Enzymes in organic synthesis: oxidoreductions

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This article reviews the use of several oxidoreduction enzymes, including monooxygenases, chloroperoxidase, arene dioxygenase, lipoxygenase, galactose oxidase and alcohol dehydrogenases, in the preparation of optically pure or enriched epoxides, halohydrins, sulfoxides, hydroperoxides, alcohols, cyclohexadienediols, lactones and α-hydroxy aldehydes. The mechanisms of some of these enzyme-catalysed transformations, and their scopes and limitations in synthetic chemistry are also discussed. Representative syntheses of a key intermediate of 5-lipoxygenase inhibitor, a seven-carbon C-glycoside and an active component of the antibiotic bialaphos have been experimentally illustrated using *Lactobacillus* alcohol dehydrogenase, chloroperoxidase and glutamate dehydrogenase, respectively.

Oxidoreductions, especially the stereoselective oxidation of olefins and reduction of carbonyls, are important organic reactions and have been practiced extensively in the laboratory. Enzymatic oxidoreductions [1] are, however, not very common in organic synthesis, though the methods are potentially useful and environmentally compatible. We describe here the enzymatic oxidoreductions of alkenes, sulfides, ketones and alcohols that are considered to be synthetically useful and discuss the advantages and drawbacks of each of the reactions.

We focus on the use of two classes of enzymes: metallooxidases and nicotinamide-dependent oxidoreductases. Metallooxidases are generally involved in the oxidation of inactive organic molecules such as alkanes, olefins, arenes and heteroatoms, while nicotinamide cofactor-dependent oxidoreductases are involved in the oxidation of alcohols and reduction of ketones. For the oxidations of alcohols that require nicotinamide adenine dinucleotide (NAD) or its phosphate (NADP), or the reductions of carbonyl compounds mediated by the reduced forms of the nicotinamide cofactors NADH or NADPH, regeneration of the co-factors [2] from their reaction byproducts is necessary, as it not only reduces the cost but also provides some synthetic advantages, it can: (i) drive a thermodynamically unfavourable reaction by coupling with a favourable co-factor regeneration reaction; (ii) prevent accumulation of the co-factor by-product that may inhibit the forward process; (iii) eliminate the need for stoichiometric quantities of co-factors and thus simplify the reaction work-up; and (iv) increase enantioselectivity relative to stoichiometric reactions in many cases.

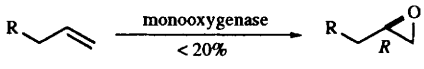
We first review the synthetic applications of metallooxidases and nicotinamide-dependent oxidoreductases, then illustrate experimentally the use of some of these enzymes in synthesis.

I. Monooxygenase

I-1. Epoxidation

A few examples of non-biological catalytic asymmetric epoxidation of olefins have been reported. With the assistance of a hydroxy group, epoxidation of the C=C double bonds of allylic alcohols, for example, can be achieved by using the Katsuki–Sharpless method which employs dialkyl tartrates as the catalysts.[3] Recent work by Jacobsen's group demonstrates a method using Mn^{III}-salen catalysts, which are especially useful for asymmetric epoxidation of aryl-substituted alkenes.[4] In these two methods, oxidants such as Bu^{t}OOH, NaOCl and PhIO are generally utilized to transfer one oxygen atom to the alkene substrate. Mukaiyama's group uses molecular oxygen and metal catalysts to effect epoxidation of certain cycloalkenes with modest asymmetric induction.[5] Asymmetric epoxidation of acyclic alkenes bearing only aliphatic substituents is, however, still a major problem. Biological catalysts [6] such as monooxygenase [7] and chloroperoxidase have been exploited for the epoxidation of protected allyl alcohol derivatives.

Enzymatic epoxidation of terminal alkenes is best carried out with *Pseudomonas oleovorans* monooxygenase (POM) to give (*R*)-epoxides (Scheme 1).[7] Either growing or resting cells of



R	% ee
$Me(CH_2)_4$	70
$CH_2{=}CH(CH_2)_3$	80
$Me(CH_2)_3O$	85
$CH_2{=}CHCH_2O$	81
$PhCH_2O$	75
PhO	92
p-$MeOC_6H_4O$	98
p-FC_6H_4O	99

Scheme 1 *Reagents and conditions: Pseudomonas oleovorans* cells, 30 °C, 12 h. See text for experimental details.

POM have been used for this transformation. More substituted alkenes are inert under these reaction conditions. Although allyl alcohols are inhibitors of this enzyme, allylic ethers are generally good substrates yielding the corresponding (*R*)-epoxides. The typically low yield (< 20%) of enzymatic epoxidation is a drawback. The NADH/O_2-dependent enzyme POM is a non-heme monooxygenase which contains iron in the active site for catalysis. The active oxygen species is a high valent iron-oxo species formally denoted as $Fe^V{=}O$.[8,9] The reaction is considered to proceed through a stepwise radical mechanism and the stereospecificity is controlled by the stereoenvironment of the enzyme's active site (Scheme 2).

The reaction with heme-containing cytochrome P-450 and non-heme *Methylosinus trichosporium* monooxygenase may proceed through a similar radical process,[10] whereas the Mn^{III}-salen mediated epoxidation [11] may involve a concerted or stepwise process depending on the alkene substrates. *trans*-2-Phenyl-1-vinylcyclopropane and related compounds have been designed and employed as sensitive probes to distinguish the reaction mechanisms.[11,12] Reaction of POM with *trans*-2-phenyl-1-methylcyclopropane gave 1-phenylbut-3-en-1-ol as a

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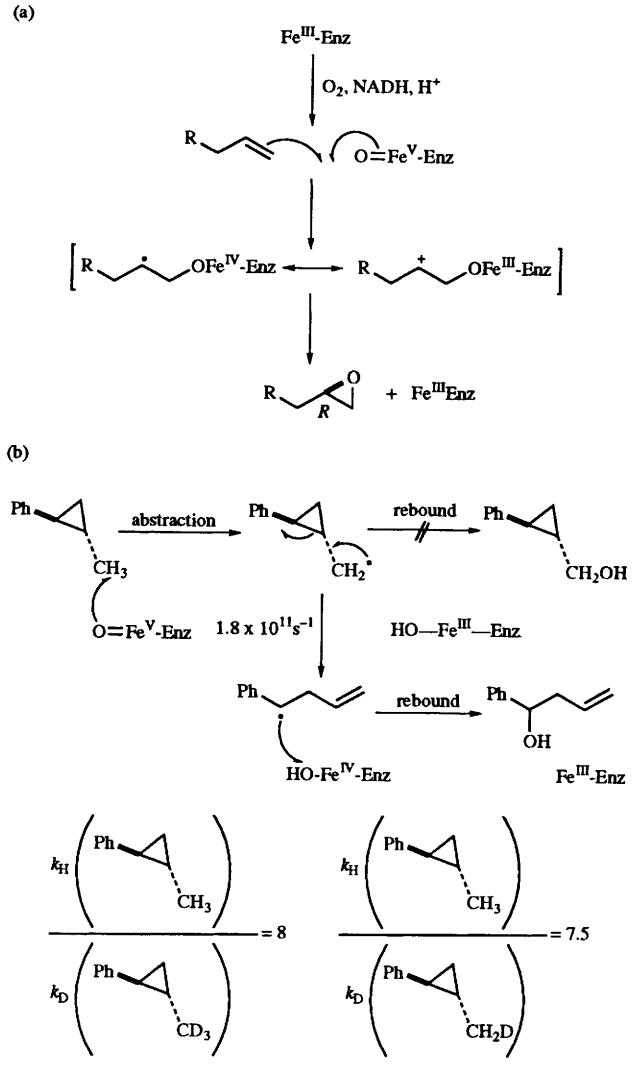
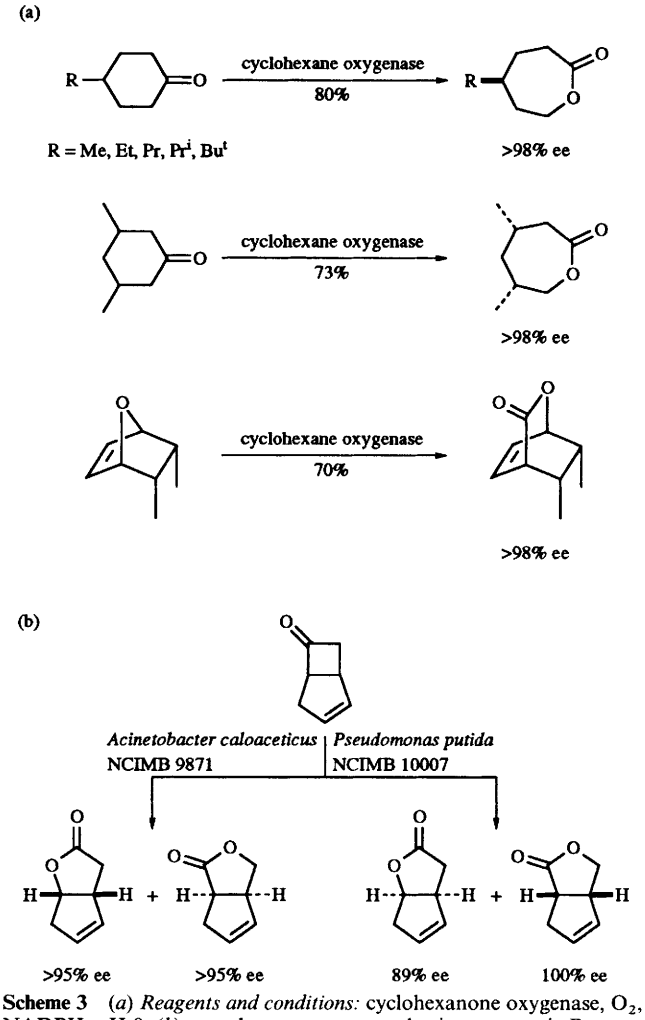


Scheme 2 Reaction mechanisms of *Pseudomonas oleovorans* monooxygenase catalysed epoxidation of alkenes and hydroxylation of alkanes

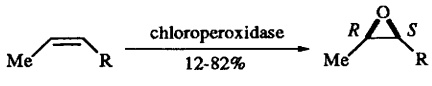


Scheme 3 (*a*) *Reagents and conditions:* cyclohexanone oxygenase, O_2, NADPH, pH 8; (*b*) complementary stereoselective enzymatic Baeyer–Villiger reactions

single product.[12] This result and the observed high kinetic isotope effect (k_H/k_D *ca.* 8) support a nonconcerted radical process. This is the first demonstration of the use of a radical probe with the radical ring-opening rate exceeding the oxygen rebound rate in the study of enzymatic hydroxylation.

I-2. Baeyer–Villiger reaction

The oxidation of ketones to esters or lactones by the Baeyer–Villiger reaction is a well established synthetic transformation. The reaction is typically effected by a peracid or a hydroperoxide in the presence of an appropriate metal ion. Enantioselective transformation of cyclic and bicyclic ketones to lactones can be carried out by enzymatic methods,[13] whereas non-enzymatic methods have not been developed. For example, cyclohexanone monooxygenase from *Acinetobacter calcoaceticus* NCIMB 9871 catalyses the Baeyer–Villiger oxidation of many cyclic and acyclic ketones in addition to cyclohexanone (Scheme 3).[14] The enzymatic reaction proceeds with the order of tertiary alkyl > secondary alkyl > primary alkyl > methyl group migration with retention of configuration at the migrating group;[15] which is similar to that of the chemical reaction. The enzymatic Baeyer–Villiger reaction requires a bound flavin co-factor in the presence of NAD(P)H and oxygen. The flavin 4α-hydroperoxide intermediate[15,16] acts as a nucleophile when reacting with the substrate carbonyl group; but becomes an electrophile when oxidizing heteroatoms.[13] Cyclohexanone oxygenase is unable to oxidize alkenes, thus, *meso*-5,6-dimethylbicyclo[2.2.1]hept-3-en-7-one is selectively oxidized to the corresponding lactone (> 98% ee) with retention of the C=C double bond.[17] The Baeyer–Villiger reaction of some bicyclic ketones is also achieved by catalysis of a monooxygenase from *Pseudomonas putida* NCIMB 10007.[18] Bicyclo[3.2.0]hept-2-en-6-one is oxidized to optically pure and enantiomeric lactones using *P. putida* and *A. calcoaceticus*, respectively (Scheme 3). These reactions require NADH or NADPH as reducing source and therefore regeneration of the co-factor *in situ* is necessary for practical synthesis in a cell-free system.



R	% ee
Pr	97
Bu	96
C_5H_{11}	92
Bu^i	94
Ph	96

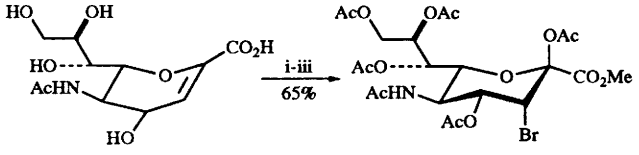
Scheme 4 *Reagents and conditions:* chloroperoxidase, H_2O_2, citrate buffer, pH 5

II. Chloroperoxidase

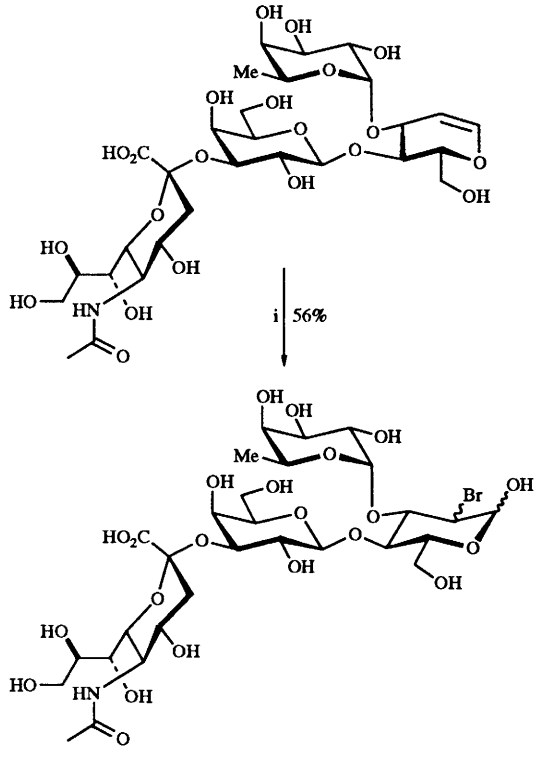
II-1. Epoxidation and halohydration

Similar to monooxygenases, the chloroperoxidase (CPO) from *Caldariomyces fumago* also catalyses the epoxidation of alkenes in the presence of H_2O_2,[19] although the reactions are very slow compared to other types of CPO reactions. Chloroperoxidase utilizes H_2O_2, whereas P-450 cytochromes use molecular oxygen and a reducing reagent such as NADH. Under controlled conditions with a slow addition of H_2O_2, CPO-catalysed epoxidation of unfunctionalized *cis* alkenes gives epoxides in variable yields with ee values ranging from ~ 10 to 97% (Scheme 4). The CPO-based system accepts certain 1,1-disubstituted and trisubstituted alkenes, but not *trans* disubstituted alkenes, as substrates. Since chloroperoxidase is commercially available and has been cloned and overexpressed,[20] it appears to be a useful 'free monooxygenase' for synthetic applications.

The chloroperoxidase from *C. fumago* catalyses the oxidation of all halide ions except fluoride.[21] In the presence of an alkene, the products formed by haloperoxidase-catalysed reactions are consistent with the products obtained from the corresponding non-enzymatic reaction with hypohalous acid.[22] When glycals are used as substrates, halohydration occurs regioselectively to give 2-deoxy-2-halo-sugars (Scheme 5).[23] The CPO-catalysed



2, 3-dehydrosialic acid



NeuAcα(2, 3)GAlβ(1, 4)[Fucα(1, 3)]glucal

Scheme 5 *Reagents and conditions:* i, chloroperoxidase, KBr, H_2O_2, citrate buffer, pH 3, room temp., 3 h; ii, Ac_2O, pyridine; iii, MeI, Cs_2CO_3

bromohydration of 2,3-dehydrosialic acid is regio- and stereospecific, giving 3-deoxy-3-bromosialic acid exclusively in 65% isolated yield. Chemical bromohydration of 2,3-dehydrosialic acid with *N*-bromosuccinimide, however, gave a 40:60 diastereoisomeric mixture of 3-deoxy-3-bromosialic acid. The enzymatic reactions with D-galactal and L-fucal in aqueous solution gave 2-bromo-2-deoxy-D-galactose and 2-bromo-2-deoxy-L-fucose, respectively, with the 2-bromo substituent equatorially orientated. The unfavourable 1,3-diaxial interaction may preclude the formation of 2-axially substituted product. The enzymatic bromohydration of glucal, Galβ(1,4)glucal, Galβ(1,3)glucal or NeuAcα(2,3)Galβ-(1,4)[Fucα(1,3)]glucal is regiospecific and gives, respectively, the corresponding 2-bromo saccharides, but no stereoselectivity is observed at the 2-position (Scheme 5). The bromohydrin obtained from NeuAcα(2,3)Galβ(1,4)[Fucα-(1,3)]glucal is an inhibitor of the cell adhesion molecule E-selectin. In the presence of high concentrations of a second nucleophile, the CPO-catalysed reaction gives dihalides.[24]

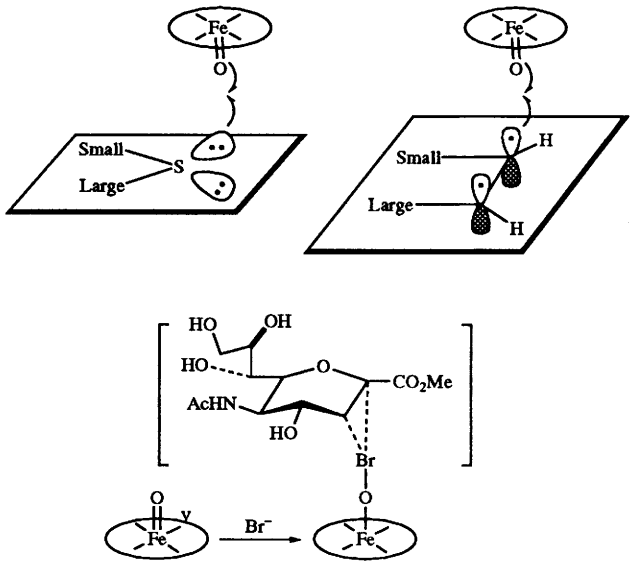
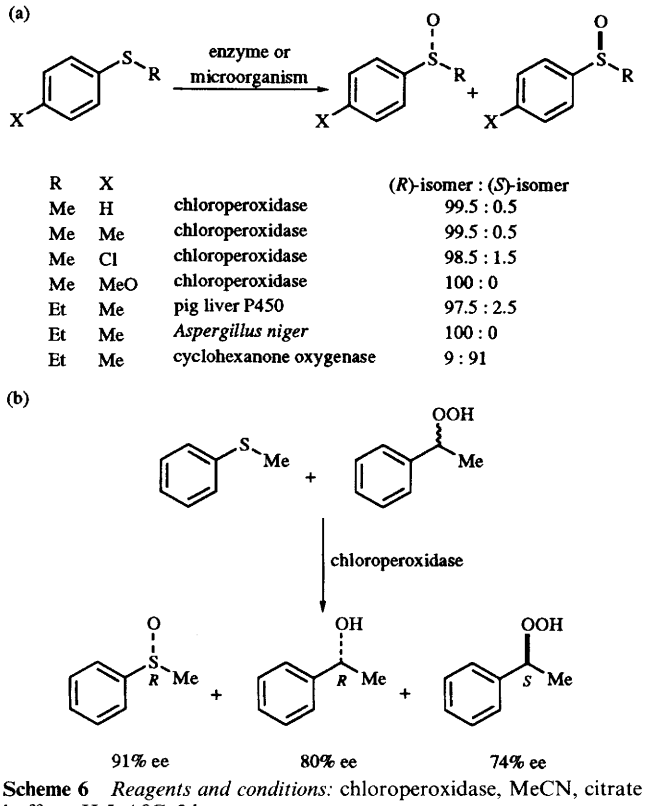


Fig. 1 Putative mechanisms of chloroperoxidase-catalysed oxidation of sulfides, epoxidation and halohydration of alkenes

In addition to monooxygenases, haloperoxidase,[25] horseradish peroxidase,[25] cytochrome C peroxidase[26] and lactoperoxidase[27] all utilize the putative iron-oxo (Fe^{V}=O) species for reaction with substrates. This reactive species perhaps forms a bound hypohalite which reacts with alkenes regio- and stereoselectively (Fig. 1). It appears that certain epoxides with well-defined absolute configuration may be obtained from the halohydrins produced *via* chloroperoxidase-catalysed addition of HOX to C=C double bonds.[25a,28]

II-2. Oxidation of sulfides

Oxidation of sulfides to chiral sulfoxides by biological and chemical methods has been reviewed.[29] Optically active sulfoxides, such as methyl *p*-tolyl sulfoxides, are versatile substrates for the synthesis of many natural products. Monooxygenases and chloroperoxidase have been used for the oxidation of sulfides, amines, phosphines and selenides.[23b,30] By choosing an appropriate organism, formation of either the *R* or *S* sulfoxides can be achieved (Scheme 6). A recent work[23b] shows that oxidation of sulfides catalysed by the chloroperoxidase from *C. fumago* is highly enantioselective, and three chiral compounds are prepared simultaneously when a chiral hydroperoxide is used as oxidant. For example, in the CPO-catalysed oxidation of phenyl methyl sulfide using racemic 1-phenylethyl hydroperoxide as oxidant, the sulfide is selectively oxidized to (*R*)-sulfoxide with a concurrent formation of (*S*)-1-phenylethyl hydroperoxide and (*R*)-1-phenylethanol. A drawback of this enzymatic process is that the undesired chemical oxidation of sulfides occurs, though it can be minimized



R	X		(*R*)-isomer : (*S*)-isomer
Me	H	chloroperoxidase	99.5 : 0.5
Me	Me	chloroperoxidase	99.5 : 0.5
Me	Cl	chloroperoxidase	98.5 : 1.5
Me	MeO	chloroperoxidase	100 : 0
Et	Me	pig liver P450	97.5 : 2.5
Et	Me	*Aspergillus niger*	100 : 0
Et	Me	cyclohexanone oxygenase	9 : 91

Scheme 6 *Reagents and conditions:* chloroperoxidase, MeCN, citrate buffer, pH 5, 4 °C, 3 h

considerably by controlling the concentration of the substrates. In a kinetic study, for example, the K_m value for a sulfide is 94 μmol dm^{-3} and that for a hydroperoxide is 104 μmol dm^{-3}. Thus, when the enzymatic conversion of the sulfide into sulfoxide is conducted at 4.0 × 10^{-4} mol dm^{-3} for both substrates, the chemical oxidation is minimized.

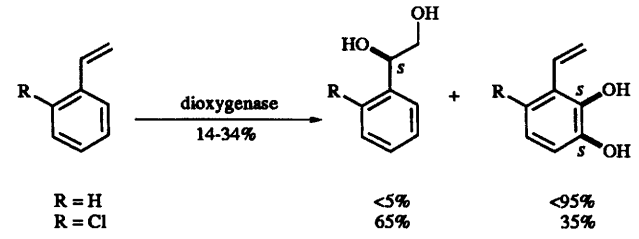
Fig. 1 shows the putative mechanism of chloroperoxidase-catalysed oxidation of sulfides that exhibits the enantiotopic preference similar to that observed in the epoxidation of alkenes.

III. Arene dioxygenase

Although a chemical method using osmium(VIII) oxide and chiral ligands such as dihydroquinine or dihydroquinidine derivatives as catalysts converts mono-, di- and tri-substituted alkenes into the corresponding *cis* diols in an enantioselective manner,[31] dihydroxylations of arenes can only be effectively achieved by biological methods.[32] A dioxygenase from *Pseudomonas putida* catalyses the oxidation of benzene, toluene, chlorobenzene, bromobenzene and other arene derivatives to the corresponding *cis*-cyclohexa-3,5-diene-1,2-diols.[33] These cell reactions gave the *cis* cyclohexadienediols with high ee values, and the diols have been used in the synthesis of many natural products such as terpenes, prostaglandins, carbocyclitols, as well as in the synthesis of polybenzenes for the manufacturing of liquid crystals, fibres and films.[34] The enzymatic dihydroxylation of styrene occurs exclusively at the arene moiety, whereas the reaction of *o*-chlorostyrene gives a major product with dihydroxylation occurring at the olefinic double bond (Scheme 7).[35]

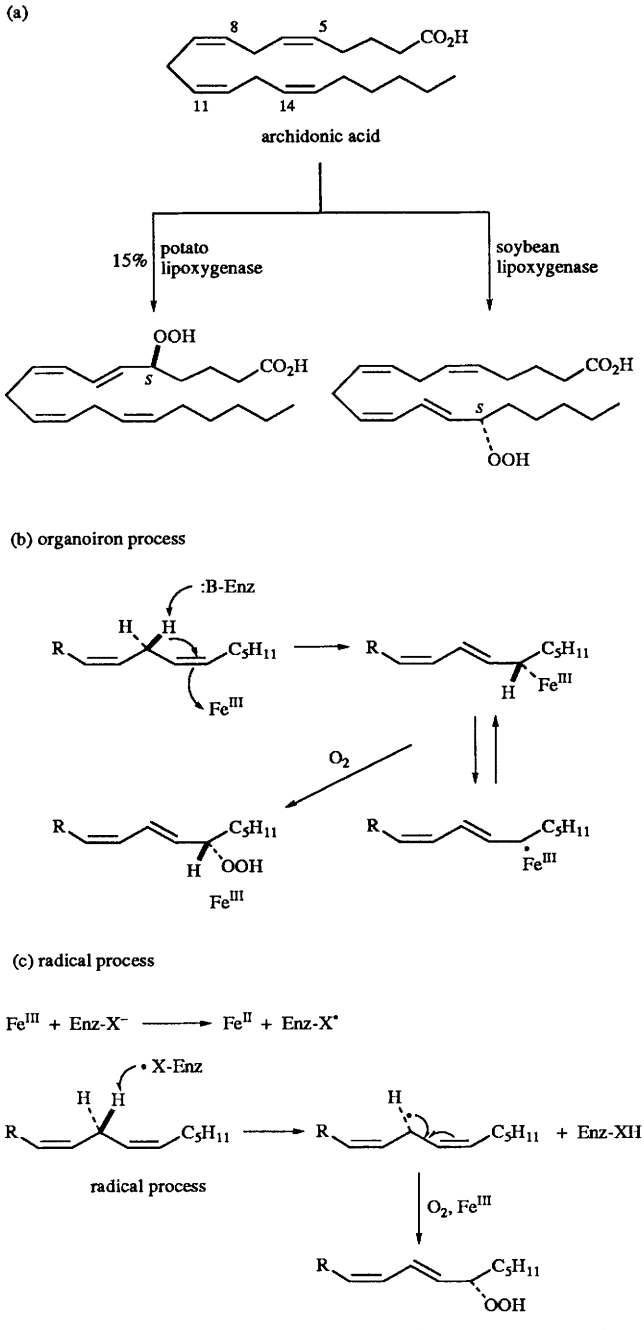
IV. Lipoxygenase

Lipoxygenase is a non-heme iron-containing dioxygenase which catalyses the oxidation of a polyunsaturated fatty acid to a hydroperoxide. In the case of arachidonic acid, the



Scheme 7 *Reagents and conditions: Pseudomonas putida* strain 39-D, O_2, pH 7.2

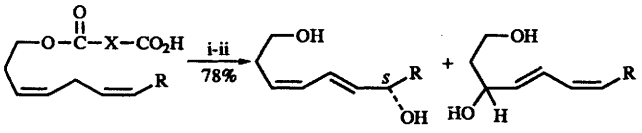
lipoxygenation can occur at carbon 5, 8, 9, 11, 12 or 15 depending on the specific lipoxygenase used. Potato lipoxygenase converts arachidonic acid on a synthetic scale into the (5*S*)-hydroperoxide (Scheme 8),[36] which is a useful intermediate for



Scheme 8 *Reagents and conditions:* potato lipoxygenase (or soybean lipoxygenase), O_2, NH_4OH, pH 6.4, 20 °C, 30 min

the synthesis of leukotrienes. The 5-lipoxygenase contains a high-spin Fe^{III} centre in the active form which specifically abstracts the 7*S* hydrogen atom from the substrate. Soybean lipoxygenase converts arachidonic acid into the (15*S*)-hydroperoxide and linoleic acid into the (13*S*)-hydroperoxide.[37] The reaction is considered to proceed through a free radical intermediate which reacts directly with oxygen or a δ-organoiron intermediate.[38] Based on the results of kinetic isotope effects, stereochemical outcomes, and inhibition studies, it seems that the organoiron process is more likely.

Recent investigation of soybean lipoxygenase regarding the synthesis of chiral diols (Scheme 9) indicates that a decrease of



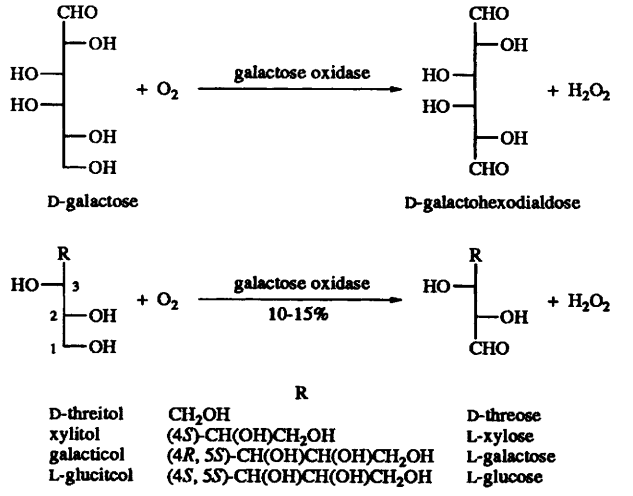
R	X	
C_5H_{11}	$(CH_2)_4$	95:5
C_6H_{13}	$(CH_2)_4$	87:13
C_7H_{15}	$(CH_2)_4$	73:27
C_8H_{17}	$(CH_2)_4$	50:50
C_8H_{17}	$(CH_2)_5$	66:34
C_8H_{17}	$(CH_2)_3$	36:64
C_8H_{17}	$(CH_2)_2$	26:74

Scheme 9 *Reagents and conditions:* i, soybean lipoxygenase, O_2, borate buffer, pH 8.5, 0 °C, 1 h; ii, $MeSCH_2CH_2OH$, 23 °C, 7 h; iii, KOH, 23 °C, 12 h

hydrophobicity for the R group and an increase of methylene units between the two carboxy groups (indicated by X) will lead to the increase of the product with the OH group next to R.[39] The reactions are best performed with sonication of whole cells. The enzymatic reaction is formally a stereospecific ene reaction of alkenes with molecular oxygen, whereas chemical preparation of chiral allylic hydroperoxides[40] is often conducted with singlet oxygen. In the chemical process, the stereoselectivity can be obtained only by steric bias, as with steroid substrates, or by the directing effect of a hydroxy group, as in the epoxidation of allylic alcohols.

V. Galactose oxidase

The galactose oxidase (GO) from *Dactylium deudroides* catalyses the oxidation of D-galactose at the C-6 position in the presence of oxygen to give D-galactohexodialdose and hydrogen peroxide (Scheme 10).[41] The enzyme contains one atom of



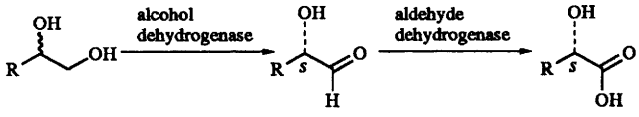
R		
D-threitol	CH_2OH	D-threose
xylitol	(4*S*)-$CH(OH)CH_2OH$	L-xylose
galacticol	(4*R*, 5*S*)-$CH(OH)CH(OH)CH_2OH$	L-galactose
L-glucitcol	(4*S*, 5*S*)-$CH(OH)CH(OH)CH_2OH$	L-glucose

Scheme 10 *Reagents and conditions:* galactose oxidase, catalase, phosphate buffer, pH 7, room temp., 5 days

Cu^{II} per molecule as co-factor. Recent investigations indicate that the enzyme catalyses the stereospecific oxidation of glycerol, 3-halogenopropane-1,2-diols and polyols to the corresponding aldehydes.[42] Several unusual L-sugars have also been prepared using this enzyme. It appears that a substrate alcohol with configuration similar to that of D-galactose from C-4 to C-6 would be oxidized to an aldehyde. The by-product hydrogen peroxide usually must be destroyed with catalase to avoid inactivation of the enzyme. One problem with this enzyme reaction is the product inhibition resulting in low yields (10–15%).

VI. Alcohol dehydrogenase

The nicotinamide-dependent alcohol dehydrogenases have been extensively studied, especially that from horse liver.[43a] The enantioselective oxidation of primary alcohols catalysed by horse liver alcohol dehydrogenase (HLADH) provides a useful route to chiral aldehydes. A series of racemic α-hydroxy and α-amino alcohols were oxidized to the corresponding aldehydes with good yields and high ee values (Scheme 11).[43b] The



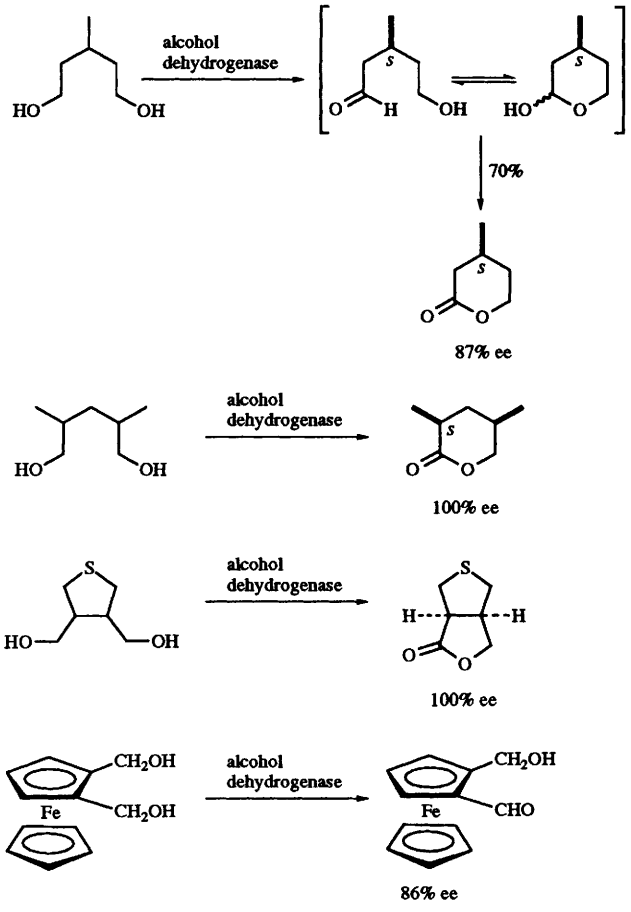
R = CH_3, $MeCH_2$, FCH_2, $ClCH_2$, $BrCH_2$, $HOCH_2$, H_2NCH_2, CH_2=CH >97% ee

Scheme 11 *Reagents and conditions:* horse liver alcohol dehydrogenase, aldehyde dehydrogenase, glutamate dehydrogenase, NAD, ammonium α-ketoglutarate, pH 8.0

reaction is thermodynamically unfavourable and requires NAD. This problem is circumvented in certain cases by using a coupling enzyme system consisting of HLADH and an aldehyde dehydrogenase. The α-hydroxy or α-amino aldehyde formed is irreversibly converted *in situ* into an α-hydroxy or α-amino acid. Oxidation of 2-aminopropane-1,3-diol using HLADH, for example, gives L-α-amino-β-hydroxypropionic acid with 96% ee.

Oxidation of an enantiotopic group of prochiral *meso* diols catalysed by HLADH yields lactones with a high degree of *pro-S* enantioselectivity (Scheme 12).[44] The enzymatic reaction presumably proceeds *via* the intermediate hydroxy aldehyde and the hemiacetal, which undergoes further oxidation to give the (*S*)-lactone. These reactions are thermodynamically favourable and provide a method to obtain valuable 5- and 6-membered chiral lactones, which are useful synthetic intermediates. Monocyclic *meso* diols are also transformed into chiral lactones with the same enantioselectivity. The HLADH-catalysed oxidation of *meso* diols linked with a tetrathiophene ring also provides optically pure lactones.[44d] In addition, stereospecific oxidation of 1,2-bis(hydroxymethyl)ferrocene gave (1*R*,2*S*)-2-(hydroxymethyl)ferrocenecarbaldehyde.[45] The alcohol dehydrogenase isolated from yeast (YADH) oxidizes allenic alcohols to allenic aldehydes,[46] which may be difficult to obtain chemically. Enzymatic oxidation of alcohols to aldehydes are, however, generally difficult due to the problem of product inhibition.

Alcohol dehydrogenases also catalyse the reductions of carbonyl compounds to the corresponding alcohols in the presence of NADH or NADPH as the co-factor. For example,[45] ferrocene-1,2-dicarbaldehyde was reduced with HLADH and NADH to give (1*S*,2*R*)-(hydroxymethyl)ferrocenecarbaldehyde, which is an antipode of the product obtained from the HLADH-catalysed oxidation of 1,2-bis(hydroxymethyl)ferrocene. The reversible hydride transfer from a reduced substrate to NAD(P) and that from NAD(P)H to an oxidized substrate is



Scheme 12 *Reagents and conditions:* horse liver alcohol dehydrogenase, glutamic dehydrogenase, NAD, ammonium α-ketoglutarate, pH 8.3, 30 °C, 9 h

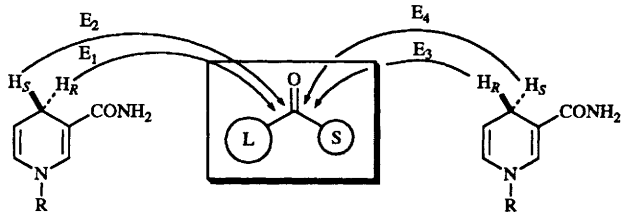


Fig. 2 Stereospecificity of alcohol dehydrogenase catalysed reductions: E_1 = alcohol dehydrogenase from *Pseudomonas* species or *Lactobacillus kefir* having the *pro-R*/*si*-face stereospecificity; E_2 = alcohol dehydrogenase from *Mucor javanicus* having the *pro-S*/*si*-face stereospecificity; E_3 = alcohol dehydrogenase from yeast, horse liver or *Thermoanaerobium* species having the *pro-R*/*re*-face stereospecificity; E_4 = the enzyme having *pro-S*/*re*-face stereospecificity is unknown. R = adenine ribose pyrophosphate, L = large substituent and S = small substituent.

stereoselective and characteristic of individual enzymes. Each enzyme is able to transfer stereospecifically one of the diastereotopic methylene hydrogens at C-4 of NAD(P)H to a substrate carbonyl group or an equivalent sp^2 centre (C=C or C=N) with high enantiofacial or diastereofacial selectivity. In principle, any NAD(P)H dependent oxidoreduction should fall into one of these four types of stereospecificity (Fig. 2). However, most alcohol dehydrogenases catalyse the transfer of *pro-R* hydrogen to the *re*-face of a carbonyl substrate, a process known as Prelog's rule (for simplicity, the *re*-face here refers to the bottom face of a carbonyl group with a small substituent on the right and a large substituent on the left side). This includes enzymes from yeast,[47] horse liver,[47] and *Thermoanaerobium brockii*[48] (E_3 in Fig. 2). The alcohol dehydrogenase from *Mucor javanicus*[47] (E_2 in Fig. 2) is specific for the *pro-S* hydrogen of

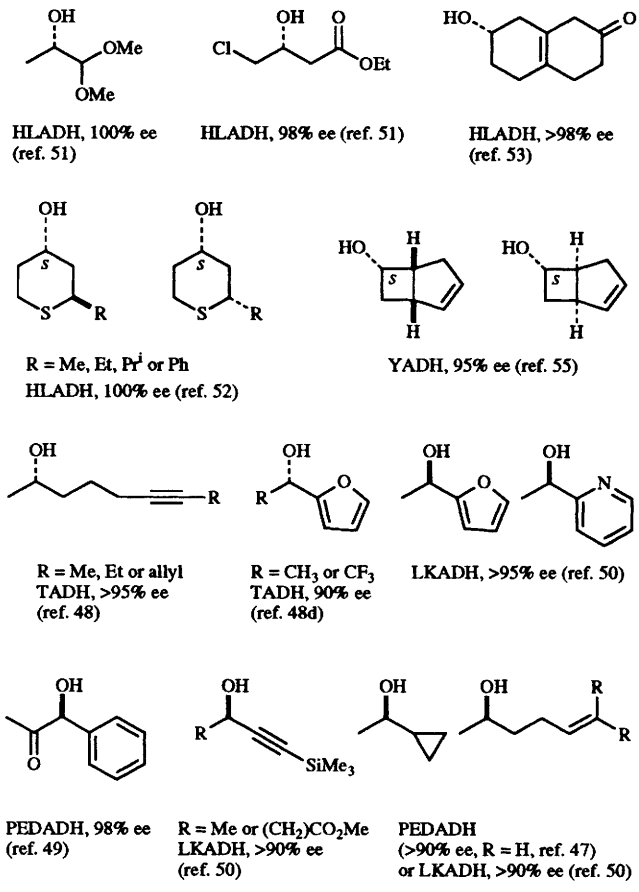
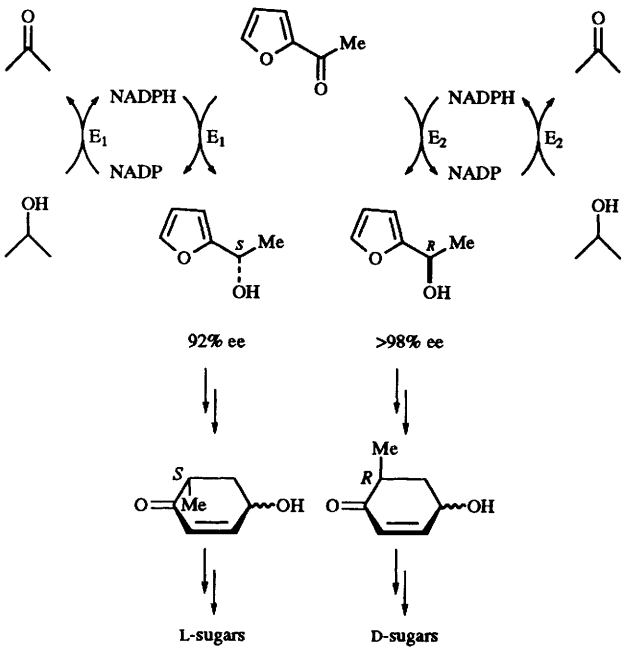


Fig. 3 Alcohol products obtained by alcohol dehydrogenase catalysed reductions of ketones: HLADH = horse liver alcohol dehydrogenase, YADH = yeast alcohol dehydrogenase, TADH = alcohol dehydrogenase from *Thermoanaerobium brockii*, PEDADH = PED alcohol dehydrogenase from *Pseudomonas* species and LKADH = alcohol dehydrogenase from *Lactobacillus kefir*

NADH and *si*-face of carbonyl substrates, and that from *Pseudomonas* species[49] or *Lactobacillus kefir*[50] (E_1 in Fig. 2) is specific for the *pro-R* hydrogen of NADH (or NADPH) and *si*-face of carbonyl substrate. The *pro-S*/*re*-face specific alcohol dehydrogenase is unknown.

Fig. 3 illustrates some representative alcohol products obtained from reduction of the corresponding ketones catalysed by different alcohol dehydrogenases.[48–55] Alcohol dehydrogenases appear to accept a large variety of side chains of different sizes, including cyclic compounds. In many cases, other functional groups such as halide, sulfide, silane, cyclopropane, alkene, alkyne, phenyl, furan, pyridine, acetal or ester do not interfere with the enzymatic reaction. Many of these optically active alcohols are either useful substrates for the synthesis of natural products, or are theoretically interesting compounds. Reductions of the racemic 2-alkylthiopyran-4-one[52] and bicyclo[3.2.0]hept-2-en-6-one[55] respectively with HLADH and YADH give diastereoisomers of alcohol products having the *S*-configurations at the carbinol centre. The reduction of 1-phenylpropane-1,2-dione with PED alcohol dehydrogenase, obtained from a *Pseudomonas* sp. strain, occurs preferably at the carbonyl group close to the phenyl group.[49] Reduction of furyl methyl ketone using the alcohol dehydrogenase from *T. brockii* gave the (*S*)-alcohol,[48d] whereas using the enzyme from *L. kefir* gave the (*R*)-alcohol.[50] These enantiomers serve as building blocks for the synthesis of L- and D-sugars (Scheme 13).

Alcohol dehydrogenase-catalysed reductions generally require regeneration of the reduced co-factor NAD(P)H by using another enzymatic system such as glucose(-6-phosphate)/



Scheme 13 Route to D- or L-sugars using enantiospecific enzymatic reductions of acetylfuran: E_1 = alcohol dehydrogenase from *Thermoanaerobium brockii* and E_2 = alcohol dehydrogenase from *Lactobacillus kefir*

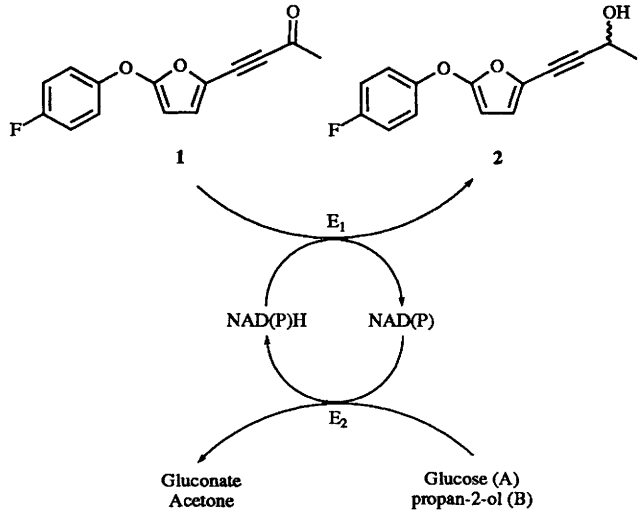


Fig. 4 (A) E_1 = horse liver alcohol dehydrogenase and E_2 = glucose dehydrogenase [10% yield of (*S*)-**2**, 96% ee]; (B) $E_1 = E_2$ = *Lactobacillus kefir* alcohol dehydrogenase [60% yield of (*R*)-**2**, >98% ee]

glucose(-6-phosphate) dehydrogenase, whereas single-enzyme systems for asymmetric reduction and co-factor regeneration have been established for the alcohol dehydrogenases from horse liver,[52] *T. brockii*,[48] *L. kefir*[50] and *Pseudomonas* species.[49] For example (Fig. 2),[49] propan-2-ol is used in the reduction of carbonyl compounds catalysed by the last three alcohol dehydrogenases to regenerate NAD(P)H from NAD(P). By this means, propan-2-ol forces the reduction of the carbonyl compound to completion. A low concentration of propan-2-ol (< 20%) also acts as a co-solvent to increase the solubility of substrates and to provide a long-term stability to PED alcohol dehydrogenase.[49]

To determine whether the enzyme is *pro-R* or *pro-S* specific for the reduced co-factor, ^{1}H NMR spectroscopy is considered to be the most convenient method.[56] The *pro-R* hydrogen has a chemical shift of 2.77 ppm and the *pro-S* hydrogen has a shift of 2.67 ppm. Using a deuteriated alcohol substrate and NAD or NADP in the presence of enzyme, one can examine the chemical shift of the isolated reduced co-factor 4-[^{2}H]-NAD(P)H to determine the stereospecificity. One can also use either (4*R*)-[^{2}H]- or (4*S*)-[^{2}H]-NAD(P)H to reduce a carbonyl substrate in the presence of enzyme. The presence or absence of the 4-H (δ 9 ppm) of the oxidized co-factor recovered will be diagnostic of deuteride or hydride transfer from the labelled reduced co-factor.

A useful application of NAD(P) dependent oxidoreductases is to prepare deuterium or tritium labelled compounds such as alcohols and α-hydroxy acids (from the corresponding keto acid precursors). For example,[57] (*S*)-1-deuteriohexanol is either synthesized from the HLADH-catalysed reduction of 1-deuteriohexanal or from the reduction of hexanal catalysed by a *Pseudomonas* sp. alcohol dehydrogenase using [$^2H_8$]propan-2-ol to regenerate the deuteriated-NADH.

It is worth noting that while enzymatic reduction of carbonyl compounds is potentially useful, non-enzymatic catalytic reductions based on chiral boranes[60] and metal-mediated hydrogenation[60b,61] are commonly practiced in the laboratory.

Results and discussion

As described above, six different types of oxidoreductases have been used in synthesis. To illustrate the use of a nicotinamide-dependent alcohol dehydrogenase in asymmetric reduction, compound **1** was converted into the reduced (*R*)-alcohol **2** in 60% yield and > 98% ee using only the alcohol dehydrogenase from *Lactobacillus kefir*[50] to catalyse the reduction of **1** and the regeneration *in situ* of NADPH from NADP by isopropyl alcohol oxidation. The solubility of the substrate is increased and the reaction equilbrium is in favour of the formation of **2** in this single enzyme system containing 10% isopropyl alcohol. Other commercially available alcohol dehydrogenases (*e.g.* the enzyme from yeast, *Thermoanaerobium brockii* and horse liver) were also tested for the reduction of **1**, and only horse liver alcohol dehydrogenase exhibited the reductive activity to give the (*S*)-alcohol **2** in 96% ee and the product was obtained in a relatively low yield (10%). A biphasic system was employed in this process in which product **2** formed was extracted into the organic phase and the co-factor regeneration occurred in the aqueous phase where NAD was reduced to NADH catalysed by glucose dehydrogenase. The alcohol dehydrogenase from *Pseudomonas* species[49] was also examined, but no reduction was observed. It is obvious that enzymatic asymmetric reduction of various ketones can be achieved by using appropriate alcohol dehydrogenases, as illustrated in many examples of reactions described in this article, whether the enzymatic processes have a clear advantage to alternative chemical reduction methods is however, not clear.‡ In any case, the (*R*)-enantiomer of compound **2** is a key intermediate used in the synthesis of a lipoxygenase inhibitor[62] which is currently produced by chemical methods.

Fig. 5 illustrates another nicotinamide-dependent enzymatic reaction for the synthesis of L-phosphinotricin from the corresponding keto acid *via* reductive amination catalysed by L-glutamate dehydrogenase. L-Phosphinotricin is the active component of the naturally occurring antibiotic bialaphos.[63] It inhibits the enzyme glutamate synthetase[64] and is widely used as a herbicide. Several methods have been described for the

‡ For example, **1** can be reduced chemically, or racemic **2** can be resolved enzymatically using lipase PS-800 as catalyst and vinyl acetate as acylating reagent. At 50% conversion, the (*R*)-acetylated product was in > 98% ee and the unchanged (*S*)-enantiomer was recovered in > 98% ee.

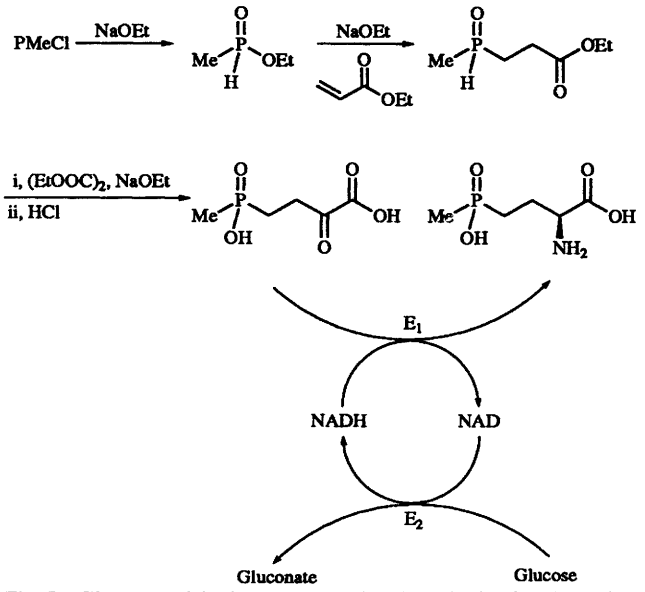


Fig. 5 Glutamate dehydrogenase-catalysed synthesis of L-phosphinothricin: E_1 = glutamate dehydrogenase and E_2 = glucose dehydrogenase

synthesis.[65] The reductive amination method described here provides the product with high enantiomeric purity and may be feasible for a large-scale process. The conversion rate is, however, relatively low (~ 0.5–1 U mg^{-1} enzyme).

In a representative metalloenzyme-catalysed oxidation, compound **5** was subjected to chloroperoxidase-catalysed bromohydration to give a mixture of two products which were acetylated and identified as **6** and **7** (Fig. 6). Our previous work with this enzyme indicates that vinyl ethers are good substrates for the enzyme as several glycals have been converted into 2-deoxy-2-halogenosugars.[23] Compound **5** also contains a vinyl ether moiety which is, as expected, also a good substrate for the enzyme. The mechanism for the formation of **7** is, however, unclear. It may arise from the spontaneous hydrolysis of substrate **5**, or from hydrolysis of the enzymatically produced bromohydrin or epoxide. To test the latter case, compound **5** was examined in the chloroperoxidase-catalysed epoxidation with the presence of H_2O_2 (Fig. 7); but no epoxidation reaction was observed. Compound **5** was, however, hydrolysed slowly at pH 3 in the presence or absence of the enzyme to a product which was acetylated and identified as **10**. Similarly, when the initial bromohydrin product was incubated at pH 3 in the absence of the enzyme followed by acetylation of the product, compound **7** was obtained. Thus, the bromohydrin product apparently undergoes further non-enzymatic hydrolysis to the diol under the reaction condition. Compounds **6** and **7** are C-linked homosugars which may be synthetically useful.

Concluding remarks

This article has described the use of enzyme-catalysed oxidoreductions of alkenes, arenes, sulfides, alcohols and ketones. It has been shown that besides natural substrates, enzymes accept a broad range of compounds and transform them into optically pure derivatives. In some instances, enzymatic oxidoreductions are not simply alternatives to chemical methods, but have an advantage in stereochemical control (such as halohydration, arene dihydroxylation and Baeyer-Villiger reactions). In other instances, non-enzymatic methods may be preferred (such as epoxidations). Reduction of ketones to either (*R*)- or (*S*)-alcohols is realized by using specific alcohol dehydrogenase and may find use in the preparation of certain alcohols. Use of enzymes in oxido-

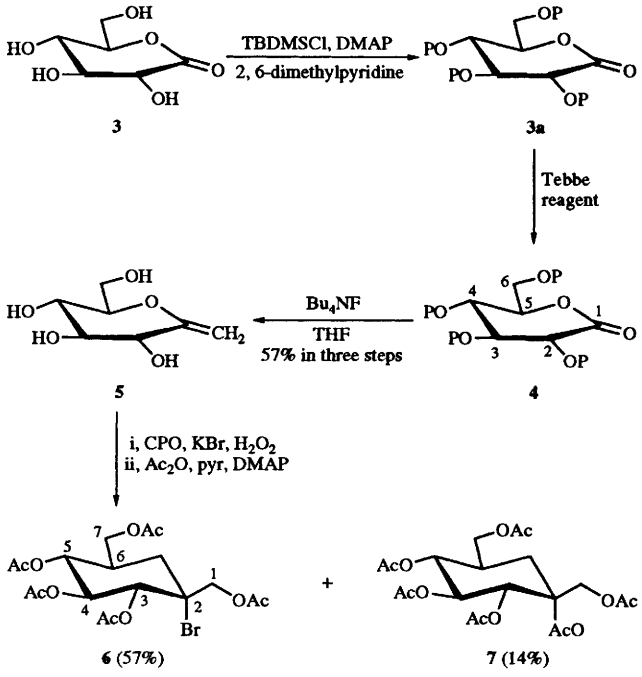


Fig. 6

reductions is, however, not limited to the current examples, many applications have been found in the activation of C–H to C–OH.[1] An impressive example[58] is the transformation of progesterone to 11α-hydroxyprogesterone in 91% yield by a mutant strain of *Aspergillus ochraceus* with an initial substrate concentration of 40 g dm^{-3}. Cyclization of tripeptide δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine to isopenicillin also involves C–H oxidative steps catalysed by the non-heme monooxygenase isopenicillin-N synthetase.[59] The practicality of many of these C–H activation processes, however, remain to be established. Though the procedures described in the Experimental section may not represent the best methods for the synthesis of those particular compounds, they may find use in the synthesis of other related compounds which are difficult to prepare by other means.

Experimental

Lactobacillus kefir alcohol dehydrogenase-catalysed reduction of 5-(4-fluorophenoxy)-2-furylbutyn-3-one 1 to the corresponding alcohol 2

The following reagents were combined and stirred at room temperature: **1** (100 mg, 0.41 mmol), NADP (10 mg), sodium phosphate buffer (pH 7.1, 950 mmol dm^{-3}; 8 cm^3) propan-2-ol (1 cm^3), the alcohol dehydrogenase isolated from *Lactobacillus kefir* (20 U),[50] magnesium chloride hexahydrate (2.5 mg) and hexane (3 cm^3). The pH was maintained at 7.1 by addition of 1 mol dm^{-3} NaOH. After 2 days the hexane layer was removed and the aqueous layer extracted with hexane (2 × 10 cm^3). The enzyme in the aqueous layer was found to retain 65% of the original activity. The organic layers were combined, dried (Na_2SO_4), concentrated under reduced pressure and purified by preparative TLC [hexane–diethyl ether (1:1) with 2% triethylamine] to give **2** (60 mg, 60%). $\delta_H(CDCl_3)$ 1.56 (d, 3 H), 1.63 (br s, 1 H), 4.57 (q, 1 H), 4.49 (d, 1 H), 6.53 (d, 1 H) and 7.10 (m, 1 H); $[\alpha]_D^{25}$ +18.2§ (*c* 0.37, $CDCl_3$), >98% ee [the specific rotation was identical with that of an authentic sample with (*R*)-configuration provided by Abbott Laboratories].

§ $[\alpha]_D$ Values given in units of 10^{-1} deg cm^2 g^{-1}.

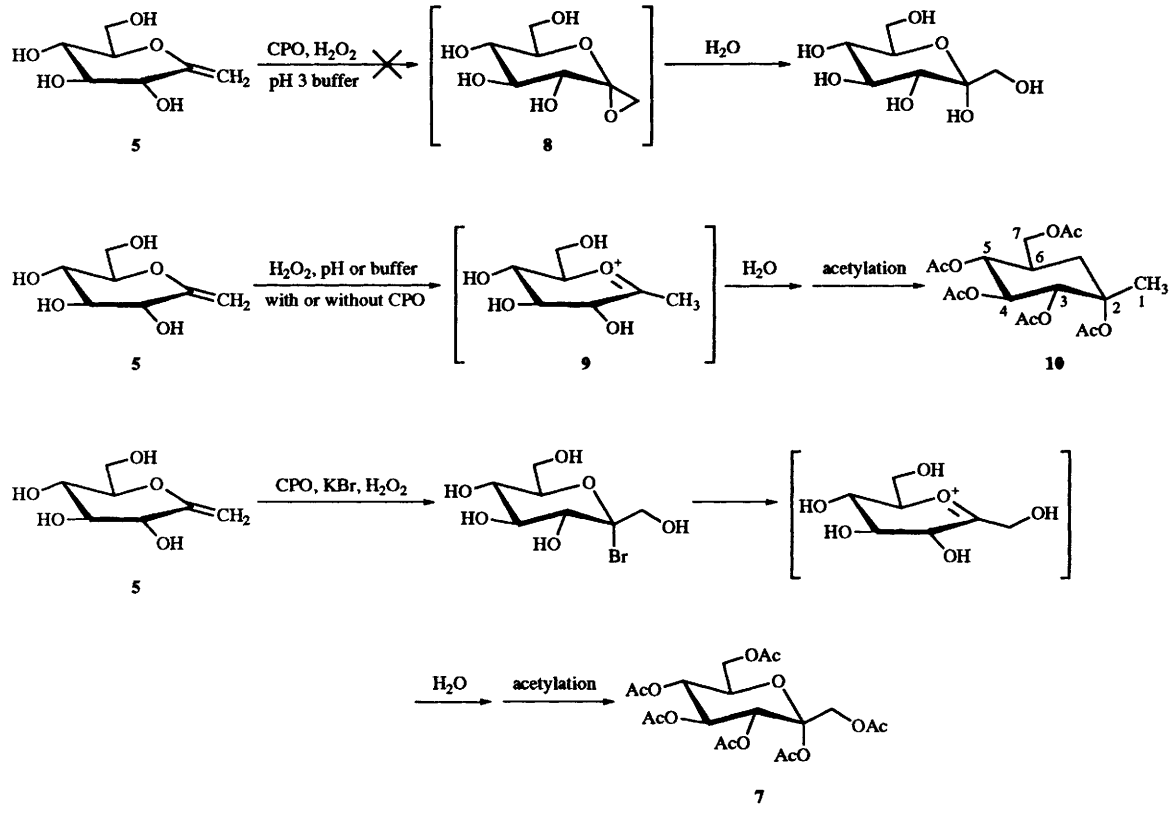


Fig. 7

Horse liver alcohol dehydrogenase-catalysed reduction of 1

The following were combined and stirred at room temperature under nitrogen: **1** (260 mg, 1.1 mmol), NAD (10 mg), glucose (240 mg, 1.3 mmol), horse liver alcohol dehydrogenase (50 mg) (from Sigma Co.), *Bacillus* glucose dehydrogenase (100 U) (from Amano Co., Japan), NaCl (0.88 g), Tris buffer (pH 7.1, 50 mmol dm^{-3}; 100 cm^3), dimethylformamide (10 cm^3) and hexane (25 cm^3). After 3 days, additional horse liver alcohol dehydrogenase (50 mg) and glucose dehydrogenase (50 U) were added. During the reaction, the pH was maintained constant by addition of 1 mol dm^{-3} NaOH. After 1 week of reaction, the hexane layer was removed and the aqueous layer extracted with hexane (2 × 25 cm^3). Analysis of the enzyme activities in the aqueous phase indicated 15 and 50% of the original activity, respectively. The organic layers were dried and evaporated and the residue chromatographed as above to give **2** in (25 mg, 10%); $[\alpha]_D^{25}$ −17.2§ (*c* 3, $CHCl_3$), 96% ee (*S*-configuration); ^1H NMR data were the same as for the (*R*)-enantiomer.

Synthesis of L-phosphinothricin

4-(Hydroxymethylphosphinoyl)-2-oxobutanoic acid was synthesized from methyldichlorophosphine as demonstrated previously.[66] Glutamate dehydrogenase (50 mg) and glucose dehydrogenase (10 mg) were added to ammonium phosphate buffer (pH 7.5, 50 mmol dm^{-3}; 50 cm^3) containing glucose (173 mg), sodium chloride (0.38 g), dithiothreitol (15 mg), ammonium chloride (0.533 g), NAD (10 mg) and 4-(hydroxymethylphosphinoyl)-2-oxobutanoic acid (150 mg). The reaction mixture was stirred at room temperature and the pH maintained constant by periodic addition of 1 mol dm^{-3} ammonium hydroxide. When the reaction was complete as determined by lack of further product formation (6 days), the solution was heated at 80 °C for 15 min, centrifuged at 15 000 rpm for 10 min and decanted. The supernatant was acidified to pH 2 with concentrated hydrochloric acid and cooled to 4 °C overnight. The resulting precipitate was filtered off and recrystallized from ethanol–water to give L-phosphinothricin hydrochloride (4.5 mg, 25%), mp 192–196 °C; $[\alpha]_D^{23}$ +24.85 (*c* 2, 1 mol dm^{-3} HCl) {lit.,[65,66] mp 194–196 °C and $[\alpha]_D^{19}$ +21.4 (*c* 1.4, 1 mol dm^{-3} HCl), 89.2% enantiomeric excess},[65,66] >99% enantiomeric excess as determined by comparison of the optical rotation. ^1H NMR data were the same as reported previously.[65,66]

Compound 3a

tert-Butyldimethylsilyl chloride (15.21 g, 0.10 mol) dissolved in dry 2,6-dimethylpyridine (30 cm^3) was added dropwise to a stirred solution of δ-gluconolactone **3** (3.00 g, 16.82 mmol) and *N*,*N*-dimethylaminopyridine (10.27 g, 84.1 mmol) in dry 2,6-dimethylpyridine (50 cm^3) at room temperature under Ar. The reaction mixture was retained at room temperature for 2 h, and then at 60 °C overnight. Ice–water (100 cm^3) was added to the reaction mixture which was then extracted with dichloromethane (4 × 30 cm^3). The collected organic layers were washed with saturated aqueous $NaHCO_3$, 0.05 mol dm^{-3} aqueous $CuSO_4$ and brine. The crude product was dried ($MgSO_4$) and purified by silica gel chromatography with hexane–EtOAc (55:1) to give the desired product **3a** (6.93 g, 65%) as white solids $[\alpha]_D^{25}$ +55.0 (*c* 5.4, $CHCl_3$); $\delta_H(CDCl_3)$ 4.56 (1 H, ddd, $J_{5,4}$ = 8.5,¶ $J_{5,6b}$ 3.5, $J_{5,6a}$ 2.0, 5-H), 4.10 (1 H, dd, $J_{4,5}$ 8.5, $J_{4,3}$ 10.0, 4-H), 4.09 (1 H, dd, $J_{3,2}$ 3.5, $J_{3,4}$ 1.0, 3-H), 3.87 (1 H, dd, $J_{6a,6b}$ 12.0, $J_{6a,5}$ 2.0, 6a-H), 3.84 (1 H, d, $J_{2,3}$ 3.5, 2-H), 3.77 (1 H, dd, $J_{6b,6a}$ 12.0, $J_{6b,5}$ 3.5, 6b-H), 0.863 (9 H, s, three CH_3 of *tert*-butyl), 0.861 (9 H, s, three CH_3 of *tert*-butyl), 0.857 (9 H, s, three CH_3 of *tert*-butyl), 0.830 (9 H, s, three CH_3 of *tert*-butyl), 0.107 (3 H, s, Si-CH_3), 0.100 (3 H, s, Si-CH_3), 0.097 (3 H, s, Si-CH_3), 0.078 (3 H, s, Si-CH_3), 0.078 (3 H, s, Si-CH_3), 0.072 (3 H, s, Si-CH_3), 0.056 (3 H, s, Si-CH_3) and 0.052 (3 H, s, Si-CH_3); $\delta_C(CDCl_3)$ 169.63, 80.76, 76.75, 73.51, 70.60, 61.21, 25.80 (three CH_3 of *tert*-butyl), 25.66 (three CH_3 of *tert*-

¶ *J* Values given in Hz.

butyl), 25.56 (three CH_3 of *tert*-butyl), 25.53 (three CH_3 of *tert*-butyl), 18.20, 17.97, 17.81, 17.76, −4.11, −4.39, −4.69, −5.05, −5.10, −5.23 and −5.45 [Found (HRMS): *m/z* 635.4008. Calc. for $C_{30}H_{67}O_6Si_4$: (M + H^+), 635.4015].

In the above reaction, there was a minor product (23%) resulting from incomplete protection (from the information of ^1H and ^{13}C NMR and HRMS, this minor product has a free hydroxy group) due to the steric hindrance of the *tert*-butyldimethylsilyl protecting group. However, the partially protected product can be converted into compound **3a** by the following procedure.

To a solution containing the partially protected δ-gluconolactone (0.76 g, 1.46 mmol) and 2,6-dimethylpyridine (1.00 cm^3, 8.68 mmol), *tert*-butyldimethylsilyl triflate (0.67 cm^3, 2.92 mmol) was added dropwise at room temperature under Ar. The reaction mixture was stirred at room temperature for 10 h after which ice–water (10 cm^3) was added to it and the whole was extracted with EtOAc (10 cm^3 × 3). The combined extracts were washed with saturated aqueous $NaHCO_3$ and brine, dried ($MgSO_4$) and purified by silica gel chromatography with hexane–EtOAc (55:1) to give **3a** (778 mg, 84%).

Compound 4

Tebbe reagent (0.5 mol dm^{-3} solution in toluene, purchased from Aldrich Inc.; 10 cm^3) was added dropwise to a THF solution (fresh distilled; 3.0 cm^3) of compound **3a** (1.85 g, 2.92 mmol) at −40 °C under Ar. The reaction mixture was stirred at −40 °C for 30 min and then at room temperature for 8 h. An aqueous solution of saturated aqueous potassium sodium tartrate (50 cm^3) was then added at 0 °C to the mixture after which it was stirred vigorously to dissolve all the insoluble precipitate. After the mixture had been neutralized by the addition of a solution of 1 mol dm^{-3} NaOH it was extracted with EtOAc (4 × 30 cm^3). The extracts were washed with brine and dried ($MgSO_4$) and the crude product was purified by silica gel chromatography with hexane–EtOAc (100:1) to give the desired product **4** (1.44 g, 78%) as clear oil; $[\alpha]_D^{25}$ +38.3 (*c* 7.83, $CHCl_3$); $\delta_H(CDCl_3)$ 4.31 (1 H, s, vinyl H-a), 4.11 (1 H, ddd, $J_{5,4}$ 9.0, $J_{5,6b}$ 3.5, $J_{5,6a}$ 1.5, 5-H), 4.00 (1 H, d, $J_{2,3}$ 3.0, 2-H), 3.88 (1 H, s, vinyl H-b), 3.84 (1 H, d, $J_{4,5}$ 9.0, 4-H), 3.81 (1 H, dd, $J_{6a,6b}$ 12.0, $J_{6a,5}$ 1.5, 6a-H), 3.73 (1 H, br m, H-3), 3.72 (1 H, dd, $J_{6b,6a}$ 12.0, $J_{6b,5}$ 3.5, 6b-H), 0.865 (9 H, s, three CH_3 of *tert*-butyl), 0.863 (9 H, s, three CH_3 of *tert*-butyl), 0.856 (9 H, s, three CH_3 of *tert*-butyl), 0.840 (9 H, s, three CH_3 of *tert*-butyl), 0.08 (3 H, s, Si-CH_3), 0.080 (3 H, s, Si-CH_3), 0.071 (3 H, s, Si-CH_3), 0.067 (3 H, s, Si-CH_3), 0.060 (3 H, s, Si-CH_3), 0.055 (3 H, s, Si-CH_3), 0.051 (3 H, s, Si-CH_3) and 0.051 (3 H, s, Si-CH_3); $\delta_C(CDCl_3)$ 157.12, 89.66, 77.18, 77.02, 73.24, 72.40, 62.42, 25.87 (three CH_3 of *tert*-butyl), 25.76 (three CH_3 of *tert*-butyl), 25.68 (three CH_3 of *tert*-butyl), 25.64 (three CH_3 of *tert*-butyl), 18.31, 17.92, 17.84, 17.84, −3.89, −4.07, −4.55, −4.63, −4.87, −4.93, − 5.03 and −5.34 [Found (HRMS): *m/z* 633.4230. Calc. for $C_{31}H_{69}O_5Si_4$: (M + H^+), 633.4222].

Compound 5

A THF solution of tetrabutylammonium fluoride (1 mol dm^{-3}; 0.50 cm^3) was added *via* a syringe to a THF solution (1 cm^3) containing compound **4** (236 mg, 0.37 mmol) at 0 °C. The stirred mixture was warmed to room temperature, concentrated, and purified by silica gel chromatography with $CHCl_3$–MeOH (6:1) to yield the desired product **5** as clear oil (60.0 mg, 92%); $\delta_H(CD_3OD)$ 4.68 (1 H, d, $J_{a,b}$ 2.0, vinyl H-a), 4.64 (1 H, d, $J_{b,a}$ 2.0, vinyl H-b), 3.86 (1 H, dd, $J_{6a,6b}$ 12.0, $J_{6a,5}$ 2.5, 6a-H), 3.80 (1 H, td, $J_{2,3}$ 9.5, *J* 3, vinyl H-a J_2, vinyl H-a J_2, vinyl H-b 2.0, 3-H), 3.70 (1 H, dd, $J_{6b,6a}$ 12.0, $J_{6b,5}$ 5.5, 6b-H), 3.45 (1 H, t, $J_{4,3}$ = $J_{4,5}$ 9.5, 4-H), 3.29 (1 H, t, $J_{3,4}$ = $J_{3,2}$ 9.5, 3-H) and 3.28 (1 H, ddd, $J_{5,4}$ 9.5, $J_{5,6b}$ 5.5, $J_{5,6}$ 2.5, 5-H); $\delta_C(CDCl_3)$ 161.98, 93.50, 83.17, 79.03, 72.49, 71.14 and 62.64.

Compounds 6 and 7

To a reaction mixture containing citrate–phosphate buffer (pH 3.0, 0.1 mol dm^{-3}; 5 cm^3), compound **5** (40.0 mg, 0.227 mmol), KBr (135.2 mg, 1.136 mmol) and chloroperoxidase (300 U) was added H_2O_2 (30%; 200 mm^3) with a syringe pump (100 mm^3 h^{-1}).‖ The reaction mixture was stirred for 3 h at room temperature and monitored by TLC with EtOAc–Pr^{i}OH–H_2O (6:4:1). After this, the mixture was neutralized by Dowex 1 (OH^- form). The resin was filtered off, and the filtrate concentrated. Some white insoluble material present at this time, could be removed by passage of the filtrate through a small amount of silica gel with MeOH as eluent. After concentration of the filtrate, the dry residue was converted into the peracetate by acetic anhydride and pyridine in the presence of a catalytic amount of *N*,*N*-dimethylaminopyridine. The product was purified by silica gel column chromatography, eluting with EtOAc–hexane (2:3), to give **6** as yellowish oil (62.5 mg, 57%) and **7** (14.7 mg, 14%).

Compound 6

$[\alpha]_D^{25}$ +36.8 (*c* 0.76, $CHCl_3$); $\delta_H(CDCl_3)$ 5.502 (1 H, t, $J_{4,3}$ = $J_{4,5}$ 10.0, 4-H), 5.140 (1 H, t, $J_{5,4}$ = $J_{5,6}$ 10.0, 5-H), 5.135 (1 H, d, $J_{3,5}$ 10.0, 3-H), 4.285 (1 H, dd, $J_{7a,7b}$ 12.0, $J_{7a,6}$ 4.0, 7a-H), 4.266 (1 H, t, $J_{7b,7a}$ = $J_{7b,6}$ 12.0, 7b-H), 4.252 (1 H, m, 6-H), 4.231 (1 H, d, $J_{1a,1b}$ 12.0, 1a-H), 3.994 (1 H, d, $J_{1b,1a}$ 12.0, 1b-H) and 2.128, 2.098, 2.091, 2.038 and 2.002 (3 H each, s, 5 × OAc); $\delta_C(CDCl_3)$ 171.1, 170.8, 170.2, 169.7, 169.6, 95.6, 71.0, 70.3, 68.4, 68.2, 66.0, 61.8, 20.8, 20.7, 20.6, 20.6 and 20.6 [Found (HRMS): *m/z* 615.0231. Calc. for $C_{17}H_{23}BrCsO_{11}$: (M + Cs^+), 615.0401].

Compound **6** is easily decomposed to give the hydrolysed product (a free OH group at C-2 position) which can be acetylated to give compound **7**.

Compound 7

$[\alpha]_D^{25}$ +51.6 (*c* 0.95, $CHCl_3$); $\delta_H(CDCl_3)$ 5.44 (1 H, t, $J_{4,3}$ = $J_{4,5}$ 10.0, 4-H), 5.32 (1 H, d, $J_{3,4}$ 10.0, 3-H), 5.18 (1 H, t, $J_{5,6}$ = $J_{5,4}$ 10.0, 5-H), 4.69 (1 H, d, $J_{1a,1b}$ 12.0, 1a-H), 4.58 (1 H, d, $J_{1b,1a}$ 12.0, 1b-H), 4.29 (1 H, dd, $J_{7a,7b}$ 12.5, $J_{7a,6}$ 5.0, 7a-H), 4.11 (1 H, dd, $J_{7b,7a}$ 12.5, $J_{7b,6}$ 2.5, 7b-H), 3.96 (1 H, ddd, $J_{6,5}$ 10.0, $J_{6,7a}$ 5.0, $J_{6,7b}$ 2.5, 6-H) and 2.20, 2.10, 2.09, 2.05, 2.05 and 2.01 (3 H each, s, 6 × OAc); $\delta_C(CDCl_3)$ 170.7, 170.2, 169.9, 169.4, 169.4, 167.6, 101.5, 70.9, 70.2, 68.7, 67.8, 62.2, 61.5, 21.5, 20.7, 20.7, 20.6, 20.6 and 20.5 [Found (HRMS): *m/z* 595.0421. Calc. for $C_{19}H_{26}O_{13}Cs$: (M + Cs^+), 595.0428].

Compound 10

To a reaction mixture containing citrate–phosphate buffer (pH 3.0, 0.1 mol dm^{-3}; 16 cm^3), compound **5** (146.0 mg, 0.829 mmol) and chloroperoxidase (700 U) was added H_2O_2 (30%; 700 mm^3) with a syringe pump (100 mm^3 h^{-1}). The reaction progress was monitored by TLC using EtOAc–Pr^{i}OH–H_2O (6:4:1) as the mobile phase. After 8 h, the reaction mixture was neutralized with Dowex 1 (OH^- form) and the resin was filtered off and the filtrate was concentrated. White insoluble material present was removed by passage of the filtrate through a small amount of silica gel with MeOH as eluent. After concentration of the filtrate, the dry residue was converted into the peracetate by acetic anhydride and pyridine in the presence of a catalytic amount of *N*,*N*-dimethylaminopyridine. The product was purified by silica gel chromatography using EtOAc–hexane (1:2) as eluent to give **10** as clear oil (260.1 mg, 78%). The same product was obtained in a separate reaction without enzyme; $[\alpha]_D^{25}$ +7.9 (*c* 14.1, $CHCl_3$); $\delta_H(CDCl_3)$ 5.623 (1 H, dd, $J_{4,5}$ 5.0,

‖ 1 mm^3 = 1 μl.

$J_{4,3}$ 3.5, 4-H), 5.481 (1 H, dd, $J_{5,6}$ 6.0, $J_{5,4}$ 5.0, 5-H), 5.266 (1 H, d, $J_{3,4}$ 3.5, 3-H), 5.067 (1 H, dt, $J_{6,7a}$ 3.5, $J_{6,5} = J_{6,7b}$ 6.0, 6-H), 4.333 (1 H, dd, $J_{7a,7b}$ 12.5, $J_{7a,6}$ 3.5, 7a-H), 4.126 (1 H, dd, $J_{7b,7a}$ 12.5, $J_{7b,6}$ 6.0, 7b-H), 2.216 (6 H, s, 2 × CH_3) and 2.092, 2.086, 2.079 and 2.066 (3 H each, s, 4 × CH_3); $\delta_C(CDCl_3)$ 170.5, 169.8, 169.7, 169.5, 169.5, 123.3, 75.8, 69.3, 68.6, 68.1, 61.5, 26.9, 20.7, 20.6, 20.5, 20.4 and 20.4 [Found (HRMS): *m/z* 537.0384. Calc. for $C_{17}H_{24}CsO_{11}$: (M + Cs^+), 537.0373].

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