solvents was also successful providing, as expected, the (R)-configured reaction products [30]: 5 g of 30b dissolved in 50 ml of hexane was acetylated with vinyl acetate (0.55 mol-eqiv.) within 2 h (45% conversion) using 0.24 g of lipase P adsorbed on porous glass beads (E > 150). Repeated batchwise use of the catalyst (seven runs) did not reveal any noticeable inactivation.

All the favorable features mentioned suggest technical potential for the present enzymatic procedure.

The enzyme transformations outlined above demonstrate that minor changes in substrate structure may entail consequences ranging from having a new optimum enzyme or acyl moiety to the necessity of a completely new synthetic route for the target compound.


Some Recent Studies into the Use of Enzymes in Organic Synthesis

Stanley M. Roberts*, Nicholas J. Turner, and Andrew J. Willetts

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There are several factors which have helped to strengthen the impact of enzyme-catalysed reactions in organic chemistry. First, a wide-range of enzymes are available from commercial suppliers. Secondly, there is a much better understanding of the chemo-, regio-, and stereoselectivity of the reactions catalysed by various enzymes and several textbooks are available [1] to help newcomers to become conversant with these data. In addition a compendium of validated procedures, with

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Fifteen years ago only a small number of academic groups and a handful of industrial companies were genuinely interested in the employment of enzymes in organic synthesis. Nowadays the situation is different. Most organic chemists are all too aware that enzyme-catalysed reactions (biotransformations) may be of potential utility in their work. Thus, it is widely appreciated that enzymes may allow chiral synthetic intermediates (synthons) to be prepared in optically active form. It is also understood that enzymes can catalyse reactions that are difficult or, at the present time, impossible to emulate using other techniques of organic chemistry.

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helpful hints and tips, has been published recently [2]. Thirdly the pharmaceutical, agricultural, flavour and fragrance industries are increasingly committed to provide new products in single-enantiomer (homochiral) form.

Hydrolase enzymes are amongst the easiest catalysts to use in organic synthesis since they require no cofactors. Indeed lipases and esterases are becoming widely employed to catalyse the hydrolysis of esters. In the majority of cases hydrolases such as lipases and esterases are used in the form of partially purified protein but in some instances purification is not carried out and the catalyst is used as part of a whole-cell system. For example the racemic acetate 1 is hydrolysed by the lipase isolated from the fungus *Mucor miehei* and by lyophilized whole-cells of bakers’ yeast to give optically active (S)-alcohol 2 (enantiomeric excess 80–97%) at ca. 50% conversion (*Scheme 1*). The alcohol 2 has been converted into the vinyltin compound 3 and coupled to the iodoalkene 4 to give a late-stage intermediate 5 to the natural product coriolic acid 6 [3]. The latter compound is associated with anti-fungal activity.

Often the enzyme-catalysed kinetic resolution of an ester involves a more complex substrate possessing multiple chiral centres. Compound 7 is such a substrate, readily derived by [2+2] cycloaddition of a ketene and cyclohexadiene, followed by reduction of the ketone function with sodium borohydride and acetylation of the alcohol group under standard conditions. The enzymes *M. miehei* lipase and porcine pancreatic lipase (ppl) each catalyse a highly enantioselective hydrolysis to give the optically pure alcohol 8 and recovered optically active ester (*Scheme 2*). The polar alcohol is easily separated from starting material and can be converted into the cyclopentenone derivative 9 in three steps [4].

There are many hundreds, if not thousands, of examples of kinetic resolutions of esters similar to those described in *Schemes 1* and 2. Likewise the hydrolysis of amides using acylase enzymes has received a considerable amount of attention and has gained commercial importance in connection with the production of 6-aminopenicillanic acid from fermented penicillins such as penicillin-G and in the large scale synthesis of some natural amino acids. In addition nonnatural amides, such as the γ-lactam 10 can be hydrolysed in a highly selective fashion. Thus, an acylase enzyme in the microorganism *Pseudomonas solanacearum* provides the γ-ami-

![Scheme 1](image1.png)

![Scheme 2](image2.png)

![Scheme 3](image3.png)

![Scheme 4](image4.png)
no acid 11 (a mimic of the neurotransmitter GABA) and recovered lactam, both compounds in optically pure form (Scheme 3). Another organism performs the hydrolysis in an enantiocomplementary fashion to provide the different enantiomers of the aminoacid 11 and the lactam 10 [5]. The optically active 10 is an important intermediate for the production of various carbocyclic nucleosides [6], such as the nucleoside analogue 12, a powerful antiherpes agent [7].

Kinetic resolutions such as those described above can give, as a maximum, a 50% yield of one pure enantiomer. There are two different ways of providing higher yields of optically pure material. Thus, a substrate of type 13 epimerizes even under the mild conditions of the reaction and enantioselective hydrolysis using immobilized M. miehei lipase leads to isolation of 14 in 95% yield and up to 70% e.e. (Scheme 4). meso-Compounds can also be useful intermediates in syntheses involving enzyme-catalysed reactions, as illustrated by the following examples. The diester 15 is hydrolysed by ppl to afford the alcohol 16 in excellent yield and, essentially, in an optically pure state (Scheme 5). The compound has been used to make aristromycin [8] and the nucleotide mimic 17 [9]. Note that 15 is made up of a complex alcohol (diol) unit and simple acyl moieties and is a typical substrate in lipase-catalysed hydrolyses. Esters comprised of complex carboxylic acid units condensed with simple alcohols are more likely to be suitable substrates for esterase enzymes. For example the diester 18 (readily obtained from 7-(tert-butoxy)norbomadiene) is hydrolysed in a highly stereocontrolled manner by pig liver esterase (ples) to give the acid 19 (e.e. > 95%), which is itself not a substrate for ples (Scheme 6) [10]. The acid 19 is a useful synthetic intermediate and has been converted into the oxazolidinone 20 en route to the natural product neplanocin 21 [11].

The stereocontrolled enzyme-catalysed hydrolysis of racemic esters and amides exploits the natural function of the proteins. A very un-natural process involves the use of a lipase enzyme in an organic
solvent to couple an acid with an alcohol or amine to give an ester or amide, respectively. Yet, such processes are readily achieved and they have rightly attracted a considerable amount of attention, since the popularization of the strategy by Klibanov in the mid-1980's. Thus, the alcohol 22 is enantioselectively acylated using cyclohexane carboxylic acid to give the ester 23 in a state of high optical purity, at ca. 50% conversion, and recovered optically active alcohol (Scheme 7). The ester and the alcohol can be separated (e.g. by distillation) and the two compounds can be converted into the two enantiomers of bicyclo[3.2.0]hept-2-en-6-one 24 both useful precursors of prostaglandins and prostanoids [12].

Inter-esterifications can also be effected using lipases and these reactions can show advantage over the corresponding esterification reaction. Thus, in the enzyme-catalysed 'double enantioselection' involving the racemic alcohol 22, racemic para-chlorophenoxypropanoic acid and immobilized M. miehei lipase, diastereoisomer 25 is formed as the major component (ca. 70%) of the diastereoisomeric mixture. By employing the ester (+)-26 and (+)-para-chlorophenoxypropanoic acid as the starting materials then 25 constitutes > 90% of the mixture of diastereoisomers formed. The reason for this is that the bicyclic entity 26 has to visit the enzyme active site twice. On the first occasion the acetyl group is removed; the so-formed alcohol then returns to an active site to pick up the aryl propanoate unit (Scheme 8) [13].

Scheme 9

![Scheme 9](image)

(i) vinyl acetate, Lipozyme®, 42° C, 10 days

Scheme 10

![Scheme 10](image)

(i) Ph₃PO₂H₃, vinyl acetate 10 h, r. t., (ii) 2-amino-6-chloropurine, NaH, Pd(PPh₃)₄, THF

Scheme 11

![Scheme 11](image)

(i) Nitrile hydratase SP 361, buffer pH 7, 30°C, 12-24h
(ii) CH₂N₂

Scheme 12

![Scheme 12](image)

R¹COR² biocatalyst

Scheme 13

![Scheme 13](image)

R¹COR² DH

DH = dehydrogenase
The inter-esterification strategy has been modified slightly to provide a very important method for the production of acetate esters. Thus, the bromohydrin 27 is available in two steps from cyclopentadiene. Reaction with vinyl acetate in the presence of Lipozyme® gives, at ca. 50% conversion, a good yield of optically pure acetate 28 and optically pure alcohol 27. The latter compound has been converted into the epoxide 29 which on photolysis provided the lactone 30 an intermediate on the route to the antihypertensive agent carbocyclic oxetanocin-A 31 (Scheme 9) [14].

The alcohol 32 is available by performing a Prins reaction on cyclopentadiene followed by triylation and purification. The alcohol is acetylated with exquisite selectivity using Pseudomonas fluorescens lipase (pfl) in vinyl acetate to give the recovered alcohol and the ester 33 [15]. The recovered alcohol was chemically acetylated and the acetate so-produced was subjected to Trost-style chemistry, using a Pd® catalyst to couple a purine base to the five-membered carbocyclic ring so as to afford a late-stage intermediate 34 to the anti-HIV agent carbovir 35 (Scheme 10) [15].

In comparison to the great volume of work involving the production and hydrolysis of esters and amides using enzymes there has been comparatively few studies centred on the bio-catalysed hydrolysis of organonitriles. Yet, the potential of utilizing nitrilases and nitrite hydratases in synthesis is very significant as the following examples will help to illustrate. meta-Dicyanobenzene 36 is hydrolysed by a nitrile hydratase to give metacyanobenzoic acid 37 (Scheme 11). The acid is not a substrate for the hydrolase enzyme. However, esterification of the acid unit followed by re-submission to the hydrolysis conditions furnished the half-ester 38. In other words the nitrile group was hydrolysed in the presence of a methyl ester, a reversal of the normal order of reactivity generally observed for acid- or base-catalysed chemical hydrolyses [16]. Furthermore, the prochiral dinitrile 39, available in bulk (by treatment of epichlorohydrin with cyanide ion followed by benzoylation) is hydrolysed to the cyanoacid 40. The acid is not a substrate for the enzyme and leads to the formation of a material of high optical purity from an achiral precursor [17].

Taken altogether, hydrolysis reactions probably contribute ca. 50% of our knowledge of those enzyme-catalysed reactions that can be considered to be of importance in the context of organic synthesis. The next most investigated series of enzymes in this connection are the oxido-reductases, enzymes that catalyse oxidation and reduction reactions.

In contrast to the hydrolases, oxidoreductase enzymes such as dehydrogenases (DHs) require a cofactor in order to effect a bioconversion. The nicotinamide nucleotide cofactors NAD+/H and NADP+/H are such cofactors and their strategic importance in a biological reduction reaction is shown in Scheme 12. Note that chiral secondary alcohols produced in this way are often optically active.

The biotransformation illustrated in Scheme 12 can be conducted using whole-cell systems or partially purified enzymes. In the former case the cofactor is present and efficiently recycled as part of the overall metabolism of the cell; in the latter case NAD(P)H must be added in addition to the enzyme to constitute the reduction system. It is not possible to use a stoichiometric amount of cofactor in this situation and an in vitro recycling system must be constructed. A typical way of doing this is by using a low-boiling sacrificial primary alcohol, allowing the dehydrogenase to oxidize this simple alcohol (used in excess) to the corresponding aldehyde (Scheme 13). Alternatively a completely different enzyme such as formate dehydrogenase can be exploited, in this instance recycling the cofactor while converting formate into carbon dioxide (Scheme 14).

Cyclic and acyclic ketones can be reduced using whole-cell systems. For instance ethyl 3-oxobutanoate can be reduced using microorganisms to give 3(S)- or (3R)-ethyl-3-hydroxybutanoate in excess. Similarly the hydroxy keto ester 41 is reduced using bakers' yeast to give the diol 42 and recovered optically active alcohol. Such a biotransformation is of significance in connection with the prepara-
tion of compounds of type 43 (Scheme 15) [18].

Similarly the ketone 44 is reduced by the fungus Mortierella isabellina to give two diastereoisomeric alcohols 45 and 46, each of these diastereoisomers being made, more or less, from one enantiomer of the starting material [19]. The alcohol (46) was transformed into eldanolide 47 a pheromone of an agricultural pest called the sugar cane borer [20] (Scheme 16).

The ketone 44 is reduced enantioselectively using 3α,20β-hydroxysteroid dehydrogenase (HSAD) to give the alcohol 46 in optically pure form and recovered optically active ketone [21]. The alcohol, after separation from residual ketone, was oxidized and both the enantiomers of the ketone 44 obtained in this way were used in an enantiocomplementary route to the naturally-occurring polyeleukotriene B4 50 (Scheme 17) [22]. Thus, the dextro-rotatory ketone provided the synthon 48 and the laevorotatory ketone furnished the synthon 49. Modification of the synthons and coupling of the two fragments provided, after deprotection, the natural product which has been implicated in diseases such as psoriasis and irritable bowel syndrome.

Many different types of oxidation reactions are catalysed by enzymes. The hydroxylation of organic compounds at positions remote from pre-existing functionality is a very important process catalysed by enzymes such as cytochrome P-450 monoxygenase. These enzymes are often exploited using whole-cell preparations of appropriate microorganisms, and some of the processes that have been discovered are of commercial significance, e.g. the hydroxylation of progesterone at the 11-position which constitutes the key step in a facile route to anti-inflammatory steroids. Aliphatic compounds (such as 

\[
\begin{align*}
\text{Scheme 17} & \quad (-) \cdot 44 \\
\text{Scheme 18} & \quad 51 & \quad 52 & \quad 53 \quad \text{Primary point of hydroxylation} \\
\text{Scheme 19} & \quad (+) \cdot 44 \\
\text{Scheme 20} & \quad 54 & \quad 55 & \quad 56 & \quad 57 & \quad 58 & \quad 59 \quad \text{Secondary point of hydroxylation}
\end{align*}
\]
cyclohexylcyclohexane 51), heterocyclic compounds (such as the spiro-compound 52) and arylalkyl species (such as the piperdine derivatives 53) are hydroxylated in a highly selective, though somewhat unpredictable, fashion using organisms such as Cunninghamamella sp. and Beauveria sulfurescens (Scheme 18) [23]. This lack of predictability concerning the point of hydroxylation limits the widespread use of this methodology at present.

The oxidation of aromatic substrates using dioxygenase enzymes present in Pseudomonas sp. is a more controlled and predictable process, giving rise to cyclohexadienediols. 3,5-Cyclohexadiene-1,2-cis-diol, after formation of the acetone derivative 54, is converted into more exotic compounds by Diels-Alder reactions [24] and [6+4] cycloadditions involving tropone (Scheme 19) [25]. When a benzene derivative such as fluorobenzene 55 or trifluoromethylbenzene 56 is used as the substrate the oxidation products are optically active. Their acetone derivatives 55 and 56 which possess the absolute configuration shown in Scheme 20 can be induced to react further by processes involving dimerization and [2+2] cycloaddition reactions involving ketenes [26].

Interestingly one of the Pseudomonas microorganisms that converts benzene into cyclohexadienediol also transforms norbornadiene 57 into the diol 58 (Scheme 21); when 7-phenylnorbornadiene is offered as a substrate the enzyme reverts to oxidizing the aromatic ring, furnishing the tetraene 59 [27].

Oxidation adjacent to the diene unit of linoleic acid 60 using immobilized soybean lipoxygenase provides the basis of another high yield route to coriolic acid 6 (Scheme 22). The reaction can be extended to the trienoic acid 61 [28].

One of the potentially most interesting biooxidation reactions involves the conversion of a ketone into an ester (or a cyclic ketone into a lactone) i.e. the Baeyer-Villiger reaction. Reactions of this type can be effected using whole-cell systems
such as *Acinetobacter calcoaceticus*. Sometimes racemic mixtures of substrates are completely oxidized by an enantiomer of the substrate 62 produces the lactone 63 while the other enantiomer of ketone 62 forms a different product namely the lactone 64 (Scheme 23) [29]. Alternatively a ketone such as the dihalogenobicycloheptanone 65 can be oxidized enantioselectively using *A. calcoaceticus* yielding, in this case, the lactone 66 and recovered optically pure ketone [30]. This recovered ketone has the correct absolute configuration for conversion into an intriguing analogue 68 of the anti-AIDS agent AZT (Scheme 24) [31]. It is noteworthy that chemical oxidation of the ketone 65 with meta-chloroperoxybenzoic acid furnishes the lactone 67, resulting from methylene group migration towards the incoming oxygen atom. Thus, the bio-Baeyer-Villiger reaction shows a different regioselectivity to the equivalent chemical process as well as displaying unprecedented stereoselectivity.

Whole-cell bioconversions of the type described in Schemes 23 and 24 rarely give high yields due to over-metabolism of the 65 monooxygenase enzyme responsible for the Baeyer-Villiger oxidations can be extracted and purified from *A. calcoaceticus*. Unfortunately, the enzyme requires NADPH as a cofactor and the in *vitro* recycling of this very expensive cofactor is notoriously difficult. One solution to this problem is to run the monooxygenase in tandem with a dehydrogenase (working in the oxidative direction) to convert a secondary alcohol such as norbornanol 69 into the corresponding lactone 70 with in *situ* recycling of the cofactor (Scheme 25) [32].

This is only a partial solution to the problem since in organic synthesis it is often a ketone, not a secondary alcohol that must be converted to the corresponding lactone. A far-reaching recent discovery was that the bacterium *NCIMB 10007* possesses a monooxygenase that utilizes NADH as the cofactor in undertaking various regio- and stereoselective Baeyer-Villiger oxidations: thus the racemic bicycloheptenone 24 furnishes two lactones 71 and 72 on oxidation with dioxygen, the enzyme, and NADH (Scheme 26, cf. Scheme 23). The NADH can be used in small quantities and recycled using either formate/formate dehydrogenase (Scheme 27A) or various cofactor-complementary alcohol dehydrogenases (Scheme 27B) [33]. The use of coupled enzyme systems such as the ones described in Scheme 27 are becoming common practice in the area of biotransformations.

Hopefully the selection of biocatalysed reactions illustrated in the above text should serve to demonstrate that enzymatic transformations of various types can be utilized in efficiently synthesizing of bioactive natural products and analogues.

The authors acknowledge the hard work and commitment of colleagues and coworkers cited in the references.

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