples, the metal center is achiral, and the chirality is caused by the presence of quaternary chiral carbon. Several different enzymes were reportedly used



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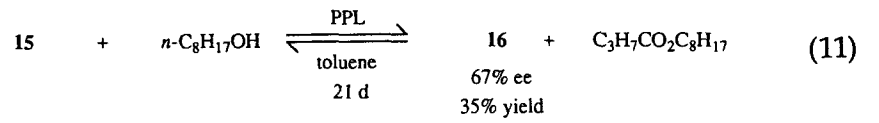


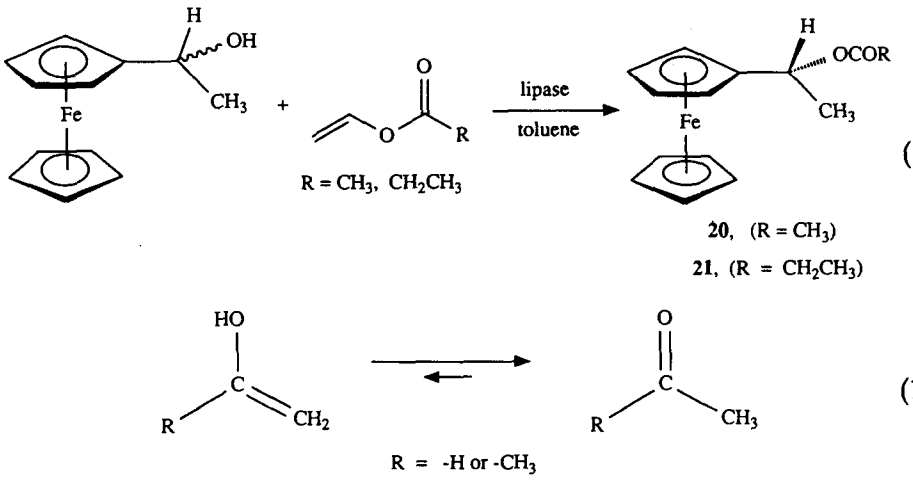
(11)

successfully, including pig liver esterase, pig pancreatic lipase, horse liver acetonic, and chymotrypsin.

The hydrolases are known to catalyze transesterifications in hydrophobic organic solvents, although the reactions are much slower than those of hydrolyses. Transesterification does not occur in aqueous media because of unfavorable thermodynamics. The strategy is usually employed for the synthesis of chiral acids but is also applicable to the synthesis of chiral alcohols. With ordinary alcohols, transesterification is reversible. However, reversible reactions are undesirable since optical yields decrease as the reverse reaction proceeds. This is because the enzyme exhibits the same stereoselectivity in both directions. The major stereoisomer in the product side of the forward reaction becomes the preferred substrate for the reverse reaction (*42*).

Our group has examined the transesterification of the substrate in Equation 7A with *n*-octanol (Equation 11). Although this transesterification is reversible, the reaction should favor the formation of the product shown in Equation 7B from the substrate in equation 7A because formation of an ester of a primary alcohol is favored over ester formation from a secondary alcohol. To further enhance the reaction rate and favor the product, a threefold excess of *n*-octanol and a ratio of 66 g/L of crude PPL/150 mmol/L of the substrate in Equation 7A were used. However, even with this amount of enzyme and substrate, the reaction was very slow. After 21 d, the reaction was 35% complete. The unreacted substrate in Equation 7A had 47% *ee*, whereas the product (Equation 7B) was obtained in 66% *ee*. This result is not sufficiently good to be useful, but it did demonstrate that transacylation is a potentially viable concept, especially if ways can be found to accelerate the reaction and increase the stereoselectivity (*43*).

To improve this reaction, we modified the strategy to make transacylation essentially irreversible (Equation 12). To accomplish this, we used vinyl esters as the acylating group. Vinyl esters have been used previously to irreversibly acylate glucose in the presence of lipase (*44*). The enol produced in the reaction readily tautomerizes to acetaldehyde, the more thermodynamically favored tautomer (Equation 13). Thus, the occurrence of reverse acylation is prevented, and the forward reaction proceeds to



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(13)

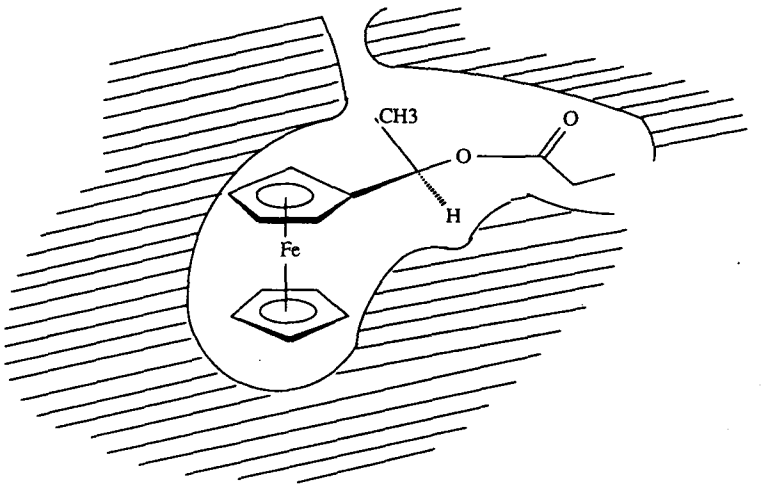


Fig. 1. Model of enzyme active site for Acylations of 16 with vinyl esters and with *Pseudomonas* and pig pancreatic lipase in organic solvents.

completion. We exploited this strategy for the irreversible acylation of the product in Equation 7B as shown in Equation 12.

Our initial studies used vinyl acetate and several readily available lipases. The results for pig pancreatic lipase, *Candida, Aspergillus,* and *Rhizopus* lipase were not very successful. The change from *n*-octanol to vinyl acetate actually led to a decrease in stereoselectivity and conversion yield (Equation 11). Using ^{1}H NMR spectroscopy and shift reagents, the highest stereoselectivity in formation of the product in Equation 12A was 19% at 33% conversion. Considering plausible models of the active site for these lipase catalyzed acylations of the production in Equation 7B, we sought to improve the vinyl ester acylation through the use of vinyl propionate in place of vinyl acetate (Fig. 1) (*45,46*). The hope was that we could achieve higher rates and stereoselectivity because of the slightly

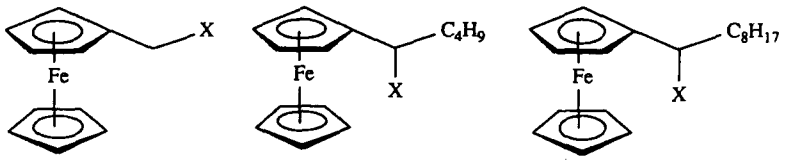
$$E = \frac{\ln [1 - (1 + K)\, c\, (1 + ee_p)]}{\ln [1 - (1 + K)\, c\, (1 - ee_p)]} \qquad (14)$$

where $K = 0$ for an irreversible reaction
c = extent of conversion
ee_p = enantiomeric excess of product

longer chain, which presumably would produce a better fit within the active site of the enzyme. This approach proved to be gratifyingly successful. Among the enzymes studied, pig pancreatic lipase exhibited the highest selectivity with optical yields of 84% for the product ester in Equation 12B and the unreacted starting material in Equation 7B. Assignments of configurations to the products of these enzymatic reactions were made by comparison with reported data for optical rotation (*14*). The optical rotation for an isolated product (shown in Equation 12B) was found to be $[\alpha]_D = -11.2°$ in comparison to the literature value of $-21.0°$ for the *R* isomer (*14*). Based on this comparison, the *R*-(-) configuration was assigned to the ester in Equation 12B. The remaining alcohol in Equation 7B obtained after 53% conversion had an $[\alpha]_D = +25.9°$ in comparison to the literature value of $+30.1°$ for the *S* isomer (*14*). THus, the *S*-(+) configuration was assigned to the production of Equation 7B.

In an irreversible enantioselective kinetic resolution such as that shown in Equation 12, the optical purity of the product decreases with extent of reaction because the less-reactive enantiomer builds up in the course of the reaction and competes with the more-reactive antipode as the substrate. Thus, if the reaction is stopped before reaching 50% conversion, one gets the maximum optical yield of the product. Conversely, as the reaction proceeds, the remaining substrate becomes increasingly richer in the less-reactive enantiomer. Stopping the reaction as the rate begins to level off, i.e., slightly beyond 50% conversion, will afford the maximum optical yield of the remaining starting material. There have been attempts to quantify the maximum optical yield of an enzymatic kinetic resolution. Sih defined a quantity, *E*, a kinetic parameter that gives the maximum enantiomeric ratio that can be attained by an enzymatic reaction (Equation 14) (*47*). Applying the equation to the reaction shown in Scheme 1 with vinyl propionate, *E* is calculated to be 95, indicating that the maximum theoretical optical yield for this reaction is 90%.

Our group never achieved these results, but a report by Boaz subsequent to our report described a similar reaction using vinyl acetate and lipase from *Pseudomonas fluorescens* in *t*-butyl methyl ether, which produced *R*-(−)- in 96% *ee* for the product in Equation 12A and *S*-(+)- in 92% *ee* for the product in Equation 7B (*46*). The reaction took 12 d to attain 50% conversion. The amount of enzyme used was not mentioned. This is an important consideration for *Pseudomonas* lipase, because the commercial preparation is highly active and expensive. On the other hand, the commercial preparation of PPL, while having low activity (< 1%/g), is very



(15)

A, X = -OH **B**, X = -OH **C**, X = -OH

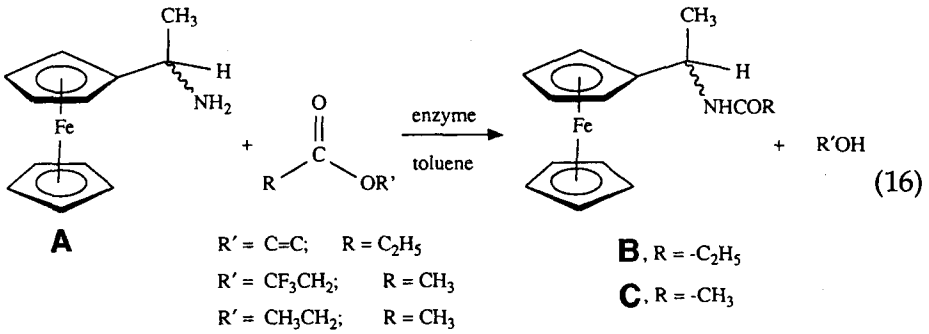
D, X = $-OCOCH_2CH_3$ **E**, X = $-OCOCH_2CH_3$ **F**, X = $-OCOCH_2CH_3$

cheap. We also found that *Pseudomonas* lipase was effective (*43*). However, in our original experiments we had to use up to 400 mg/L of *Pseudomonas* lipase in toluene and only achieved a 19% conversion to R-(−)- in 13 d for the product in Equation 12B.

The configurational assignments by our team and Boaz's seem to be in keeping with the trend noted for the action of PPL and other hydrolases on unnatural substrates. For example, in the stereospecific transesterification of chiral racemic alcohols with trichloroethyl butyrate in the presence of PPL, only the *R* isomer of the alcohol reacts to form the *R* ester. The reaction stops when none of the *R* isomer remains. Conversely, if racemic 2-halocarboxylic acids are reacted with butanol, only the (*R*)-butyl ester is formed (% *ee* > 95%) (*48*). The active site working model that has been proposed for some hydrolqases provides an explanation for this selectivity. The active site of the enzyme has been known to contain a serine hydroxyl group that is crucial to the catalytic activity of the enzyme. The active site is shaped to accommodate a hydrophobic group in a deep groove and has a small niche that will accept only an α-hydrogen of the substrate. This directs the preference of the enzyme for the *R* substrate. The enantioselectivity of the reaction indicates that the ferrocene nucleus fits into the hydrophobic groove of the active site (Fig. 1) (*46*).

The effect of chain length of the alkylferrocene on the rate and enantioselectivity of reaction in Scheme 9 was also studied (*43*). Ferrocenylmethanol (Equation 15A), ferrocenylpentanol (Equation 15B), and ferrocenylnonanol (Equation 15C) were synthesized from ferrocenecarboxyaldehyde for use as alternative substrates for enzymatic transesterification by vinyl propionate. All three alcohols were found to be substrates for PPL producing the esters in Equations 15D, E, and F, respectively. However, the reaction rate was much slower for the two higher chain analogs of Equations 15B and C.

The ester in Equation 15E was obtained only in 20% yield with 68% *ee*, whereas the reaction of Equation 15B was quenched after 15 d. The enantiomeric excess was estimated by comparison of the α-methine triplets of the enantiomeric esters in the presence of $Eu(hfc)_3$, a chiral ^1H NMR shift reagent. The compound in Equation 15C produced less than 10% of the ester in Equation 15F after 15 d. These results are consistent with the conformationally inflexible behavior of the enzyme in organic solvents.

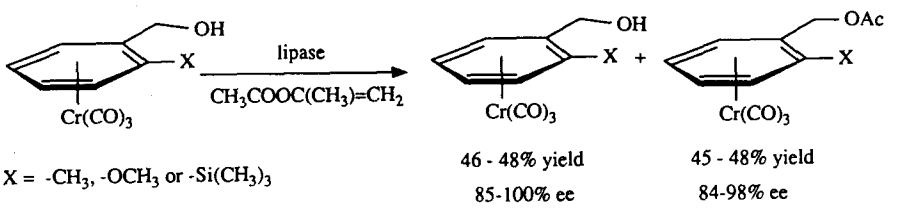


(16)

Protein folding has been attributed to four kinds of intermolecular interactions, namely: H-bonding, electrostatic interactions among charged groups, hydrophobic interactions, and aromatic–aromatic interactions. Because of the predominance of the groups involved, hydrophobic and aromatic–aromatic interactions mainly determine the catalytically active structure of the enzyme. If the interactions of the enzyme with the solvent are decidedly weaker than these forces, there is a high entropic barrier to protein unfolding in this solvent.

The potential of hydrolytic enzymes for enzymatic kinetic resolution of ferrocenylethylamine (Equation 16A) has also been examined (*43*). Hydrolysis of N-ferrocenylethyl butyramide was not successful. This amide was not significantly hydrolyzed by α-chymotrypsin, subtilisin, and papain even after 3 wk under similar conditions. Upon quenching, only the papain reaction mixture showed traces of the product amine by silica gel TLC. Acylation also proved to be less successful than the alcohol acylation described in Equation 12. Acylation reactions were carried out on the product in Equation 16A using a variety of acylating agents and enzymes. However, in all cases where activated esters were used, non-enzymatic transacylation to form the products indicated in Equations 16B and C competed significantly with the enzymatic process, precluding any possibility of achieving high stereoselectivity. Substitution of a less-active ester, ethyl acetate, led to lower synthetic yields in a slow reaction. For example, after 23 d, the compound in Equation 16A and ethyl acetate yielded from Equation 16C in about 20–25% yield using either *Candida* lipase or pig pancreatic lipase. Under the same conditions, papain resulted in only 12% conversion of Equation 16A into 16C. Moreover, the stereoselectivities seen were modest, 28% *ee* for PPL and 14% for *Candida* lipase based on ^{1}H NMR analysis using chiral shift reagents (*43*).

Subsequent to our work, several other examples have appeared in the literature certifying the generality of the vinyl ester interchange as a route to asymmetric esterification of chiral organometallics; Boaz's report has been mentioned above (*46*). In addition, a report by Nakamura shows that similar esterifications can be accomplished with planar chiral organo-



(17)

metallic alcohols (*49*). This latter work has since been independently verified by a study using vinyl esters in which a total of 16 lipases were screened for the same types of substrates shown in Equation 17 (*50*). A more recent report by Yamazaki shows that this sort of resolution is even effective when the substituent $X = -CH_3$ is shifted from the 2 position on complexed arene to the 3 position (*51*). However, in these cases, very high % *ee* values could only be obtained though the use of a double-resolution procedure (*52*).

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REFERENCES

1. Yamamoto, A. (1986), *Organotransition Metal Chemistry: Fundamental Concepts and Applications*, John Wiley and Sons, New York, pp. 1–480.
2. Noyori, R. (1990), *Science* **119**, 4.
3. Noyori, R. and Kitamura, M. (1989), *Mod. Synth. Methods* **5**, 115.
4. Collman, J. P., Hegedus, L. S., Norton, J. R., and Finke, R. G. (1987), *Principles and Applications of Organotransition Metal Chemistry*, University Science Books, Mill Valley, CA, pp. 1–989.
5. Dalton, D. M., Fernández, J. M., Emerson, E., Larsen, R. D., Arif, A. M., and Gladysz, J. A. (1990), *J. Am. Chem. Soc.* **112**, 9198.
6. Theopold, K. H., Becker, P. N., and Bergman, R. G. (1984), *J. Am. Chem. Soc.* **104**, 5250.
7. Davies, S. G., Bashiardes, G., Beckett, R. P., Coote, S. J., Dordo-Hedgecock, I. M., Goodfellow, C. L., Gravatt, G. L., NcNally, J. P., and Whittaker, M. (1988), *Philos. Trans. R. Soc. London, A* **326**, 619.
8. Liebeskind, L. S., Welker, M. E., and Fengl, R. L. (1986), *J. Am. Chem. Soc.* **108**, 6328.
9. Brunner, H., ed. (1989), *J. Organomet. Chem.* **370.**
10. Epton, R., Hobson, M. E., and Marr, G. (1977), *J. Organomet. Chem.* **134**, C23.
11. Hanzlik, R. P. and Soine, W. H. (1978), *J. Am. Chem. Soc.* **100**, 1290.
12. Cuingnet, E., Sergheraert, C., Tartar, A., and Dautrevaux, M. (1980), *J. Organomet. Chem.* **195**, 325.

13. Brunet, J. C., Cuingnet, E., Gras, H., Marcincal, P., Mocz, A., Sergheraert, C., and Tartar, A. (1981), *J. Organomet. Chem.* **216,** 73.
14. Gokel, G. W., Marquarding, D., and Ugi, I. K. (1972), *J. Org. Chem.* **37,** 3052.
15. Marquarding, D., Klusacek, H., Gokel, G., Hoffmann, P., and Ugi, I. (1970), *J. Am. Chem. Soc.* **92,** 5389.
16. Yamada, H. and Shimizu, S. (1988), *Angew. Chem. Intl. Ed. Engl.* **27,** 622.
17. Jones, J. B. (1986), *Tetrahedron* **42,** 3351.
18. Whitesides, B. M. and Wong, C.-H. (1985), *Angew. Chem. Intl. Ed. Engl.* **24,** 617.
19. Klibanov, A. M. (1990), *Acc. Chem. Res.* **23,** 114.
20. Russell, A. J. and Klibanov, A. M. (1989), *Biochem. Soc. Trans.* **17,** 1145.
21. Johnson, R. A. and Murray, H. C. (1971), *J. Chem. Soc., D.* 989.
22. Ratajczak, A. and Misterkiewicz, B. (1976), *Chem. Ind.* 902.
23. Sokolov, V. I., Troitskaya, L. L., and Reutov, O. A. (1977), *Doklady Akad. Nauk SSSR* **237,** 1376.
24. Yamazaki, Y. and Hosono, K. (1989), *Tetrahedron Lett.* **29,** 5769.
25. Top, S., Jaouen, G., Gillois, J., Baldoli, C., and Maiorana, S. (1988), *J. Chem. Soc., Chem. Commun.* 1284.
26. Gillois, J., Jaouen, G., Buisson, D., and Azerad, R. (1989), *J. Organomet. Chem.* **367,** 85.
27. Izumi, T., Hino, T., Shoji, K., Sasaki, K., and Kasahara, A. (1989), *Chem. Ind. (London)* 457.
28. Izumi, T., Murakami, S., and Kasahara, A., (1990), *Chem. Ind.* 79.
29. Tacke, R., Linoh, H., Stumpf, B., Abraham, W.-R., Kieslich, K., and Ernst, Z. (1983), *Z. Naturforsch, B: Anorg. Chem.* **38B,** 616.
30. Tacke, R., Hengelsberg, H., Zilch, H., and Stumpf, B. (1989), *J. Organomet. Chem.* **379,** 211.
31. Syldatk, C., Andree, H., Stoffregen, A., Wagner, F., Stumpf, B., Ernst, L., Zilch, H., and Tacke, R. (1987), *Appl. Microbiol. Biotechnol* **27,** 152.
32. Syldatk, C., Stoffregen, A., Wuttke, F., and Tacke, R. (1988), *Biotechnology Lett.* **10,** 731.
33. Tacke, R., Fritsche, K., Tafel, A., Wuttke, F. (1990), *J. Organomet. Chem.* **388,** 47.
34. Zaks, A., and Klibanov, A. M. (1985), *Proc. Natl. Acad. Sci.* **82,** 3192.
35. Zaks, A., and Klibanov, A. M. (1984), *Science* **224,** 9.
36. Kagan, H. B. (1985), *Asymmetric Syntheses,* vol. 5, Morrison, J. D., ed., Academic Press, New York, p. 1.
37. Wang, Y. F., Lalonde, J. J., Momongan, M., Bergbreiter, D. E., and Wong, C.-H. (1988), *J. Am. Chem. Soc.* **110,** 7200.
38. Dastoli, F. R., Musto, N. A., and Price, S. (1966), *Arch. Biochem. Biophys.* **115,** 44.
39. Alcock, N. W., Crout, D. H. G., Henderson, C. M., and Thomas, S. E. (1988), *J. Chem. Soc. Chem. Commun.* 746.
49. Yamazaki, Y. and Hosono, K. (1990), *Anals. N.Y. Acad. Sci.* **613,** 738.
41. De Jeso, B., Belair, N., Deleuze, H., Rascale, M.-C., and Maillard, B. (1990), *Tetrahedron Lett.* **31,** 653.
42. Langrand, G., Baratti, J., Buono, G., and Triantaphylidis, C. (1986), *Tetrahedron Lett.* **27,** 29.

43. Momongan, M. (1991), *Ph. D. Dissertation,* Texas A&M University.
44. Sweers, H. M. and Wong, C.-H. (1986), *J. Am. Chem. Soc.* **108,** 6421.
45. Ohno, M., Kobayashi, S., Adachi, K. (1986), *Enzymes as Catalysts in Organic Synthesis,* Schneider, M. P., ed., Reidel, Dordrecht, Holland, pp. 123–142.
46. Boz, N. (1989), *Tetrahedron Lett.* **30,** 2061.
47. C.-S. Chen. and Sih, C. J. (1989), *Angew Chem. Intl. Ed. Engl.* **28,** 695.
48. Kirchner, G., Scollar, M. P., and Klibanov, A. M. (1985), *J. Am. Chem. Soc.* **108,** 7072.
49. Nakamura, K., Ishihara, K., Ohno, A., Uemura, M., Nishimura, H., and Hayashi, Y. (1990), *Tetrahedron Lett.* **31,** 3603.
50. Yamazaki, Y. and Hosono, K. (1990), *Tetrahedron Lett.* **31,** 3895.
51. Yamazaki, Y., Morohashi, N., and Hosono, K. (1991), *Biotechnology Lett.* **13,** 81.
52. Drueckhammer, D. G., Barbas, C. F., III, Nozaki, K., Wong, C.-H., Wood, C. Y., and Ciufolini, M. A. (1988), *J. Org. Chem.* **53,** 1607.