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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**

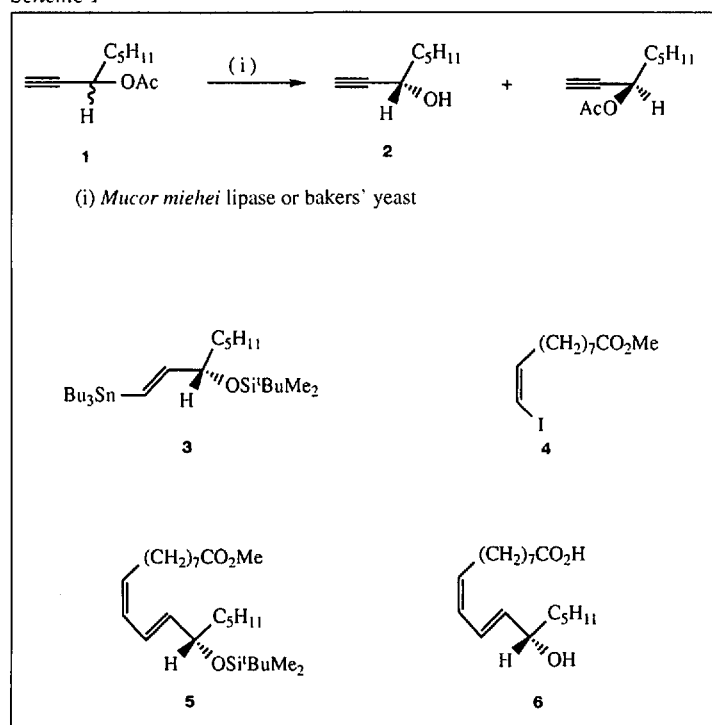
(enantiomeric excess 80–97%) at ca. 50% conversion (*Scheme 1*). The alcohol **2** has been converted into the vinyltin compound **3** and coupled to the iodoalkene **4** to give a late-stage intermediate **5** to the natural product coriolic acid **6** [3]. The latter compound is associated with anti-fungal 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

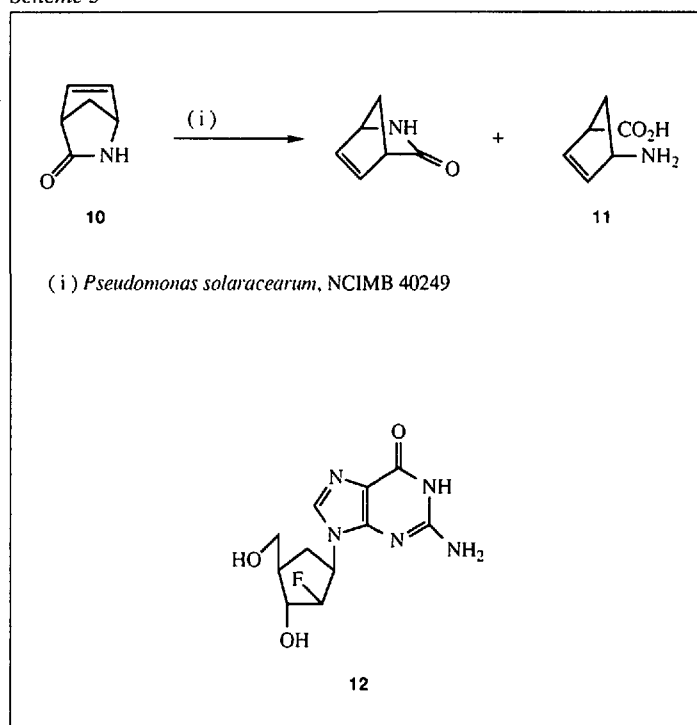
recovered optically active ester (*Scheme 2*). The polar alcohol is easily separated from starting material and can be converted into the cyclopentenone derivative **9** in three steps [4].

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-

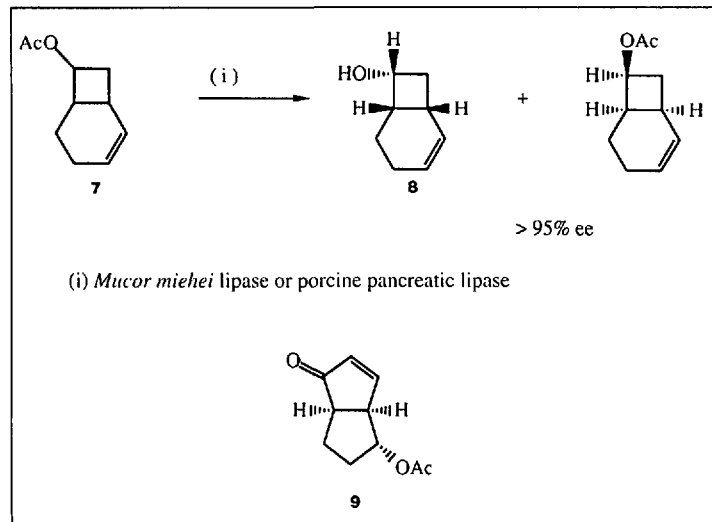
Scheme 1



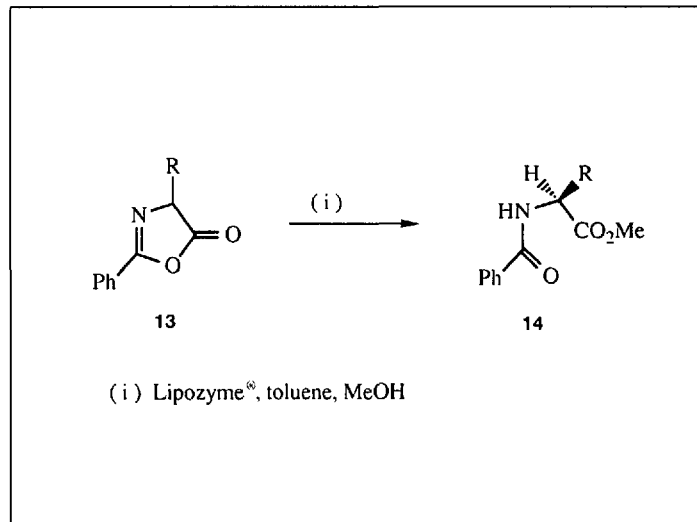
Scheme 3



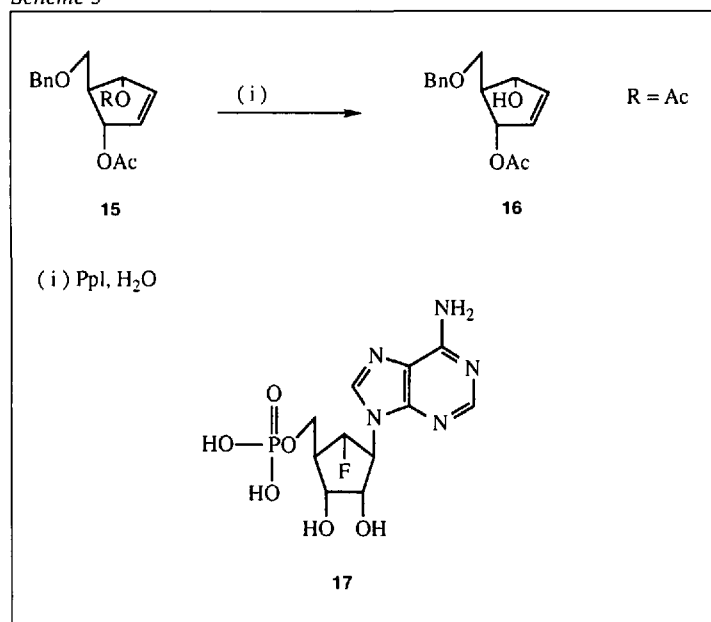
Scheme 2



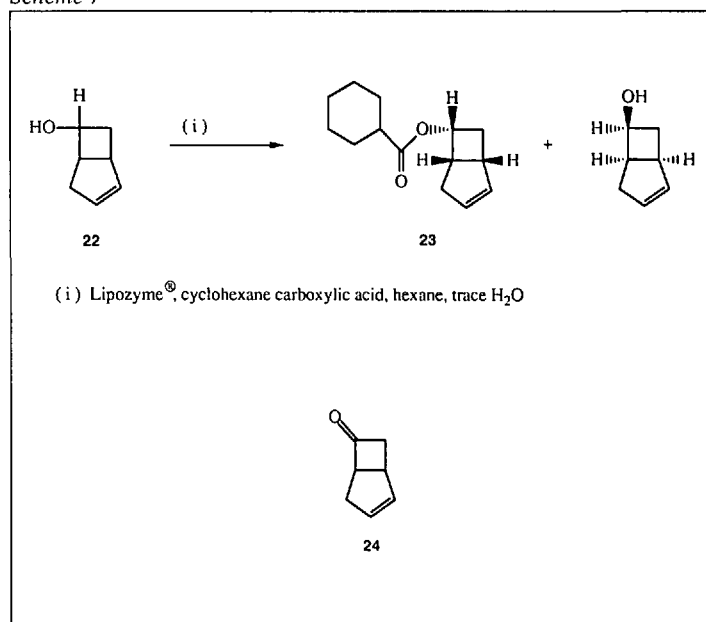
Scheme 4



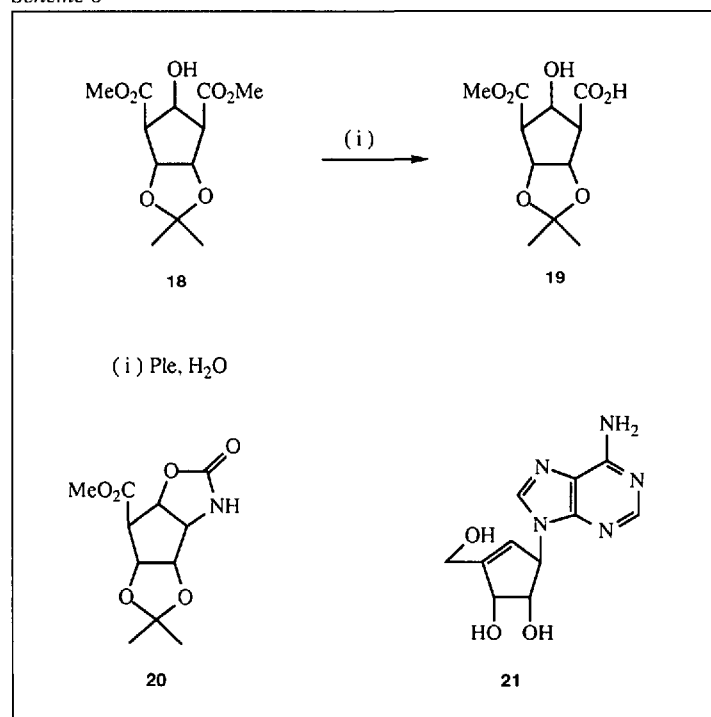
Scheme 5



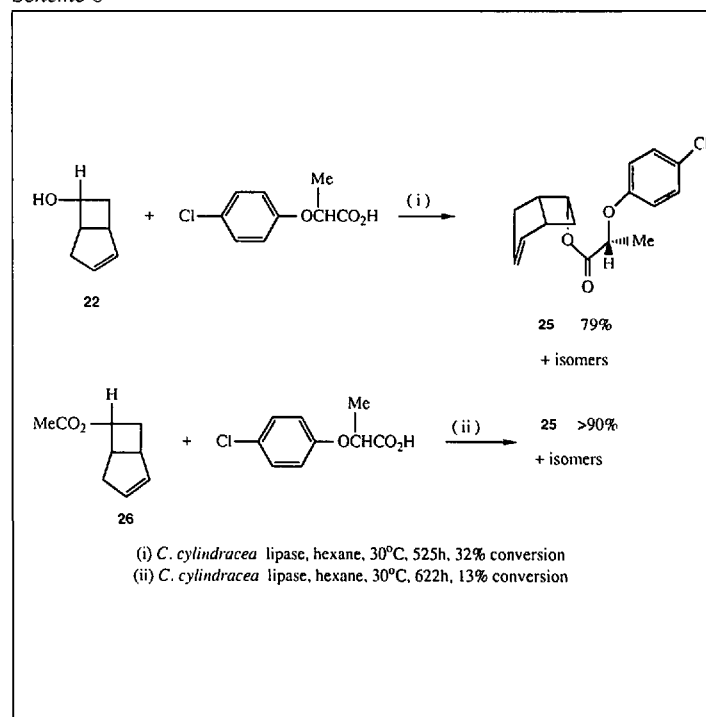
Scheme 7



Scheme 6



Scheme 8



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 amino acid **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 anti-herpes 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 aristeromycin [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)norbormadiene) is hydrolysed in a highly stereocontrolled manner by pig liver esterase (ple) to give the acid **19** (e.e. >95%), which is itself not a substrate for ple (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

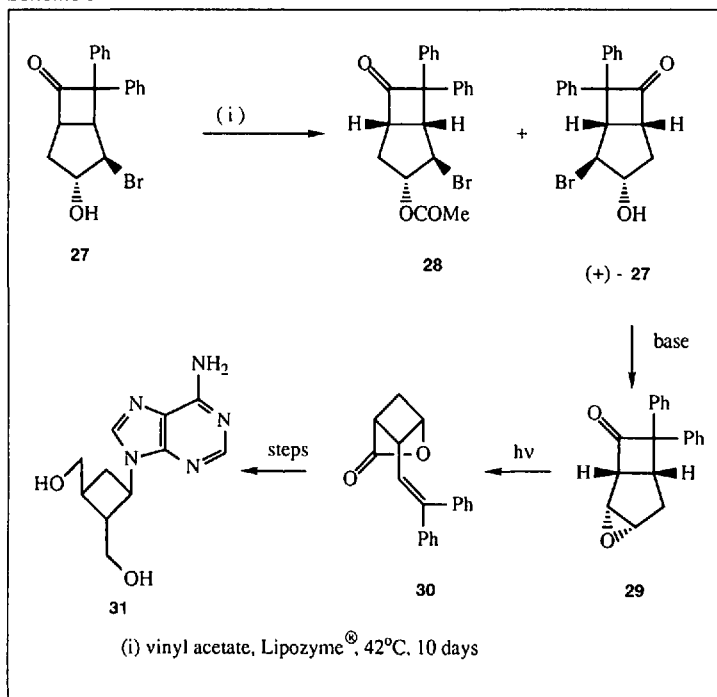
solvent to couple an acid with an alcohol or amine to give an ester or amide, respectively. Yet, such processes are readily achieved and they have rightly attracted a considerable amount of attention, since the popularization of the strategy by *Klibanov* in the mid-1980's. Thus, the alcohol **22** is 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].

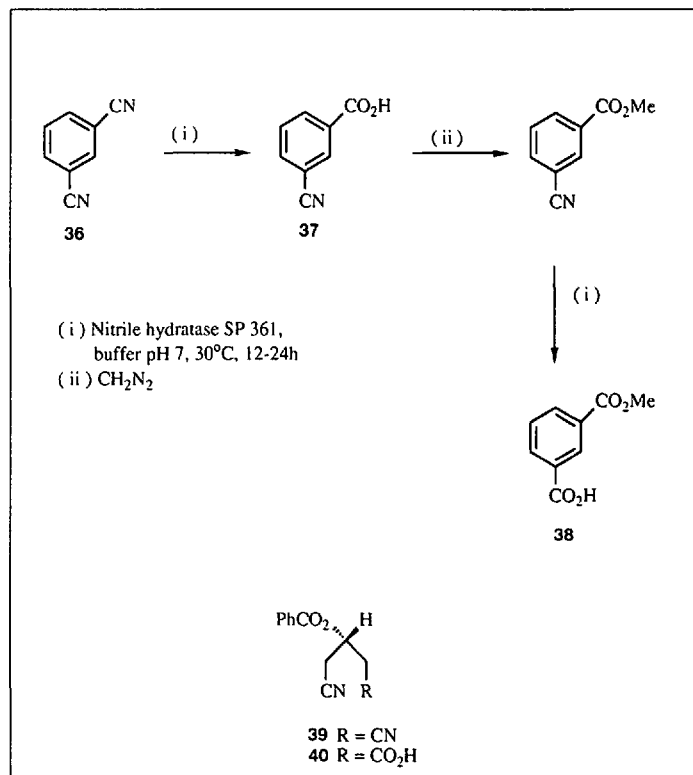
Inter-esterifications can also be effected using lipases and these reactions can show advantage over the corresponding esterification reaction. Thus, in the enzyme-catalysed 'double enantioselection' involving the racemic alcohol **22**, racemic *para*-chlorophenoxypropanoic acid and immobilized *M. miehei* lipase, diastereo-

isomer **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].

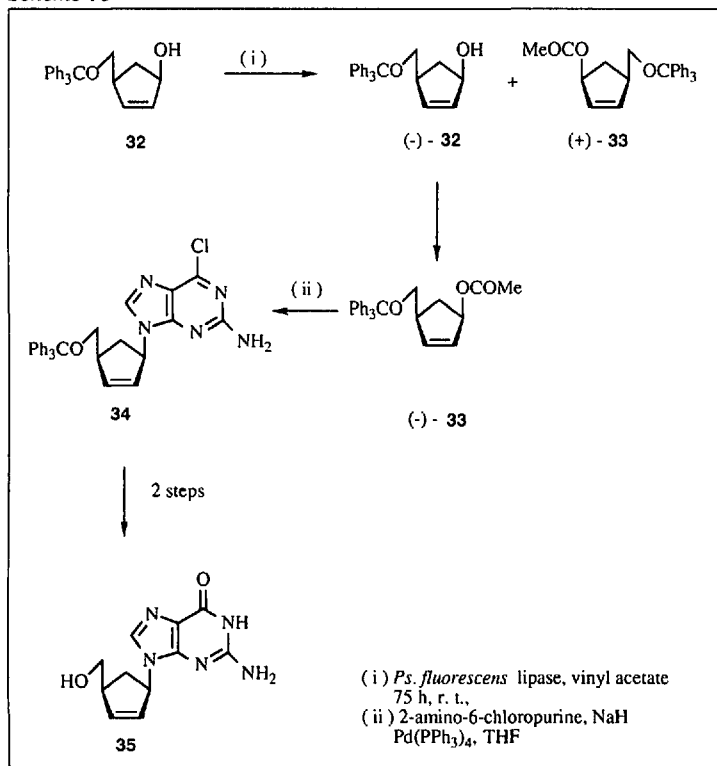
Scheme 9



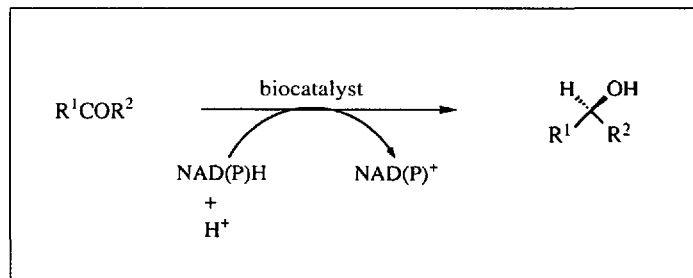
Scheme 11



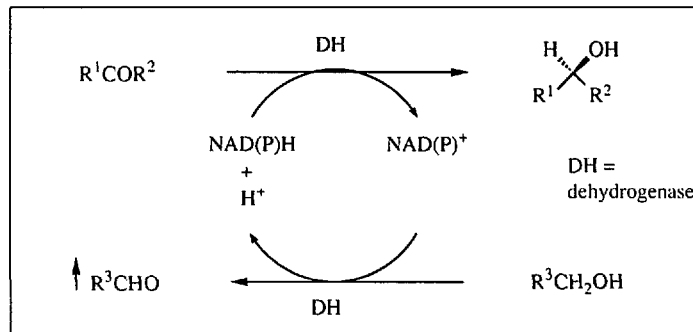
Scheme 10



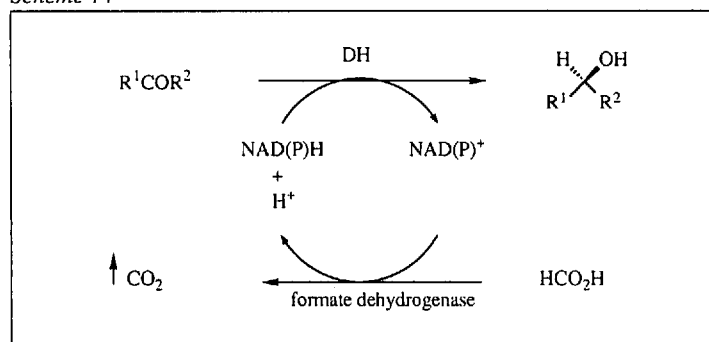
Scheme 12



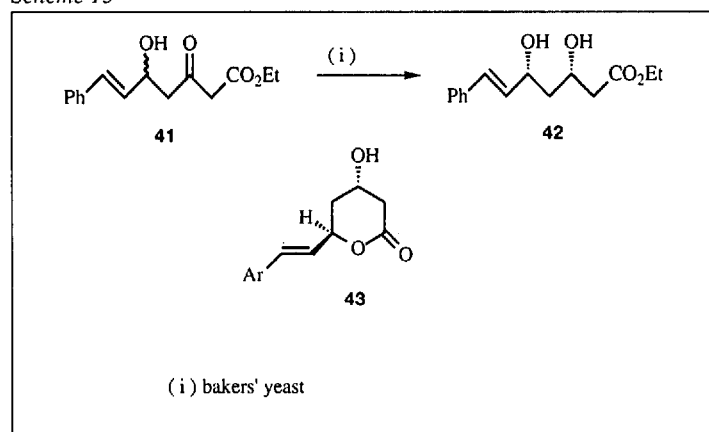
Scheme 13



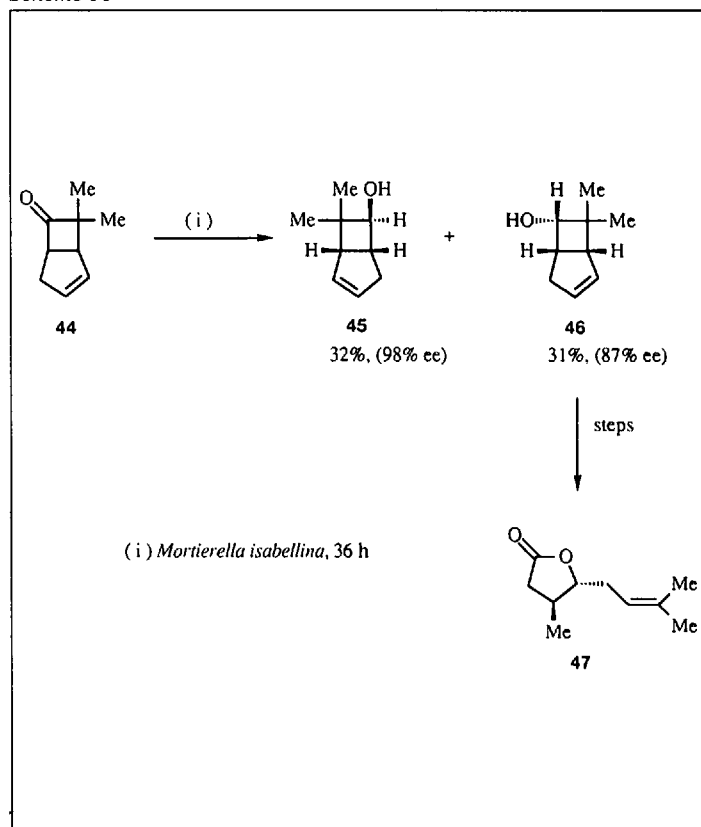
Scheme 14



Scheme 15



Scheme 16



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 antiherpetic agent carbocyclic oxetanocin-A **31** (Scheme 9) [14].

The alcohol **32** is available by performing a *Prins* reaction on cyclopentadiene followed by tritylation 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 Pd⁰ 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 nitrile hy-

dratases in synthesis is very significant as the following examples will help to illustrate. *meta*-Dicyanobenzene **36** is hydrolysed by a nitrile hydratase to give *meta*-cyanobenzoic 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 benzylation) 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 3(*R*)-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].

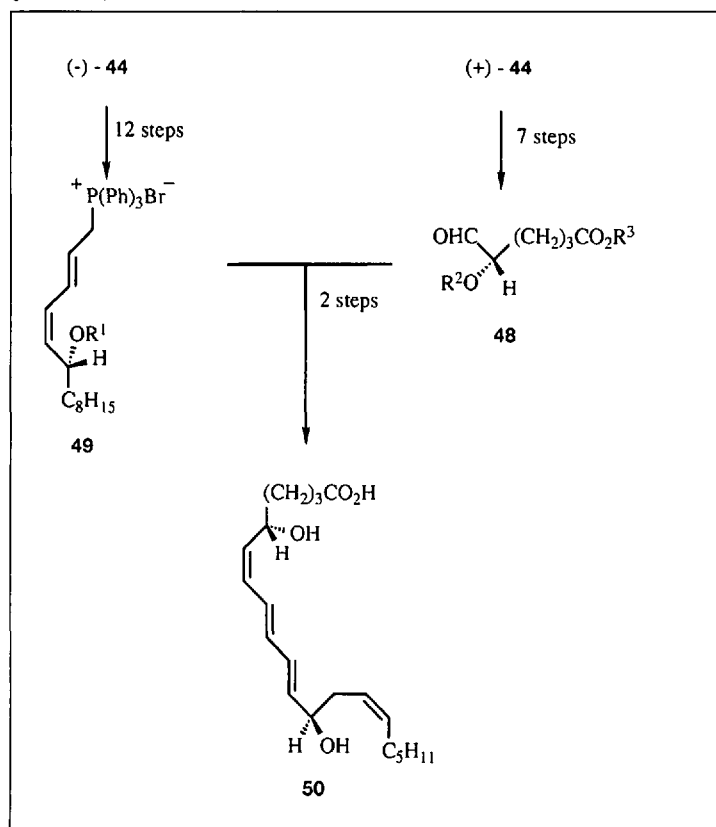
Similarly the ketone **44** is reduced by the fungus *Mortierella isabellina* to give two diastereoisomeric alcohols **45** and **46**, each of these diastereoisomers being made, more or less, from one enantiomer of the starting material [19]. The alcohol (**46**) was transformed into eldanolide **47**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

The ketone **44** is reduced enantioselectively using 3 α ,20 β -hydroxysteroid dehydrogenase (HSAD) to give the alcohol **46** in optically pure form and recovered

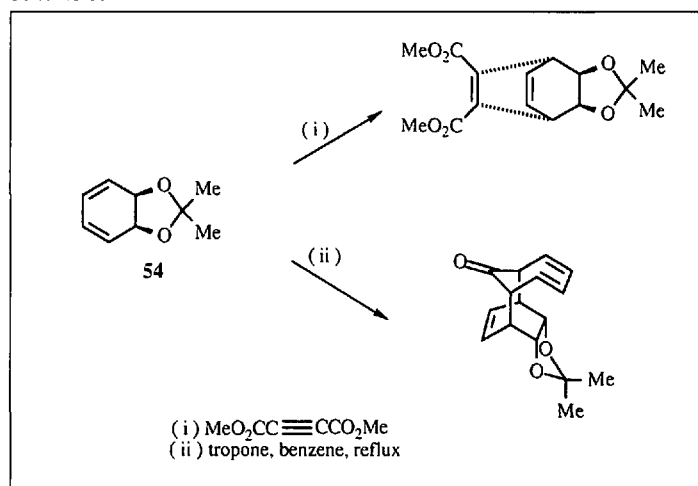
optically active ketone [21]. The alcohol, after separation from residual ketone, was oxidized and both the enantiomers of the ketone **44** obtained in this way were used in an enantiocomplementary route to the naturally-occurring polyene leukotriene-**B**₄ **50** (Scheme 17) [22]. Thus, the dextro-rotatory 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 monooxygenase. 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

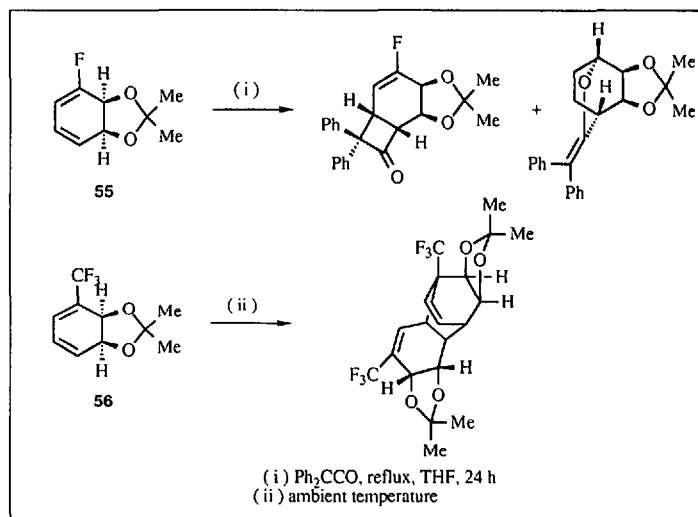
Scheme 17



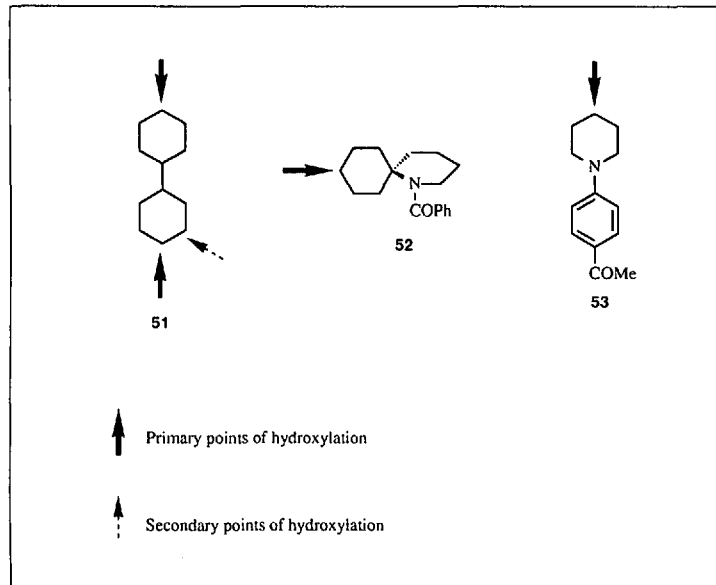
Scheme 19



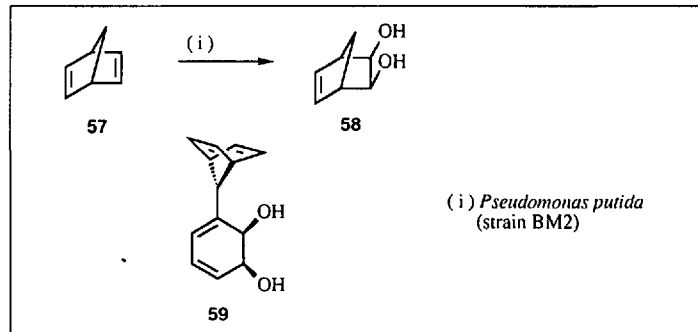
Scheme 20



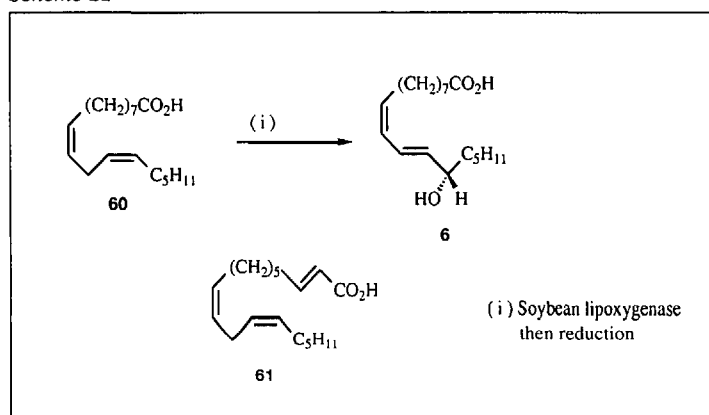
Scheme 18



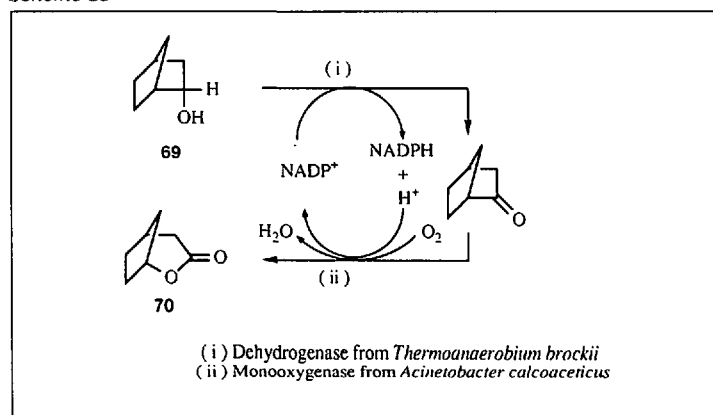
Scheme 21



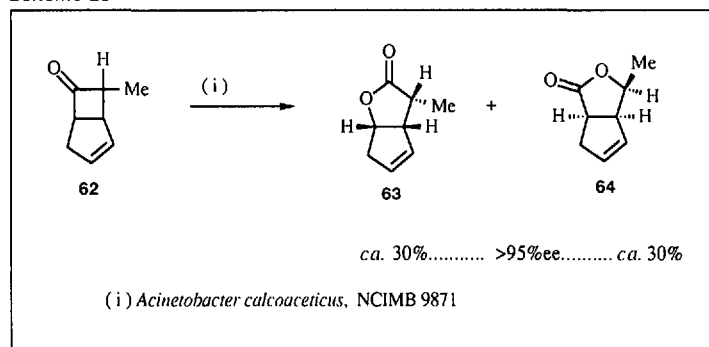
Scheme 22



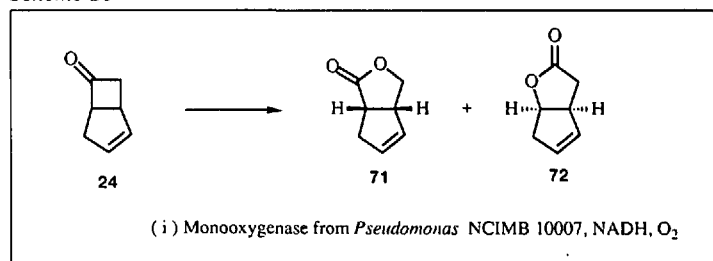
Scheme 25



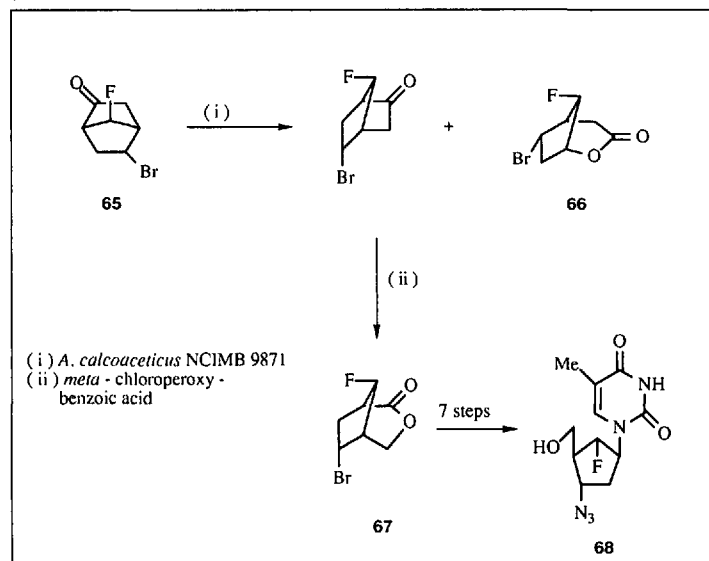
Scheme 23



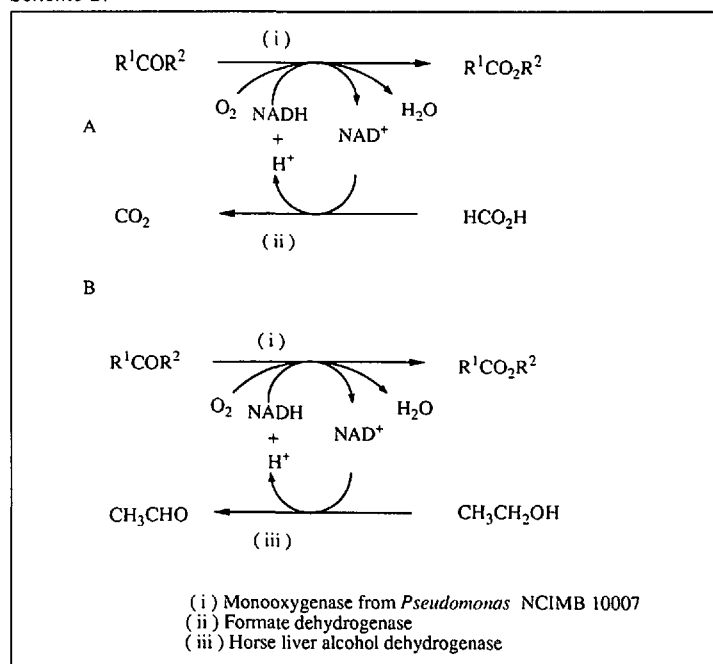
Scheme 26



Scheme 24



Scheme 27



cyclohexylcyclohexane **51**), heterocyclic compounds (such as the spiro-compound **52**) and arylalkyl species (such as the piperidine derivatives **53**) are hydroxylated in a highly selective, though somewhat unpredictable, fashion using organisms such as *Cunninghamella* sp. and *Beauveria sulforescens* (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 aceto-

nide 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 acetonide 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 nor-

bornadiene **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 enantiodivergent biotransformation; for example one 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 first-formed product(s). To preclude this, the 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 *Pseudomonas* 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 enzymic transformations of various types can be utilized in efficient syntheses of biologically active natural products and analogues.

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