Biocatalysis and enzymes in organic synthesis

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1 Introduction

This review is written as an update to Nick Turner's excellent review of 1994 and so adopts the same format based on enzyme type rather than transformation type. Given the breadth of reaction types catalyzed by particular enzymes and the inherent reversibility of catalysis such a distinction will always, of course, be arbitrary. Since 1994 a wealth of excellent work has been published in the area of biocatalysis. This is illustrated by examining the number of publications per year using the word "biocatalysis" in their titles, keywords or abstracts, which rose

Ben Davis got his BA (1993) and DPhil (1996) from the University of Oxford. During this time he learnt the beauty of carbohydrate chemistry under the supervision of Professor George Fleet. He then spent two years as a postdoctoral fellow in the laboratory of Professor Bryan Jones at the University of Toronto exploring protein chemistry and biocatalysis. In 1998 he returned to the UK to take up a lectureship at the University of Durham; in autumn 2001 he moved to the Dyson Perrins Laboratory, University of Oxford and a fellowship at Pembroke College, Oxford. His group's research centres on chemical biology with an emphasis on carbohydrates and proteins. In particular, their interests encompass synthesis and methodology, inhibitor design, biocatalysis, enzyme mechanism, protein engineering, drug delivery, molecular modelling, molecular biology and glycoscience. This work has been recognised by the RSC Meldola Medal and Prize; AstraZeneca Strategic Research Award; DTI Smart Award; and Mitzutani Foundation for Glycoscience Award.

Viviane Boyer received her PhD in 1999 from the Université Joseph Fourier (Grenoble, France) under the direction of Dr Hugues Driguez. Her PhD involved the use of a mutated enzyme or glycosynthase as an efficient tool for the synthesis of complex oligosaccharides. Since January 2000 she has been a Senior Research Associate at the Universities of Durham and Oxford with Dr Benjamin G. Davis, working in the area of carbohydrate synthesis with minimal protection.

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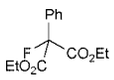
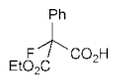
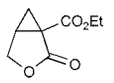
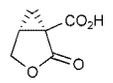
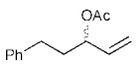
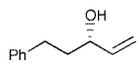
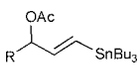
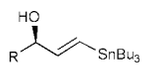
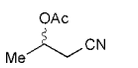
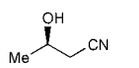
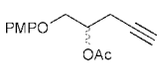
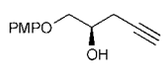
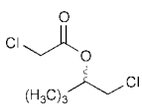
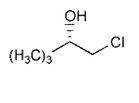
Table 1 Lipase-catalyzed enantioselective hydrolyses: as *Pseudomonas cepacia* lipase = PCL

Substrate	Lipase	Product	Ee (%)	Reference
	PPL		96	50
	Lipase PS		98	51
	PCL		99	47
	PCL		98	47
	Lipase PS (+ crown ether)		76	52
	Lipase PS		92.2	53
	Lipase PS		91	54

almost 4-fold over the period covered by this review from 33 in 1993 to 123 in 1999. Similarly, there were 279 papers in 1999 that contained both of the concepts "organic synthesis" and "enzym-" from 174 in 1993.

It is not the intention of this review to comprehensively catalogue all biotransformations during this period but to instead describe selected highlights. We hope therefore that this review will act more as a primer or overview for this period. Many other good reviews have also covered parts of this period.[1–42] In particular the reviews of Stan Roberts have provided a wonderfully detailed review of 1996,[43] 1997[44] and 1998.[45] More details of particular biotransformations can be found in several excellent specialized reviews that have appeared and these have been highlighted at the start of the relevant sections below.

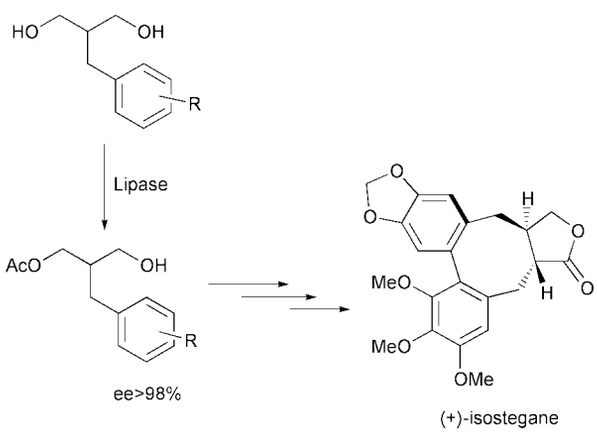
2 Acyl transferases

2.1 Lipases

Among the biocatalysts in organic synthesis, lipases are the most frequently used.[5,46–48] In particular, this class of enzyme is able to perform enantioselective hydrolytic reactions (Table 1) and catalyzes the formation of a wide range of ester and amide bonds (Table 2) and it is in this role that they are most frequently employed.[49]

A number of unusual substrates have featured recently; including resolutions of cyclophanes,[62] thioesters,[63] ferrocenes,[64] phosphonates,[65,66] helicenes,[67] and calixarenes.[68] Even lipoic acids in which the chiral centre is 5 carbon atoms from the acylated centre can be resolved.[69] Hydrolytic decarboxylation of a tetraester allows a very interesting resolution to provide a diester carbocycle as a valuable synthetic precursor.[70] In addition, secondary amines can be resolved.[71] Even fullerene derivatives have been acylated.[72] Amano PS lipase catalyzed hydrolysis of acyl diacetoxypropionate is nicely chemo-, regio- and stereo-selective.[73] Similarly, chemoselective deacetylation over the hydrolysis of an ethyl ester present in phenolic peracetates has been described.[74]

In this way, lipases have been extensively utilized in the synthesis of many biologically active compounds and natural products. For example, optically active tertiary carbinols such as (+)-isostegane were obtained *via* acylation (Scheme 1).[75] The facile asymmetric preparation of (+)-hydroxylactone allowed its use as a starting material in the synthesis of plant bioregulators (+)-strigol and (+)-orobanchol.[76] Components of the pheromone of *Microdiprion pallipes* were obtained by

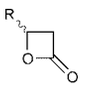
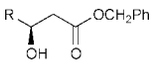
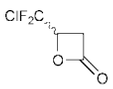
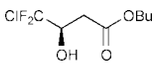
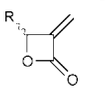
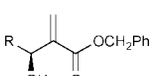
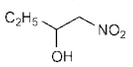
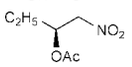
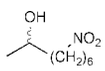
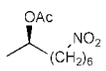
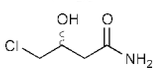
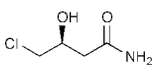
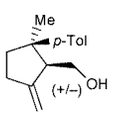
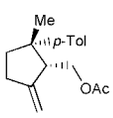


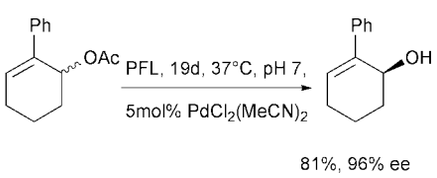
Scheme 1

employing lipase-catalysed kinetic resolution as a late step.[77] The transesterification of (±)-*trans*-2-phenylcyclohexan-1-ol with *Candida rugosa* lipase (CRL) gave in pure form (1*S*,2*R*)-(+)-*trans*-2-phenylcyclohexan-1-ol, a useful chiron in the preparation of many optically pure pharmaceutical intermediates.[78] Enantiopure carbohydrate mimics such as 1-azafagomine were prepared through the enantioselective acylation of a primary hydroxy group.[79]

Typically kinetic resolutions are limited to a maximum yield of 50% of desired enantiomer. However, by coupling the method with epimerization of the starting materials, dynamic kinetic resolution (DKR) results.[80] For example, OH groups at lactol or hemiacetal centres mutarotate and this can be exploited for DKR through acylation.[81,82] The Williams group has been instrumental in developing several novel strategies in the area of DKR. Epimerization of the allylic acetate starting material in Scheme 2 using Pd(II)-catalysis coupled with PFL-catalyzed hydrolysis yielded alcohol in 81% yield and 96% ee[83] and Rh-catalyzed hydrogen transfer to and from *sec*-phenethyl alcohol coupled with PFL-catalyzed acetylation give the corresponding acetate in 60% yield and 98% ee.[84] This method was later adopted for DKR of *sec*-phenethyl alcohol that instead used Ru-catalysis to epimerize the starting material and halophenol acetates as acyl donors.[85] A nice additional adaptation is the lipase-catalyzed hydrolysis of an α-bromoester which is epimerized by a solid supported bromide ion source more rapidly than its acid hydrolysis product.[86]

Table 2 Enantioselective lipase-catalyzed transesterifications: porcine pancreatic lipase = PPL; PFL = *Pseudomonas fluorescens* lipase (same as *Pseudomonas cepacia* lipase = PCL); *Candida antarctica* lipase = CAL; *Candida rugosa* lipase = CRL (same as *Candida cylindracea* lipase = CCL)

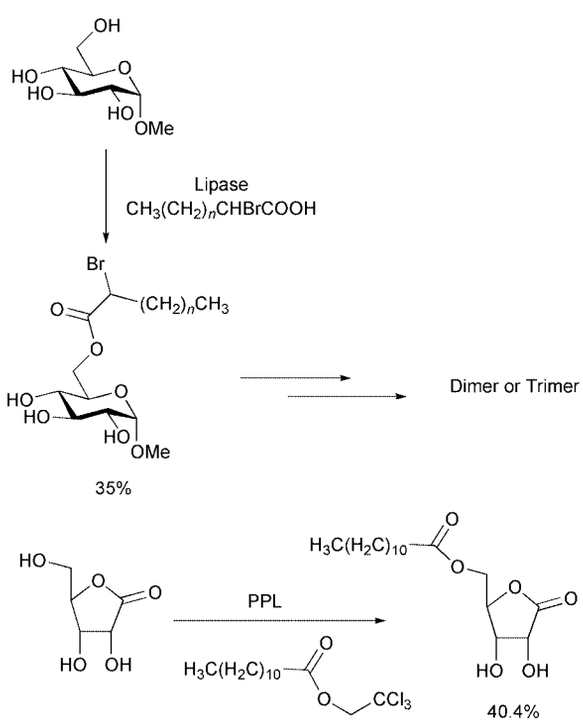
Substrate	Lipase	Co-reactant	Product	ee (%)	Reference
	PPL	$PhCH_2OH$		85	55
	Lipase PS	BuOH		84	56
	PFL	$PhCH_2OH$		85	57
	Lipase PS	Vinyl acetate		88	58
	Goat liver lipase	Vinyl acetate		91	59
	CAL	NH_3		81	60
	CRL	Vinyl acetate		99	61



Scheme 2

Desymmetrization of *meso* or prochiral compounds also offers routes into single enantiomers with a yield >50%. Desymmetrization methods have long been exploited in biocatalysis and with other enantioselective reagents as a valuable tactic for asymmetric induction. A review on this topic has appeared.[87] The excellent work of the Johnson group, largely exploiting acyltransferases, illustrates the power of both biocatalysis and desymmetrization in the synthesis of homochiral target molecules and some of this work has also been reviewed.[88] A nice trick which extends ideas of *meso* desymmetrization allows the preparation of single enantiomers from racemic 1,2-diol precursors.[89] Essentially, since *meso* desymmetrization relies on picking an *R* centre over an *S* or *vice versa*, it works just as well on *rac*-diol systems followed by configurational inversion to allow what is termed an "enantiomeric convergence". In this way the preparation of single enantiomeric *trans*-epoxides were prepared form *cis*-olefin precursors *via Pseudomonas* lipase-catalyzed enantioselective hydrolysis of diacetates and then conversion to mono acylated, mono mesylated diols prior to inversion.

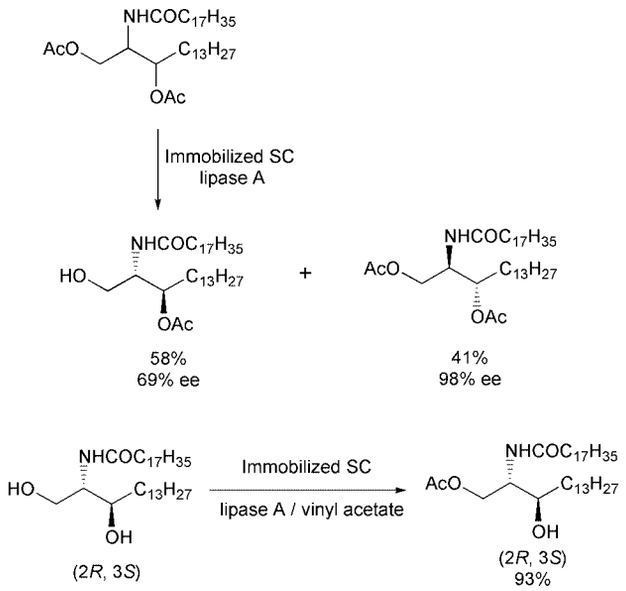
The alcohol groups in polyhydroxylated natural products such as steroids or glycosides can be readily differentiated[90] through acylation or deacylation using *e.g.*, *Candida antarctica* lipase (CAL) in organic solvents.[91–93] Typically lipases are selective for primary hydroxy groups. For example, in complex glycosylated saponins like ginsenoside Rg1, the secondary hydroxy groups were totally unaffected.[94,95] Systematic studies of glycosides have shown that the aglycone and the stereochemistry of the glycosidic bond can have a deep influence on the regioselectivity of acylation. Such acylations are compatible even with complex glycosides possessing large aglycones and with polysaccharides. These acylations are usefully applied in the synthesis of fatty acid esters of carbohydrates for use in the food, detergent or cosmetic industries as surfactants and emulsifiers (Scheme 3). For example, Vulfson *et al.* have developed



Scheme 3

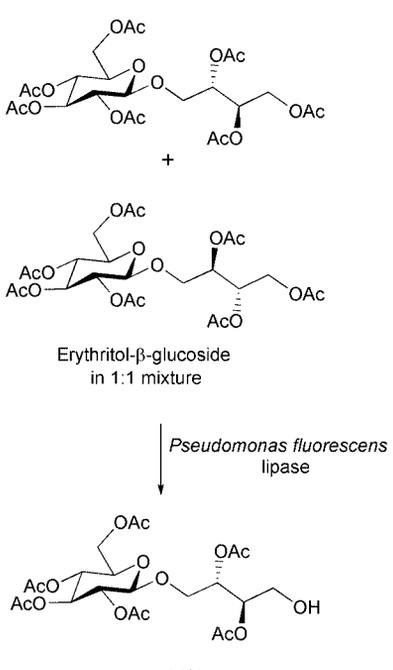
Porcine pancreatic lipase (PPL)-catalyzed syntheses.[96] Several aldonolactones were regioselectively esterified on the primary hydroxy group using 2,2,2-trichloroethyl alkanoates as acyl donors (Scheme 3).[97,98] Lipases have also been used for the acylation of nucleosides,[99] where the 5′-*O*-acyl derivatives were isolated as the major products. The lipase-catalyzed acylation of ceramides also showed complete regioselectivity for the primary hydroxy group (Scheme 4).[100] Deacylation selectivity can also be exploited. A recent survey of the deacylation of peracylated D-galactals showed that variations in 6-*O* and 3,6-*O* selectivity may be achieved depending upon catalyst and acyl group.[101] Isobutyryl and CCL proved a particularly effective and selective combination for 6-*O*-deacylation.

If the primary hydroxy groups are already protected or otherwise differentiated in glycosides then interesting



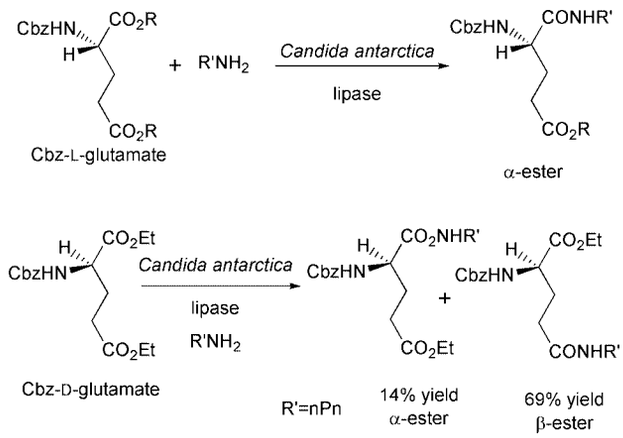
Scheme 4

regioselectivities may arise. For example, in lactosides, the 2′-OH can be selectively levinuylated when 6′-OH is protected[102] and the hydrolysis of acetylated D-galacturonic acid derivatives displays selectivity for the 2-*O*-acetyl group.[103] The use of *Pseudomonas cepacia* lipase (PCL) for the regioselective acylation of 6-*O*-protected glycosides has shown that α-glycosides were acylated at the *O*-2 position, whereas the β-glycosides were acylated on the *O*-3 position.[104] The same method has been used to differentiate *O*-2 and *O*-3 in various benzylidene-protected glucosides.[105] Interestingly, when peracetylated erythritol- or glycerol-β-glucosides were hydrolyzed by *Pseudomonas fluorescens* lipase (PFL), the sugar moiety was not affected and only one product of hydrolysis was obtained [106] (Scheme 5).



Scheme 5

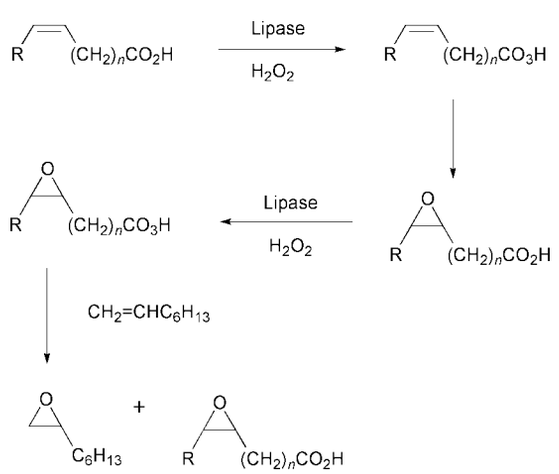
Lipases are powerful catalysts in other regioselective acylations. For example, CAL catalyzes the transfer of divinyl carbonate as an acyl donor to primary amines. The remaining enol group can then be displaced by glycine to create a carbamate.[107] Lipases also catalyze acyl transfer to other amines, for example, the amidation of Cbz-glutamic acid diesters to give the corresponding monoamide derivatives (Scheme 6).[108] More recently, an enantioselective aminolysis was described for the preparation of pyrrolidine derivatives containing stereogenic centres.[109] Lipase-catalyzed aminolysis and ammoniolysis, including carbamate formation, have been thoroughly reviewed.[5]



Scheme 6

Lipases may also be used as tools for the removal of protecting groups from delicate substrates. This utility is well illustrated by the removal of the acetyl groups from the side chain carbohydrates and the *C*-terminus methoxyethyl ester of a glycopeptide using wheat germ and *Mucor javanicus* lipases, respectively.[110]

In 1995, a lipase-catalyzed self-epoxidation of unsaturated carboxylic acids was described[111] (Scheme 7). The epoxy acid obtained was also used again to perform epoxidations of other unsaturated compounds. More recently, the same authors have described lipase-catalyzed conversions of unsaturated trimethylsilyl ethers;[112] deprotection, acetylation and epoxidation reactions took place in one-pot.



Scheme 7

Vinyl esters, and less frequently propenyl esters, are often used as acyl donors in lipase-catalyzed esterifications due to the formation of non-nucleophilic and therefore non-competing aldehyde. It has been suggested that the aldehyde released increases the rate of enzyme denaturation, perhaps through browning-type reactions following lysine imine formation. To avoid this potential problem 1-ethoxyvinyl esters[113,114] or mixed carbonic anhydrides[115] have been suggested. Another nice trick along similar lines involves the use of enol esters as substrates, which not only form ketones on hydrolysis that do not compete in transesterifications but can also be re-used. [116] Very elegantly

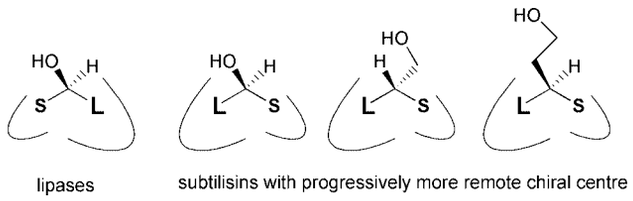


Fig. 1

this trick can be used on achiral α-alkyl- [117] or prochiral bis- enol esters.[118]

A major development in the use of lipases has been the valuable introduction by Reetz and co-workers of sol–gel encapsulated lipases.[119] The porous nature of sol–gels bestows the enzyme with enhanced longevity and mechanical stability and allows recovery and re-use. These reagents were named Reagent of the Year in 1997. The use of cross-linked enzyme crystals (CLECs)[120] has also been widely adopted.

Ester hydrolysis may be strongly influenced by co-solvents and their use may enhance enantioselectivity, it is proposed by interaction with the active site.[121] The addition of sulfur-containing crown ethers also has a beneficial effect.[122,123] The use of lipases has also been demonstrated in supercritical carbon dioxide.[124,125] Pretreatment with propan-2-ol can also increase selectivity.[126]

It has been suggested that microwave irradiation can also enhance rates and selectivities in PPL-catalyzed acylations.[127] The addition of an aqueous LiCl solution to a diisopropyl ether transesterification also enhanced enantioselectivity.[128] The addition of Et_3N in certain situations also increases the rates of lipase-catalyzed transesterifications[129] or ensures that reactions go to completion.[130]

The redesign of lipases to increase enantioselectivity has been powerfully illustrated by Reetz. Forced evolution of a *P. aeruginosa* lipase increased enantioselectivity *for the selected ester* from 2% to a remarkable 81% ee in just 4 cycles of point mutation and selection.[131,132] However, in such studies it is important to recognise the stringent specificity that often results; summed up by the adage "you get what you screen for". Such studies can be aided by rapid screening using thermographic imaging[133] or mass spectrometric detection[134] of *e.g.*, isotopic "pseudoenantiomers".[135] An ingenious colorimetric screening method of high utility has also been described; "Quick E" provides a rapid assessment of enantioselectivity.[136] Remarkably low temperatures have been described in the *P. cepacia* lipase catalyzed resolutions. Interestingly optimal resolution was observed at −40 °C.[137]

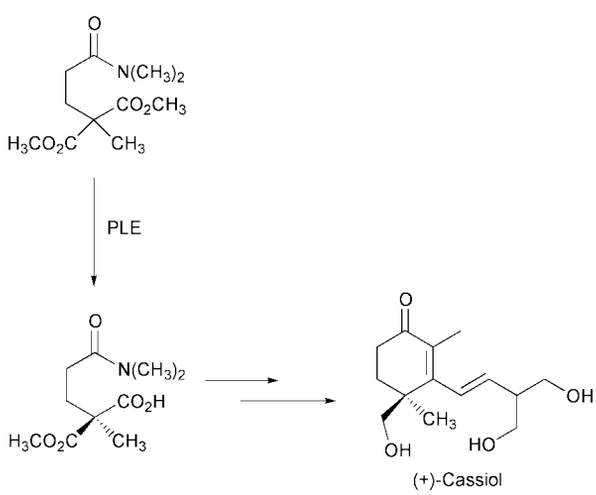
Active site models are very useful provided that they are used as a guide rather than a prescription and many papers get bogged down in the minutiae of proving this or that model correct. By definition, even when the 3D structure of the active site is precisely known through *e.g.* crystallography, additional considerations outside of simply the shape of the site are also relevant to *e.g.* resolutions that rely on the effect of diastereomeric differences in transition states. Nonetheless, certain models have very much proved their worth as valuable heuristic tools – the archetype being the powerful Jones pig liver esterase (PLE) model. Several recent models also fit into the "useful" category.[138] Kaslauskas and co-workers[139,140] amongst others[141] have started the process of unifying models of both subtilisins and lipases of different types. Examples of the type of models that result are illustrated in Fig. 1 for alcohol transesterification.

By using lipases as catalysts for acyl transfer, bifunctional monomers may be assembled, *e.g.* mixed monomers (diol with diacyl) or single monomer (hydroxyacyl), into polymers.[142] For example, CAL catalyzed the polymerization of a cyclic carbonate.[143]

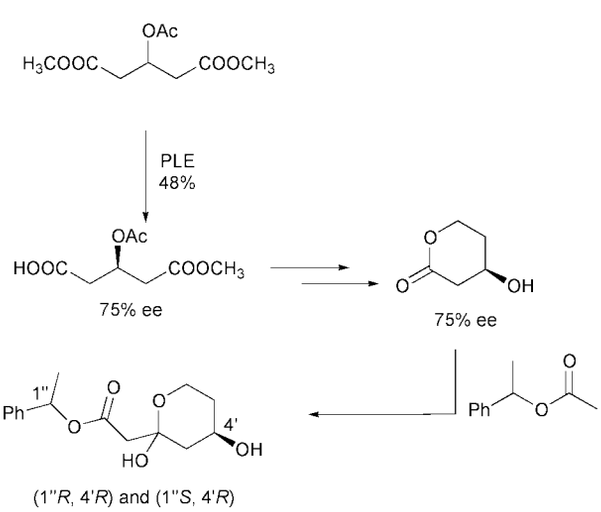
2.2 Esterases

As hydrolytic enzymes, esterase applications are numerous; one of them being the removal of protecting groups such as choline ester with butyrylcholine esterase,[156–159] *p*-acetyloxybenzyl ester with acetyl esterase[160] or heptyl[161,162] or 2-methoxyethanol[163] esters with *Aspergillus niger* lipase (ANL).

As for lipases, their use in enantioselective hydrolyses is an important way to obtain chiral building blocks as intermediates in asymmetric syntheses (Table 3): pig liver esterase (PLE) being most widely used. For example, the synthesis of (+)-cassiol, an antiulcerogenic agent, is based on the enantioselective hydrolysis of a prochiral diester by PLE (Scheme 8).[164] It was also applied to the preparation of the southern-half subunit of the fungicidal macrolide soraphen A (Scheme 9).[165] Sledeski and co-workers have described a rapid synthesis of potent antiviral agent, cyclobut-A, from the inexpensive starting material maleic anhydride (Scheme 10).[166] The same approach was used to synthesize (−)-pumiliotoxin C,[167] the segment of the antibiotic curacin A[168] and β-lactams[169] with good enantioselectivities. The plant hormone, (+)-abscisic acid, was also prepared with an esterase, which catalysed the enantioselective synthesis of the key phorenol intermediate (Scheme 11).[170] PLE was used to obtain versatile intermediates for the synthesis of alkaloids such as (−)-Kishi lactam (Scheme 12).[171] Some very valuable PLE-catalysed resolutions of α-substituted β-ketoesters were published in 1993.[172] These β-ketoesters are building blocks possessing an asymmetric quaternary carbon centre that is present in several natural products including (+)-nitramine and (−)-isonitramine.

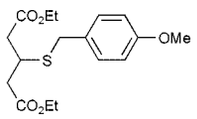
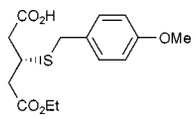
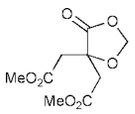
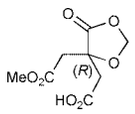
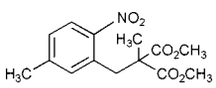
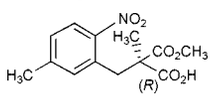
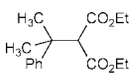
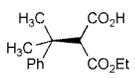
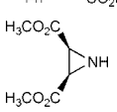
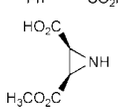
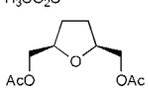
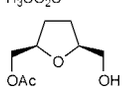
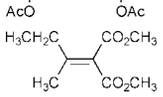
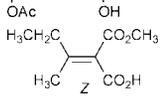
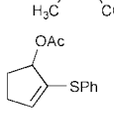
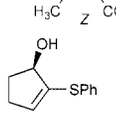
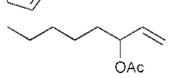
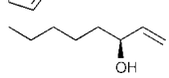
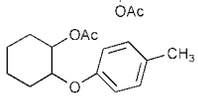
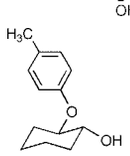
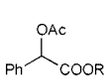
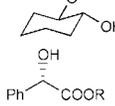
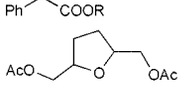
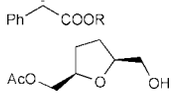


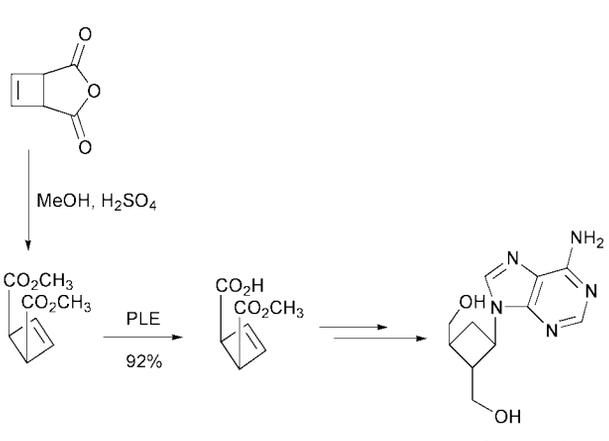
Scheme 8



Scheme 9

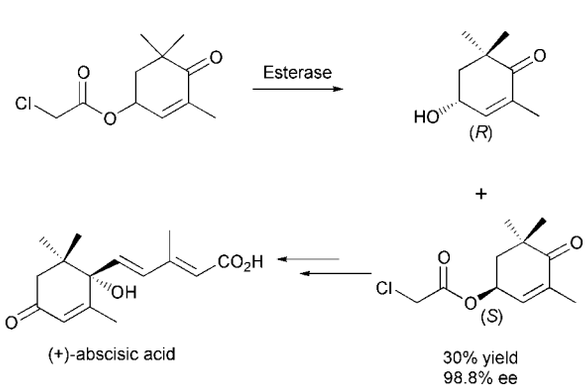
Table 3 Esterase-catalyzed stereoselective hydrolyses. PLE: pig liver esterase; PLAP: pig liver acetone powder; BLAP: bovine liver acetone powder

Substrates	Enzymes	Products	Ee (%)	References
	PLE		71	144
	PLE		90	145
	PLE		80.6	146
	PLE		94	147
	PLE		92	148
	PLE		71	149
	PLE		100	150
	PLAP		70	151
	BLAP		77	152
	PLAP		99	153
	PLAP		75	154
	PLE		100	155

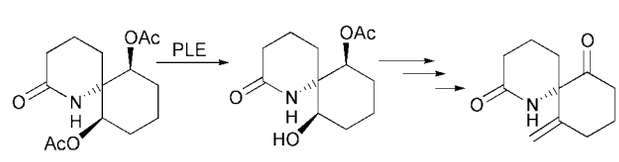


Scheme 10

Cholesterol esterase (CE) has been used in the kinetic resolution of chiral sulfoxides, which are useful chiral auxiliaries in carbon–carbon bond formation (Scheme 13).[173] Similarly, enantioselective acetate hydrolysis yields enantiomerically enriched chiral phosphines or phosphine oxides (Scheme 13).[174] Aliphatic, aromatic or cyclic amino esters or amino acids have been obtained in enantiomerically pure form using *Humicola* amino esterase[175] (Scheme 14).

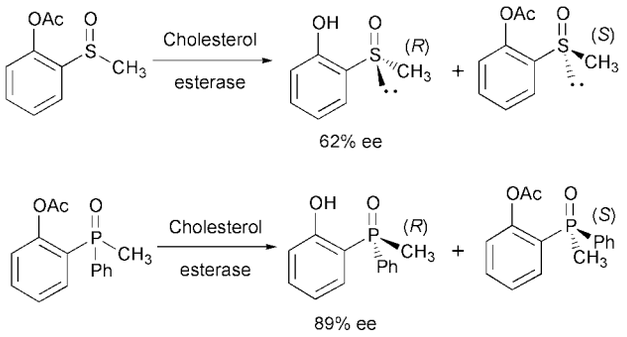


Scheme 11

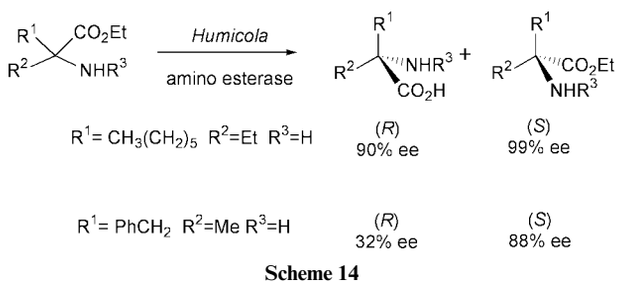


Scheme 12

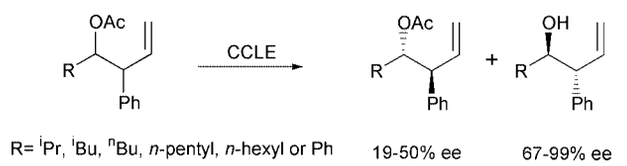
Crude chicken liver esterase (CCLE) is useful for the preparation of enantiopure *anti*-homoallyl alcohols (Scheme 15).[176]



Scheme 13



Scheme 14



Scheme 15

As for lipases, esterases display regioselectivity towards esters of polyhydroxylated compounds. In a nice example of the use of hydrolases in carbohydrate functionalization, the esterase from *Rhodosporidum toruloides* has been used to selectively hydrolyse *O*-6 esters. Sulfation of the product alcohols and acid-catalyzed migration followed by sulfation allowed the regioselective sulfation of *O*-6 and *O*-4, respectively.[177] In other regioselective hydrolyses of peracylated monosaccharides a high degree of regioselectivity is observed, which is dependent on the structure of the acyl-protecting group.[178] The authors also described products of enzymic hydrolysis obtained by subsequent intramolecular migrations of acetyl groups.

2.3 Peptidases and amidases

As described in Section 2.1, the regioselective esterification of polyhydroxylated compounds such as sugars is a very useful enzyme-catalyzed technique. However, one of the problems is finding a solvent in which the sugar is soluble and yet in which the enzyme is still active. Very valuably, pyridine and DMF are solvents in which several peptidases are comfortable. This has allowed, for example, the selective subtilisin-BPN′-catalyzed acetylation of *N*-acetylneuraminic acid (NeuAc) on its primary OH to give the natural product 9-*O*-Ac-(NeuAc).[179] To exploit this type of useful reaction and others relying on organic environments for success, the Arnold group have created a subtilisin E variant through forced evolution; an enzyme that is significantly more stable in DMF than its wild type parent.[180]

Double resolutions in which racemic mixtures of both the acyl donor and acceptor are resolved are possible. The use of CLEC-subtilisin and naphthylalanine as acyl donor and 1-naphthylethylamine as acceptor has been described as a nice example.[181] Tertiary alcohols and their esters are rarely substrates for hydrolases, indeed they can often be used as solvents. However, the cysteine protease papain has been used to resolve the tertiary acetate shown in Fig. 2.[182]

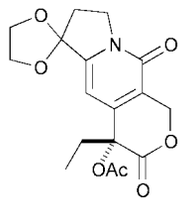


Fig. 2

The Wong group has extended the use of carbonate donors to include commercially available diallyl carbonate.[183] The use of subtilisin BPN′ in phosphate buffer provides conditions which avoid the background non-enzyme mediated reaction that can occur for primary amines in organic solvents. In this way several amines were simultaneously resolved and protected as their Aloc derivatives including an impressive regio and enantioselective carbamylation of the *meso*-diamine 2-deoxystreptamine.

Chymotrypsin (CT) has been used in the DKR of a phenylalanine iminoester in which 1,4-diazabicyclo[2.2.2]octane (DABCO) was used to epimerise the α-centre.[184] Similarly, the use of electron-withdrawing trifluoroethyl thioesters allows DKR *via* base-catalyzed epimerization of the thioester α-chiral centre coupled with subtilisin Carlsberg-catalyzed transesterification to give the corresponding less acidic *n*-butyl ester.[185]

Thermolysin featured as the key peptidase in the regioselective enzyme-catalyzed formation of an adipate tether between paclitaxel and *O*-6 of glucose.[186] The thermolytic/proteolytic stability and the utility of thermolysin-CLEC has been described including its use in the ligation of aspartate,[187] phenylalanine and alanine acyl donors in a variety of solvent systems.[188] In stunning examples of selectivity, subtilisins have also been used to catalyze the ligation of protein fragments to give lysozyme[189] and a pure, non-natural glycoform of ribonuclease.[190] They are also powerful ligating catalysts for a range of non-natural amino acids[191] and glycopeptides.[192] As for most biocatalytic systems, overly stringent substrate specificity can limit synthetic utility. A nice strategy for circumventing this problem, utilizes the concept of substrate mimicry.[193] Thus, the incorporation of a 4-guanidinophenyl leaving group into the acyl donor reactant allows the use of trypsin, thrombin and clostripain, all peptidases with a P_1-specificity for positively charged sidechains. Clostripain, a cysteine protease, proved most efficient in the synthesis of a number of polypeptides and advantageously this enzyme displays a wide P_1' specificity for ligation to a number of acyl acceptors. A phthalyl amidase has been isolated from *Xanthiobacter agillis* and used in selective deprotections.[194]

The washing of CLEC-subtilisin with organic solvents has been studied in detail to ensure a reliable reproducible source of solid-supported enzyme. The washing method and solvent affects CLEC activity in acetonitrile and it has been suggested that this importance of hydration history and a hysteresis in activity is potentially due to "locking" of the enzyme structure in an active conformation.[195] This work was extended to proteases supported on silica and then washed with *n*-propanol.[196] In addition, the use of a solid acid and its conjugate base in organic solvents increases activity by a suggested buffering effect.[197] Similar effects have been observed in other systems[198] and with dendritic buffers.[199] The use of added salts controlled water activity to allow penicillin amidase-catalyzed peptide ligations of phenylacetyl acyl donors.[200] Desolvation effects are proposed as critical in determining the difference in both level *and* sense of enantioselectivity in resolutions in organic solvents.[201] In desymmetrization reactions, enhancements of selectivity observed for CT in certain organic solvents have been shown to be due to an increase in activity towards a particular prochiral face rather than as a result of a decrease in activity

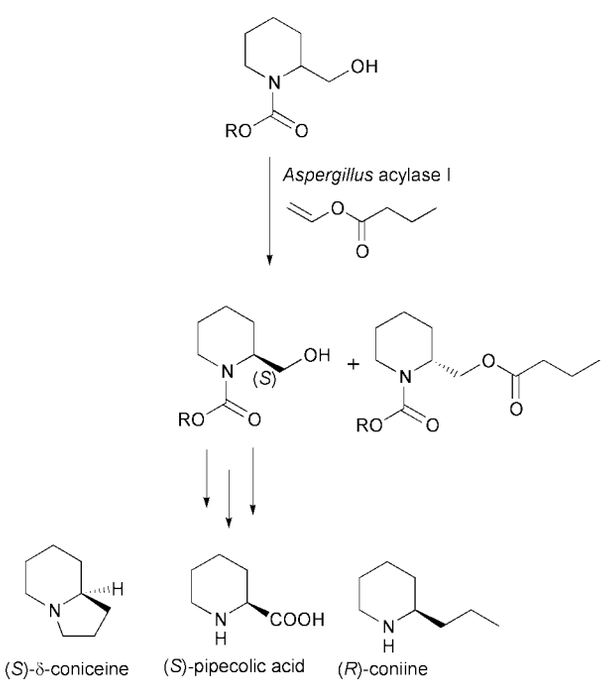
for the other.[202] For example, CLEC-α-CT preferentially transesterifies the (*R*)-acyl donor PhCH(CH$_2$OH)COOMe in acetone and the (*S*) in cyclohexane. KCl matrices[203] and lyophilization from phosphate buffer[204] create subtilisin preparations with higher activities than from non-salt or other buffer solutions. Rates are also increased by pretreatment with MeOH.[205] Soaking of subtilisin Carlsberg CLECs with crown ether increases the rates of peptide ligations.[206] The use of surfactants allows the creation of enzyme ion pairs that are valuably soluble in organic solvents.[207] Ion pairing of substrates may also enhance reaction outcomes, such as enantioselectivity.[208] The effects of pressure on subtilisins in supercritical solvents is very solvent dependent with greatest effects being observed in supercritical carbon dioxide scCO_2.[209]

A combined site-directed mutagenesis and chemical modification strategy using the subtilisin from *Bacillus lentus* allows the generation of enzymes with better than wild-type kinetic activity[210] which is matched by enhanced transesterification yields.[211]

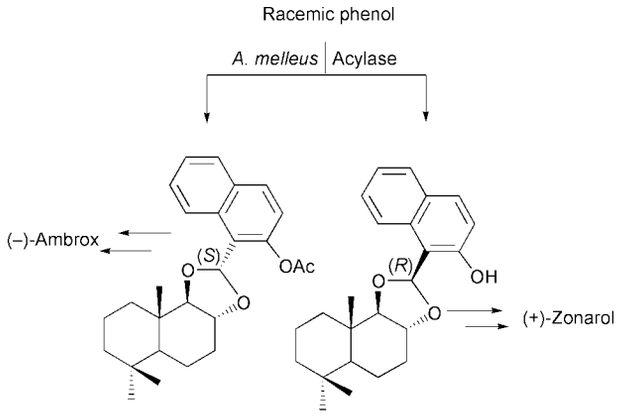
Various peptidases have been used in a novel method of encoding tentagel beads for use in the synthesis of peptide libraries.[212] This so-called enzyme "shaving" method cleaves off peptide caps on a small proportion of surface (and therefore protein and enzyme accessible) sites. These sites can then have putative target sequences constructed on them before being screened against proteins. The remaining interior "unshaved" sequences, which do not interact with proteins, can be used as a coding portion through Edman degradation for identification of the surface exposed sequences.

2.4 Acylases

Acylases are mainly used as catalysts in transesterification reactions. For example, their use has been described in kinetic resolutions of racemic piperidine derivatives (Scheme 16).[213] The resulting (*S*)-configuration building blocks were used for the short syntheses of the alkaloids (*S*)-pipecolic acid (*S*)-δ-coniceine and (*R*)-coniine. A similar approach was applied to the preparation of drimane sesquiterpenes (−)-ambrox and (+)-zonarol;[214] thus, the enantioselective hydrolyses by acylase I from *Aspergillus melleus* of a phenolic acetal derivative were used to obtain both of the required enantiomeric intermediates (Scheme 17).

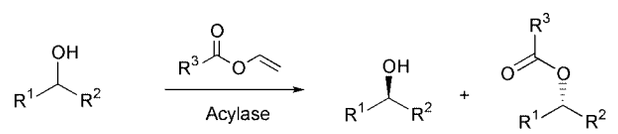


Scheme 16



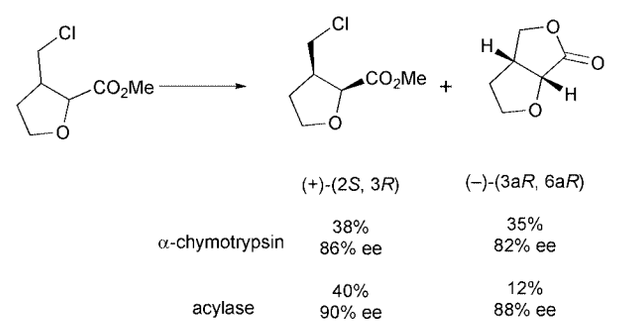
Scheme 17

The resolution of secondary alcohols was performed using the acylase from *Aspergillus niger* with different vinyl esters as acyl donors (Scheme 18).[215] The enzymatic hydrolysis by α-chymotrypsin or acylase I was also applied to the resolution of racemic esters such as methyl 3-chloromethyltetrahydrofuran-2-carboxylate (Scheme 19).[216] In this case, hydrolysis was accompanied by spontaneous lactonization.



R^1= aryl, alkyl
R^2= Me, Et, vinyl, allyl, propargyl
R^3= Me, $CH_2CH_2CH_3$

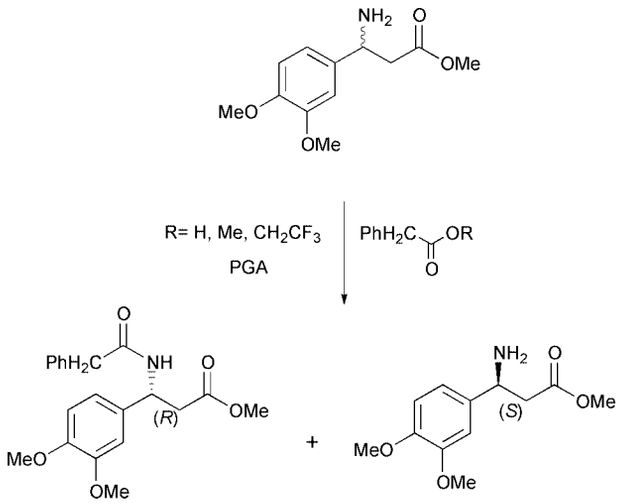
Scheme 18



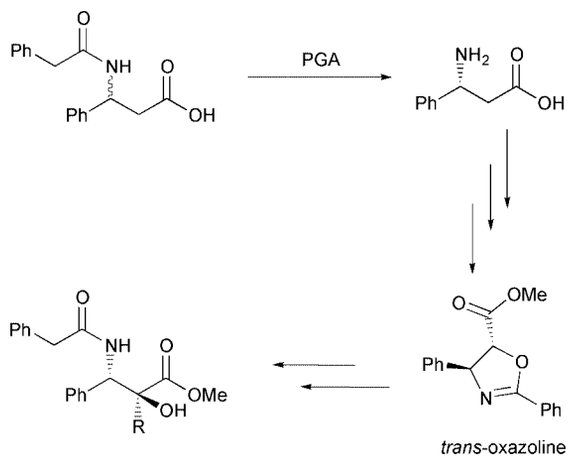
Scheme 19

Recently acylases have found particular use in the selective protection and liberation of amino functionalities. For example, Roche *et al.* resolved racemic β-amino esters using penicillin G acylase (PGA) (Scheme 20).[217] Similarly (2*S*,3*S*)-(+)-hydroxyleucine in 99% ee[218] and (2*R*,3*S*)-*N*-benzoylphenylisoserine methyl ester, a Taxol side-chain analogue,[219] have also been prepared. More recently, Cardillo *et al.* have published the preparation of *anti*-α-alkyl-α-hydroxy-β-amino acids.[220] PGA also allowed the formation of an enantiopure amino acid, which then gave a useful *trans*-oxazoline intermediate (Scheme 21). Alkylation of the oxazoline, followed by its hydrolysis, furnished the corresponding *anti*-α-alkyl-α-hydroxy-β-amino acid. Soloshonok *et al.* have resolved various β-fluoroalkyl-β-amino acids with PGA (Scheme 22).[221–223] The resolution of racemic NHAc-protected amino acids can also be performed using acylases. For example a ferrocenyl side-chain amino acid was resolved in this way using *Aspergillus melleus* acylase[224] and the same method was used to prepare enantiopure neoglycolipid *C*-glycoside templates (Scheme 23).[225]

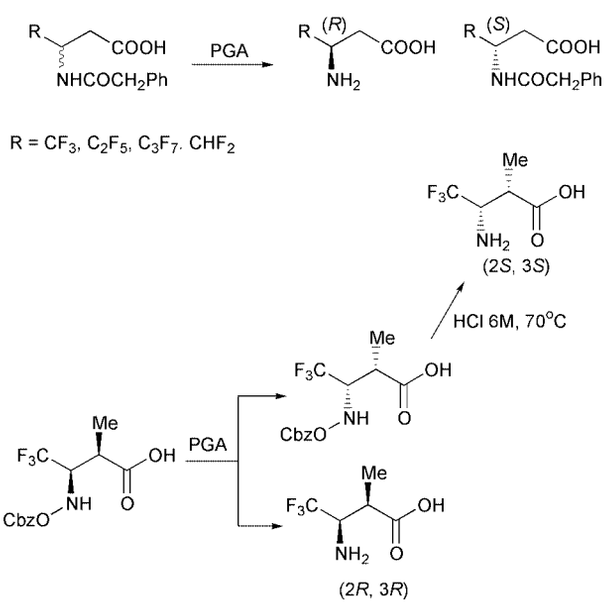
PGA has also been largely used in enzymatic protecting group strategies.[226,227] Waldmann and coworkers have described the application of PGA to remove the phenylacetyl[161,228] or



Scheme 20



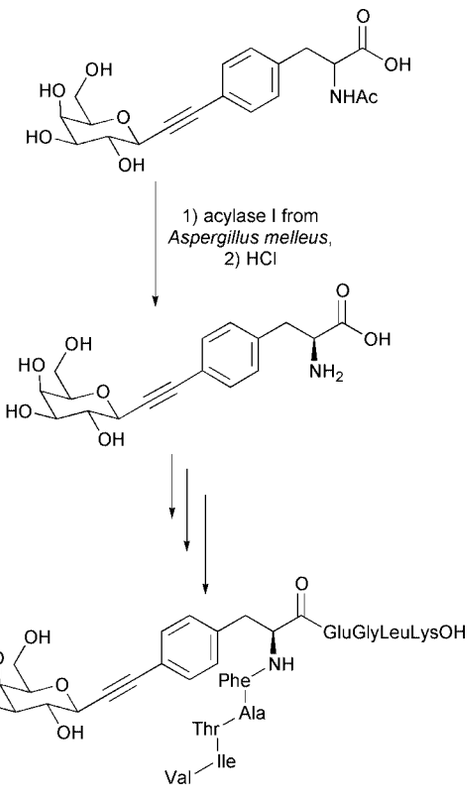
Scheme 21



Scheme 22

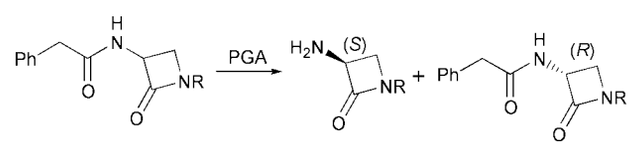
p-acetoxybenzyloxycarbonyl[229,230] protecting groups. Both deprotections rely on the very useful preference of this enzyme for a P_1 phenylacetyl moiety. An extension of this concept allows the use of an acylase cleavable *p*-phenylacetoxybenzyloxycarbonyl solid-phase linker unit which spontaneously fragments following cleavage of the acetyl group.[231]

Enantiopure (*S*)-3-aminoazetidin-2-one, the core substructure of most important β-lactam antibiotics (penicillins,

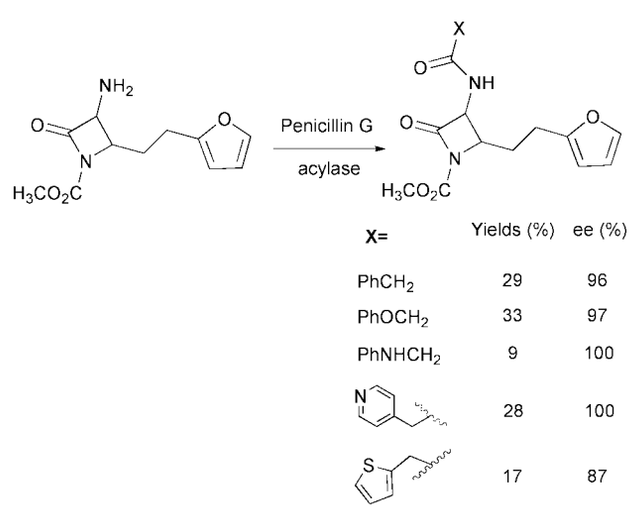


Scheme 23

cephalosporins[232,233] and monobactams), was obtained by deprotection of the amino group with PGA (Scheme 24).[234] The action of PGA on α-amino-β-lactams has been studied by Briggs *et al.*; the enzyme was able to transfer a variety of different acyl donors onto the free amino group (Scheme 25).[235] Pyrroline derivatives have been synthesized by the liberation of an amino group using immobilized PGA followed by spontaneous ring closure.[236]



Scheme 24



Scheme 25

3 Carbohydrate processing enzymes

Enzymatic methods are now widely accepted as being as much a part of oligosaccharide synthesis as more traditional chemical

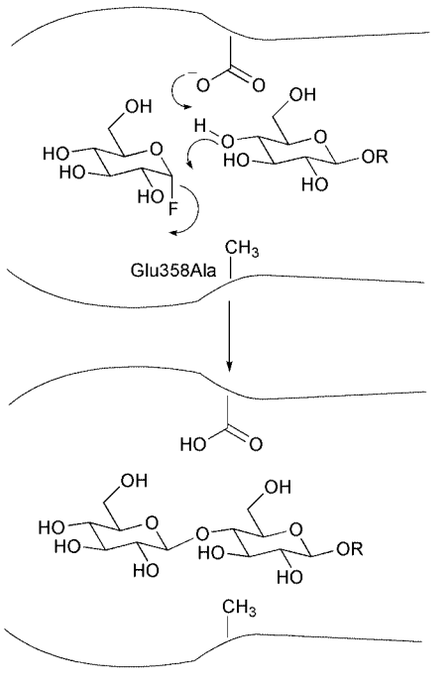
methods. Given the often long-winded protection regimes required for most chemical glycosylations, the use of enzyme-catalyzed one step systems is clearly attractive. Thus, sialyltransferases or transialidases typically provide a superior method for sialylation to any chemical techniques. However, there will also be occasions when the complexity of enzyme systems, low yields, stringent specificities or the lack of an available catalyst will favour chemical systems. For example, until relatively recently examples of enzyme catalyzed β-mannosylations have been limited in number and/or effectiveness. Of course, as their use widens, these potential limitations of carbohydrate-processing enzymes will diminish and their utility continues to rapidly expand. Many of the exciting recent developments in this field have been covered in three excellent reviews,[237–239] and aspects of the use of glycosyltransferases and glycosidases applied to the synthesis of sLe^x have also been discussed.[240] In addition, two classic reviews on the use of biocatalysis in carbohydrate chemistry and biology have appeared from the Wong group: Part I[241] describes the broad uses of aldolases (see section 7.1) and Part II[237] the use of biocatalysis for the construction of the glycosidic linkage.

3.1 Glycosidases

An exemplary screening study using various *p*-nitrophenyl (pNP) glycosides as donors and carbohydrates as acceptors, shows the sort of thoroughness that is required for establishing the true synthetic utility of glycosidases.[242] In this excellent work approximately 60 crude preparations were screened for 7 activity types and once identified, regioselectivities then defined.

In a highly elegant strategy Withers and co-workers have obtained very high yields for glycosylations using glycosyl fluoride donors with glycosidase mutants devoid of the nucleophilic active-site carboxylate.[243] These so-called glycosynthases allow oligosaccharide synthesis but are unable to hydrolyse *O*-glycosidic linkages. Thus, the Glu358Ala mutant of the glucosidase from *Agrobacterium sp.*, in which the $–CH_2CH_2COO^-$ of glutamate is chopped back to just $–CH_3$, was created. This leaves an intact active site shape and general base catalyst but no natural activity. This mutant was then used with activated glucosyl fluoride donors and various acceptors to give high to excellent (66–92%) yields of oligosaccharides without concomitant hydrolysis (Scheme 26). In fact, the reaction is so efficient that the major problem of this method is oligomerization to tri-, tetra- and higher saccharides. The potential of this method was further illustrated by the use of the 2-deoxy-2-fluoroglucosides as acceptors that would have irreversibly inhibited a wildtype enzyme.

β-Galactosidases from *Bacillus circulans*[244,245] or *Bacillus singularis*[246] allow the synthesis of Galβ(1,4) disaccharides. *Bacillus circulans* galactosidase also shows this β(1,4) regioselectivity in the glycosidation of a wide range of acceptors[247] including thioglycosides.[244] This selectivity increases with the number of saccharide residues in the acceptor as demonstrated by the synthesis of the trisaccharide Galβ(1,4)GlcNAcβ(1,6)GalNAc in 48% yield.[247] Interestingly, enhanced β(1,3) regioselectivity is seen for this enzyme with β-1-*N*-acetamido-D-glucopyranose, a simple model for *N*-linked glycopeptides, as an acceptor: a result attributed to enhanced potential for hydrogen bonding by the acceptor aglycon.[248] This same study also thoroughly investigated the relative activities of *B. circulans* β-galactosidase with various organic cosolvents: acetonitrile was by far the most detrimental and 30% acetone v/v was finally chosen as optimal. Non-covalent coating of β-galactosidases with a polyhydroxy headgroup lipid allowed the use of these enzymes in non-polar organic solvents such as diisopropyl ether.[249] With simple alcohol acceptors the specificities of these systems appear to reflect those of the native unmodified enzymes in aqueous systems and conversions as high as 62% were reported, although oligosaccharide synthesis was not mentioned. The same lipid-coated galactosidase also showed superior activities in supercritical carbon dioxide ($scCO_2$) relative to diisopropyl ether and over non-coated enzyme in $scCO_2$.[250]



Scheme 26

Regioselective β(1,4)-galactosylation of GlcNAcα(1,2)-ManαSPh using a galactosidase from *Bifidobacterium bifidium* proved a key step in the first synthesis of the serine tetrapeptide from α-dystroglycan, which contains a rare Man–Ser linkage.[251] Chemical glycosylation of the serine side chain through NIS, TfOH activation of a protected form of the thioglycoside that is the product of enzymatic galactosylation followed by sialyltransferase mediated sialylation gave the final product in just 9 steps.

Screening of recombinant thermophilic glycosidases from the Diversa Corporation allowed the identification of high yielding β(1,4) galactosidases that formed *N*-acetyllactosamine from *N*-acetylglucosamine in 61% in 30 minutes using a pNP galactoside donor.[252] Ion exchange chromatography and the use of the thermophilic β-galactosidase Gly001-09 (operating temperature 85–90 °C) also allowed the synthesis of the lactosamine using lactose and glucosamine as starting materials on a gram scale with good 1,4 regioselectivity.[253,254] Notably both the normally good 1,4 selectivity of *B. circulans* galactosidase, which gave 10% 1,4 and 6% 1,6, and the activity of bovine β(1,4)-galactosyltransferase, the latter due to its stringent substrate specificities, failed in this system.

It should be noted that the 1,4 selectivities shown by these β-galactosidases complements the 1,6 selectivity of *E. coli* β-galactosidase and the 1,3 selectivity of bovine testes β-galactosidase. For example, the Galβ(1,3)GalNAcαSer TF determinant has been synthesized using a β-galactosidase from bovine testes.[255,256] Due to its broad donor specificity the β-glycosidase from the thermophilic microorganism *Thermus thermophilus* allows Galβ(1,3) autocondensation and Glcβ(1,3) transglycosylation.[257] Reduced hydrolysis product and increased rates of conversion have been reported in transglycosylations to simple alcohols catalyzed by other thermophilic β-galactosidases in microwave irradiated, dry media.[258]

The importance of the so-called α-Gal epitopes to immunoregulatory therapies has prompted a number of enzymatic syntheses of Galα(1,3)Gal containing oligosaccharides. Crout and co-workers have described a practical (and simpler alternative

to galactosyltransferases, see below) α-galactosidase approach which gives yields of α-Gal epitope saccharides of 42–48% with exclusive α(1,3) selectivity using the readily available α-galactosidase from *Penicillium multicolor.*[259] This has improved a previous sequential use of the β-galactosidase (*Bacillus circulans*) and an α-galactosidase (*Aspergillus oryzae*) to give a linear α-Gal epitope trisaccharide; until the use of the *P. multicolor* enzyme, lack of regioselectivity in the α-galactosylation had necessitated separation from the α(1,6) side product.[260] The α-galactosidase from *A. niger* gave only α(1,6) products whereas those of *Coffea arabica* and *A. oryzae* gave mixtures of Galα(1,6) and Galα(1,3) products.[261] A second galactosidase from *A. oryzae* interestingly gave only a α(1,6) *branched* trisaccharide product from lactose. Furthermore increasing the size of the anomeric substituent in the thioglycoside acceptor gave better yields.

The first use of a readily available β-mannosidase using *p*-nitrophenyl β-mannoside as a donor provides a vital enzyme-catalyzed alternative to the variety of chemical methods for this difficult to form linkage.[262] The enzyme was isolated from the crude extract of hexosaminidase available from *A. oryzae* and gave 26% yield of the β-mannotrisaccharide Manβ(1,4)GlcNAcβ(1,4)GlcNAc.

The β-*N*-acetylhexosaminidase from *Aspergillus oryzae* shows a β(1,4) selectivity for D-gluco substrates but a β(1,6) selectivity for D-galacto substrates.[247] Unusually, the same hexosaminidase catalyzes the transfer of *N*-acetylglucosamine from pNP-β-GlcNAc to the OH-1 of mannose to give as the major product a trehalose-type derivative and this is the first example of a galactosidase catalyzed glycosylation of an anomeric hydroxy.[263] The properties, *e.g.* sequence alignments, specificity and catalytic properties of microbial β-hexosaminidases have been reviewed.[264]

The often broad acceptor specificity of glycosidases, whilst providing an advantage in terms of general utility, may lead to low regioselectivities. To overcome this problem, protease catalyzed regioselective acylation of *O*-6′ of lactose allowed selective α-galactosylation of *O*-3′ using the α-galactosidase from *Talaromyces flavus*, by blocking the competing *O*-6′ glycosylation site.[265] Simple glycosides may be synthesised by reverse hydrolysis from the corresponding parent sugars using an appropriate glycosidase in a reaction solution containing a high (up to 90%) proportion of the acceptor alcohol.[266] It was found that unlike the use of lipases, where almost all water can be excluded, at least 5% is required for the optimal activity of glycosidases. By drawing an analogy with acyltransferase biocatalysis, where the use of, for example vinyl acetate, is common due to the formation of non-nucleophilic and therefore non-competing acetaldehyde, vinyl galactosides have been suggested as donors for β-galactosidase catalysed glycosylations.[267] Although at room temperature standard NP donors gave better yields, at −7 °C yields of up to 80% were reported for vinyl glycosides. This reversal is attributed to a reduction in hydrolytic activity and a concomitantly lower reduction in transglycosylation activity. The use of nitropyridyl (3- and 5-) leaving groups offers the advantages of higher solubility over concentrated pNP glycoside donors.[268] Saturated β-galactosyl, glucosyl and *N*-acetylglucosaminyl donor concentrations of 600, 300 and 50 mM as compared with 100, 100, and 5 mM for pNP glycosides allowed greater yields than pNP controls in the hands of the authors. Furthermore, increased reactivity allowed shorter reaction times. Transglycosidation reactions of nucleosides have been performed using *Bacillus stearothermophilus* and allow the introduction of non-natural bases.[269]

3.2 Glycosyltransferases

Nine diantennary glycodelin oligosaccharides ranging from hepta- to undeca-saccharides have been synthesised using a combination of conventional chemical glycosylation and glycosyltransferase catalyzed elaborations.[270] Fucα(1,3)GlcNAc and all sialyl links were introduced using fucosyltransferases and an α(2,3)sialyl transferase. Remaining Fucα(1,2)Gal links were achieved chemically. This synthesis demonstrates both the strengths (excellent strategic planning) and weaknesses (unpredictable steric mismatches and over specificity of glycosyltransferases) of current approaches and so illustrates well the challenges and opportunities for integrating biocatalysis into oligosaccharide synthesis.

Ingenious modifications of enzymatic systems often circumvent problems of inhibition by products. For example, the problem of inhibition by nucleotide diphosphate leaving group of glycosyltransferases has been solved by *in situ* regeneration of glycosyl nucleotide diphosphates (also an economic benefit as these are expensive and difficult to prepare). A new three enzyme system uses sucrose synthase driven in reverse to give glucosyl UDP followed by epimerization with epimerase to give galactosyl UDP which is in turn the substrate for the third and key synthetic enzyme, galactosyltransferase.[271] Another excellent example is that of a combined β-galactosidase–α(2,3)-sialyltransferase system, which still operates well at pH 7.5 despite the nearly 3 pH unit difference from the galactosidase optimum.[272] This allowed the synthesis of sialyl T-antigen Siaα(2,3)Galβ(1,3)GalNAc in 36% overall yield.

A thiol spacer arm linked *N*-acetylglucosamine was loaded onto a thiopyridyl sepharose matrix and β(1,4)galactosyltransferase-mediated galactosylations revealed optimal efficiencies for the longest linker length (78 atoms).[273] Sequential β(1,4)Gal-T, Sial-T and Fuc-T reactions allowed the synthesis of SLe^x in 57% overall yield after cleavage from the support with 1,4-dithio-DL-threitol (DTT). *N*-Acetylglucosaminyltransferase and galactosyltransferase have also been extended to the use of sepharose-acceptor conjugates linked by a squarate linker.[274] Bovine β(1,4)galactosyltransferase has also been used to galactosylate OH-4 of C-glucosides bearing hydroxymethyl and protected aminomethyl substituents at the pseudoanomeric centre.[275] Notably, the use of *meso*-compounds opens up opportunities for enzymatic desymmetrization.

With the aim of preparing a potential α-Gal epitope antagonist, the Wang group has used a recombinant bovine α(1,3)galactosyltransferase to prepare α-Gal epitope containing structures.[276] To ensure solubility of this naturally membrane-bound protein a truncated domain was designed and the corresponding gene sequence was cloned into a readily available pET vector and then expressed in *E. coli.* This gave high specific activities well above those from extracting such enzymes from natural sources and therefore ready access to α(1,3)Gal bond formation with a range of C-1 and C-2 modified lactose and lactosamine acceptors. A novel galactose epimerase α(1,3)galactosyltransferase fusion protein has also been described which utilizes the cheaper substrate UDP-Glc.[254]

A truncated yeast β(1,4)-mannosyltransferase has been expressed in *E. coli.*[277] Unlike, the membrane bound native form, this novel form is devoid of a hydrophobic membrane-anchor region and is therefore soluble. Furthermore, His-tagging (addition of a multihistidine sequence to a terminus of the protein) allowed its easy purification with a nickel affinity column. This also allowed immobilization and thereby stabilization. This enzyme catalyzed the transfer of a β-mannosyl unit to the OH-4′ of natural and mimetic chitobiosyl phospholipid acceptors from donor GDP-mannose. A Rhaα(1,3)Galβ-chromophore acceptor has been β(1,4)-mannosylated using a recombinant mannosyltransferase from *Salmonella* thereby expanding the substrate specificity from the native acceptor which is very specific and difficult to synthesise.[278]

3.3 Other carbohydrate processing enzymes

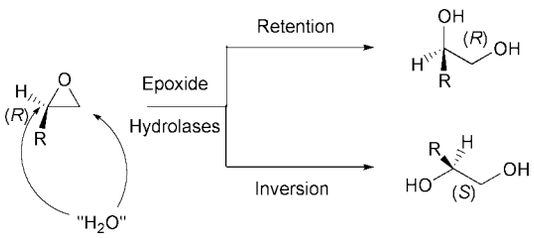
Galactosides can be oxidised to C-6 aldehydes using galactose

oxidase[279] and nucleosides to C-5 acids using a nucleoside oxidase.[280]

4 Other hydrolytic enzymes

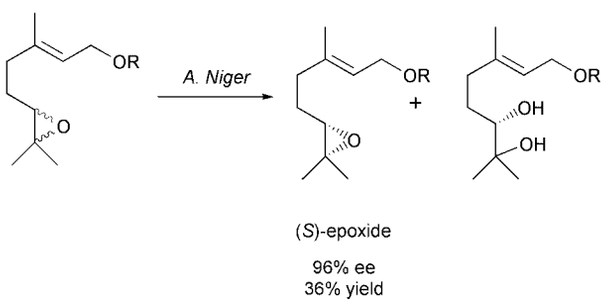
4.1 Epoxidases

Epoxidases are enzymes that catalyse the hydrolysis of an epoxide to furnish the corresponding vicinal diol.[281,282] The configuration of the resultant diol can be retained or inverted, depending upon the regioselectivity of the enzyme and the substituents on the carbon atom involved (Scheme 27). For example, epoxidases have been used for the hydrolysis of different mono-[283,284], di-[285] or tri-[286] substituted and styrene-oxide-type epoxides.[287] Several reviews of the field of enzyme catalysed epoxide hydrolysis have been published, highlighting, in particular, the use of enantiopure epoxides and diols as valuable chiral building blocks.[288,289] All of the following examples are based on kinetic resolutions each leading to an optically active 1,2-diol and the recovery of an unprocessed epoxide.



Scheme 27

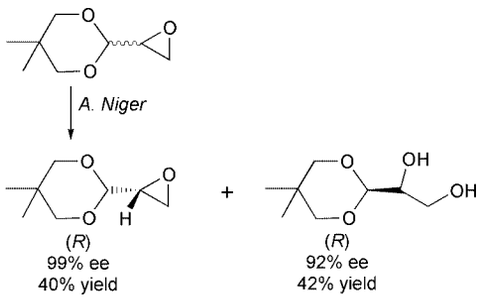
The enantioselective hydrolysis of racemic epoxides has been investigated for the synthesis of Bower's compound.[290] The epoxidase from *A. niger* gave the enantiopure (*S*)-epoxide and (*S*)-diol (Scheme 28). Furstoss *et al.* have employed the same approach for the hydrolysis of various stereoisomers of the exocyclic limonene epoxide.[291] More recently the epoxidase from *A. niger* was used for the preparative hydrolysis of glycidyl acetal derivatives, which are difficult to obtain by purely chemical methods (Scheme 29).[292] Faber *et al.* have also used this strategy for the preparation of *trans*-(2*R*,5*R*)- or *cis*-(2*R*,5*S*)-5-(1-hydroxy-1-methylethyl)-2-methyl-2-vinyltetrahydrofuran (linalool oxide) (Scheme 30).[293] Similarly, enantioselective hydrolysis by the epoxidase from *R. equi* of racemic epoxide was used to obtain the pheromone (*S*)-(−)-frontalin (Scheme 31).[294] Other pheromone analogues have been synthesised by hydrolysis of *meso*-epoxides.[295] In the chemoenzymatic synthesis of (*R*)-nifenalol, a β-adrenergic blocker (Scheme 32),[296] an *A. niger* epoxidase-catalysed hydrolysis of racemic *p*-nitrostyrene oxide was the key step.



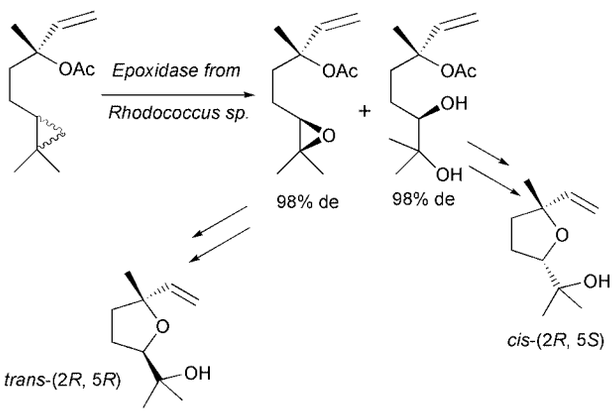
Scheme 28

4.2 Nitrilases and nitrile hydratases

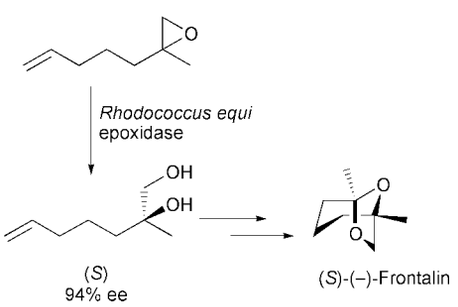
These enzymes are responsible for the biodegradation of nitriles, which can proceed *via* two different enzymatic pathways. Nitrilases catalyse the direct hydrolysis of nitriles to the corresponding acids; whereas nitrile hydratases catalyse the hydration of nitriles to the corresponding amides.[297] These



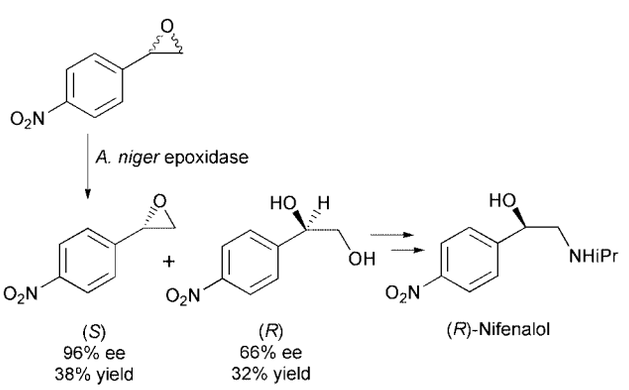
Scheme 29



Scheme 30



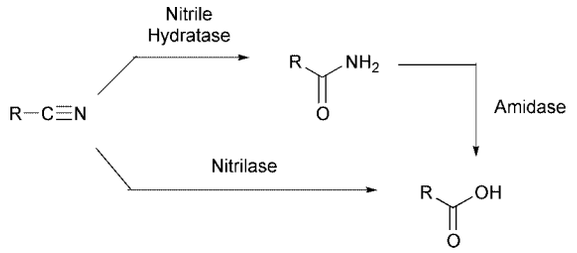
Scheme 31



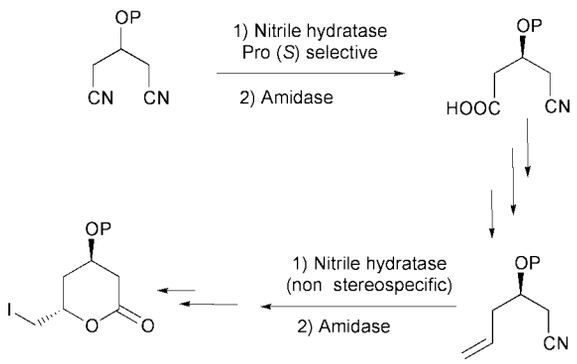
Scheme 32

amides can then be converted to acids by *e.g.*, an amidase in a second step (Scheme 33).[298] Several systematic studies have examined different racemic or prochiral nitriles.[299] Regioselective hydrolyses of aliphatic dinitriles,[300] aromatic dinitriles,[301] and heterocyclic nitriles[302] have also been published. The high functional group flexibility of nitriles as intermediates in synthesis has driven much of this work as exemplified by the use of these enzyme-catalyzed hydrolyses for the preparation of various enantiomerically enriched targets.

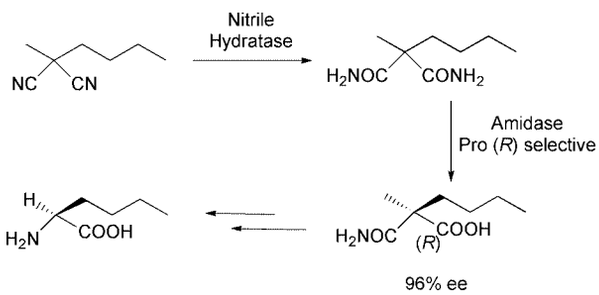
For example, a stereoselective *meso*-desymmetrization followed later in the route by a non-specific hydrolysis were applied to the preparation of the lactone moiety of mevinic acids, hypocholesterolemic agents (Scheme 34).[303] In the prepar-



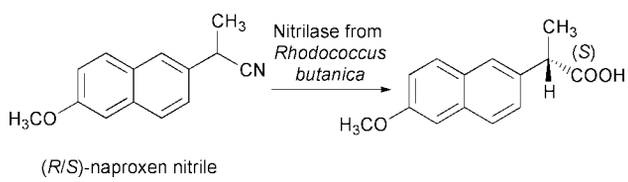
Scheme 33



Scheme 34



Scheme 35



Scheme 36

ation of α,α-disubstituted α-amino acids from prochiral dinitriles (Scheme 35),[304] the enantioselectivity came solely from the action of the amidase in the second step. Effenberger and co-workers have described the synthesis of the non-steroidal anti-inflammatory (*S*)-naproxen[305] in 99% ee (Scheme 36) through the hydrolysis of the racemic naproxen nitrile.

4.3 Other hydrolases

In a striking example of the general utility of biocatalysis in broad applications, an organophosphorus hydrolase has been incorporated into firefighting foam without dramatic loss of activity.[306] This provides an effective way of decontaminating areas that have been affected by nerve agents: the agent is extracted from the surface into the foam and then hydrolysed.

5 Reduction

5.1 C=O, C=N reduction

Baker's yeast, because of its availability and cheapness, is probably still the most popular reducing biocatalyst. In particular it shows great usefulness in the stereoselective reduction of β-ketoesters to (*S*)-alcohols.[307,308] More complex substrates including ketosulfones[309] also serve as good substrates. In these reductions very good chemoselectivity can be observed, such as in the stereo- and chemo-selective reduction of a ketone α to an oxime,[310] that would be difficult to achieve by other methods. 1,3-Diones may also be stereoselectively reduced to diols.[311] Models for the reduction of certain substrates, such as cyclic β-ketoesters,[312] are also being developed. Interestingly, stereoselectivities and conversions increase in certain baker's yeast reductions when organic solvents are employed.[313] Cofactor NADH recycling can be achieved using methylviologen and electrolysis[314] amongst other methods,[315] in addition to more usual enzyme couples. Reductases isolated from baker's yeast also give very good results.[316]

Stereoselective microbial reduction by *Geotrichium candidum* is also a popular method.[317] For example, use of an acetone powder with $NADP^+$ and propan-2-ol reduces a broad range of ketones to (*S*)-alcohols[318] and in the reduction of racemic mixtures of α-substituted-β-ketoesters is typically equally specific for both enantiomers. In certain circumstances, *anti* selectivity can be increased by the addition of methyl vinyl ketone or chloroacetone which inhibit *syn*-selective reductases.[319] Such inhibition has been demonstrated by isolation of the reductases concerned.

For anti-Prelog selectivity to give (*R*)-alcohols, *Pichia farinosa* or *Yarrowia lipolytica*[320] have been recommended as biocatalysts. Their sometimes low selectivities can be enhanced by enantioselective biocatalytic oxidation of the unwanted (*S*)-alcohol.[321] Similarly, appropriate choice of organism allows stereoselective reductions of other substrates, *e.g.*, nitroketones, in both Prelog and *anti*-Prelog senses.[322]

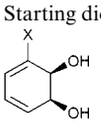
The lactate dehydrogenase from *Bacillus stearothermophilus* (BSLDH) shows a very good L-stereospecificity that makes it a good model for the study of the origins of stereospecificity. Some very interesting engineering work has revealed some key elements of the source of this specificity.[323] Thus, double point mutation to create I240K/R171Y-BSLDH reverses the positions of hydrophobic and electrostatic binding points, respectively and is highly effective in causing a partial reversal to create a 2.3% D-lactate leakage through inverse binding and opposing facial hydride delivery. To achieve full D-lactate-type selectivity the lactate dehydrogenase from *Staphylococcus epidermidis* (SELDH) can be used, however this enzyme can show a restricted substrate specificity towards bulky substituents. This problem was interestingly circumvented by the use of a H205Q mutant of the dehydrogenase from D-hydroxyisocaproate.[324]

Willis and co-workers have cleverly exploited a variety of amino acid dehydrogenase-catalyzed reductive aminations in some expeditious syntheses of labelled amino acids.[325] In many cases, this was combined with a CCL-catalyzed racemization-free ester hydrolysis to give the appropriate keto-acid substrate. For example, leucine dehydrogenase was used to create both diastereotopically CD_3 and $^{13}CH_3$ labelled leucines[326] and labelled L-valines, L-isoleucines and *allo*-isoleucine[327] from Evans oxazolidinone derived keto-acid precursors. The two epimers of MOM-protected-3-hydroxy-2-oxo-butanoate were stereoselectively reductively aminated using leucine or phenylalanine dehydrogenase which show different stereospecificities for the C-3 stereocentre.[328] This difference in stereospecificity was investigated and showed that leucine dehydrogenase allows the first example of a combined reductive amination and kinetic resolution of $CH_3(CH_3XCH_2Y)C^*HC(O)COOH$ where X,Y = O,O (de = 60%).[329] However, use of X,Y = CH_2,CH_2 or CH_2,O or phenylalanine dehydrogenase resulted in 1 : 1 epimeric mixtures.

5.2 Other reductions

Choice of organism can allow the selective reduction of the C=O alone or saturative reduction in certain stereoselective α,β-unsaturated ketone reductions.[330] Similarly, the use of

Table 4 The use of enantiopure dienediols in asymmetric syntheses

Starting diol 	Products	References
X = Cl	Deuterated hexoses	349
	L-Ascorbic acid	350
	Nonulosonic acids	351
	Deoxyfluorohexoses	352
X = Br	Inositols	353
	Aza-*C*-disaccharides	354
X = CN	Shikimic acids	355
$X = CH_3$	Taxol AB-ring system	356
$X = CH_2CH_2Br$, Me, Ph	Morphinoids	357, 358

Pichia farinosa-catalyzed reduction followed by PCC oxidation allows the overall preparation of cyclohexanones from α,β-unsaturated counterparts with fair to good stereoselectivity.[331] The apparent baker's yeast-catalyzed stereoselective reduction of the C=C double bond of an allylic alcohol probably proceeded *via* the enal.[332]

Aryl azides,[333] aromatic *N*-oxides[334], azide over aldehyde reduction[335] and even unusual Ar-C=C bond reductions[336,337] have been demonstrated using baker's yeast or enzymes isolated from it. Sulfoxides can be reduced stereoselectively using *Rhodobacter sphaeroides*.[338] Hilvert's selenosubtilisin, the ingenious product of site-selective chemical modification of subtilisin, is not a peptidase but a peroxidase. Its use in a highly enantioselective reduction of a hydroperoxide has been demonstrated.[339]

6 Oxidations

6.1 Hydroxylation

Phenylcyclohexane has been used as a substrate to investigate the selectivities of C–H bond oxidation of wild-type (WT) and mutant monooxygenases.[340] Exclusive aliphatic chemoselectivity, moderate regioselectivity and low (Y69F mutant and WT) to zero (Y69A) stereoselectivity were observed in preparative scale incubations. Dioxygenases can also show monooxygenase-type activity to allow stereoselective hydroxylation.[341] Benzoxazoles have been ingeniously used as directing groups in enzymatic hydroxylations.[342] Taxol precursors may be regioselectively hydroxylated.[343] Pea leaf (*Pisum sativum*) produces an oxidase that can α-hydroxylate carboxylic acids.[344,345]

6.2 Dihydroxylation

Two very popular sources of dihydroxylation are *Pseudomonas putida* strains and *E. coli* JM109 (a strain that expresses toluene dioxygenase).[346] Stereoselectively dihydroxylated arenes give rise to diols that are valuable synthetic precursors to a wide variety of products (Table 4).[347] For example, an elegant chemoenzymatic route, based on the selective hydrogenation of a dienyl bromide, that is, the product of a dioxygenase, has allowed access to typically fleeting arene oxide and diol metabolites of monooxygenases.[348]

6.3 Baeyer–Villiger oxidations

Various cycloalkanone monooxygenases display parallel stereoselectivities in Baeyer–Villiger oxidations,[359] although whole cell transformations give more variable results and this reflects the activity of multiple monooxygenases in such systems. The broad substrate specificity of such Baeyer–Villigerases suggests that binding of the carbon framework is unlikely and hence hydroxy binding is implicated and a trioxane-like transition state has been proposed.[360] In an excellent comparative study, parallel selectivities were observed for microbial Baeyer–Villiger oxidations and those using enzymes isolated from the same source.[361] The expression of cyclic monooxygenases in baker's yeast allows more reliable stereoselective Baeyer–Villiger reactions,[362–364] although they are less efficient at oxidising cyclopentanones than cyclohexanones.[365]

6.4 Other oxidations

The broad utility of flavin-dependent cycloalkanone monooxygenases (CMOs) in the enantioselective oxidation of organic sulfur compounds has been highlighted[366] and an excellent model of sulfoxidation by *Helminthosporium* based on nearly one hundred sulfide substrates has been compiled.[367] Dithianes are also stereoselectively oxidized by the CMO from *Acinetobacter calcoacetius*.[368] The same enzyme can be used to resolve sulfites.[369]

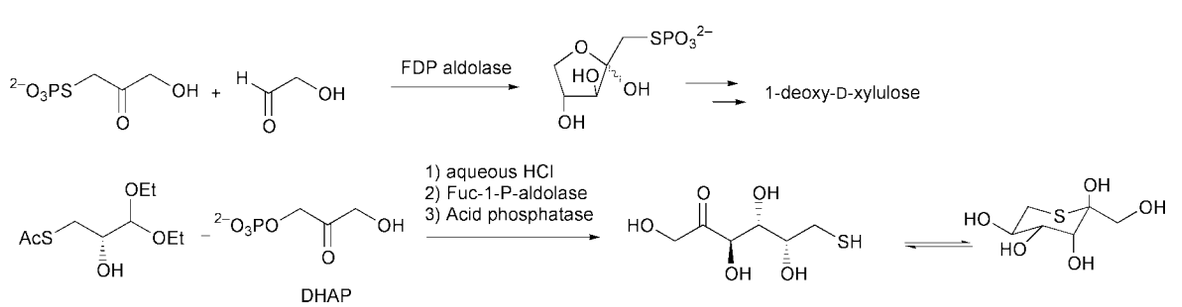
The use of peroxidases has been reviewed.[370] Chloroperoxidases, which are commercially available, and cyclohexanonemonooxygenases make complementary catalysts for the stereoselective oxidation of sulfides to sulfoxides.[371] Chloroperoxidases can also oxidise oximes to α-bromonitro compounds,[372] and can be used to form enantiomerically enriched epoxides.[373]

Galactosides can be oxidised to C-6 aldehydes using galactose oxidase[279] and nucleosides to C-5 acids using a nucleoside oxidase.[280]

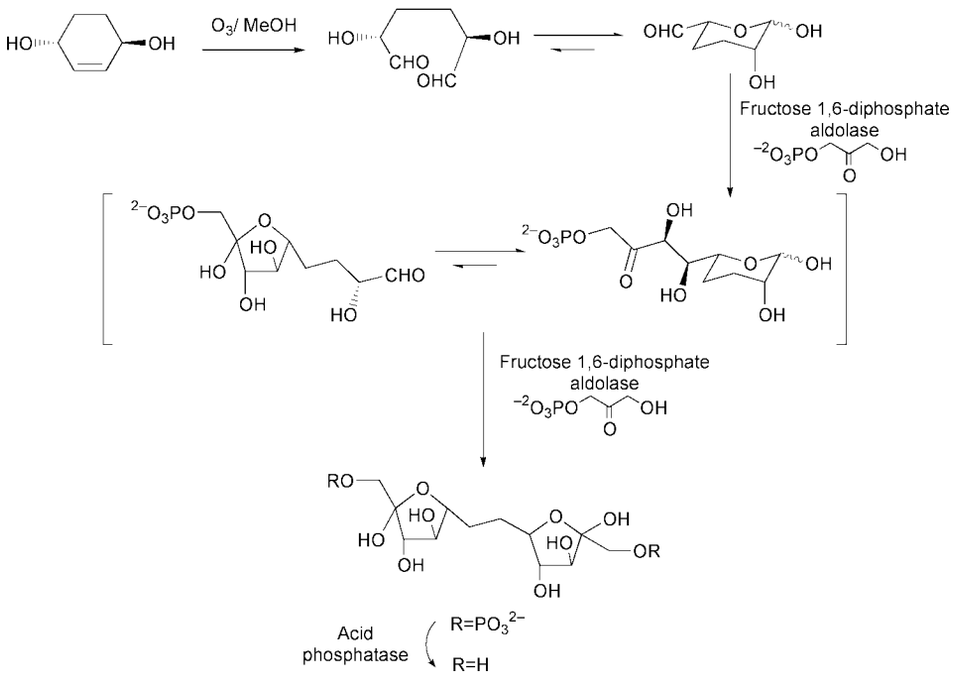
7 C–C bond formations

7.1 Aldolases

Aldolases catalyse carbon–carbon bond formation through aldol condensation with typically good stereocontrol, the sense of which is a function of the aldolase type used.[374] They have been widely applied to the synthesis of carbohydrates and carbohydrate mimetics.[375–377] An excellent review in 1995 by the Wong group described the use of aldolases in the synthesis of carbohydrates and in particular the use of fructose diphosphate aldolase.[241] For example, the condensation of dihydroxyacetonephosphate (DHAP) with a broad range of α-hydroxy aldehydes allows the construction of aza-sugar and thio-sugar carbohydrate mimetics *via* polyhydroxyketones. The same trick can be played with other aldolases to accordingly give access to a variety of stereochemistries of the diol unit formed. The use of *trans*-ketolases and -aldolases is also highlighted. Deoxysugars have also been prepared *via* aldolase-catalysed synthesis.[378] For example, Chou *et al.* have described the synthesis of deoxythiosugars[379] (Scheme 37).

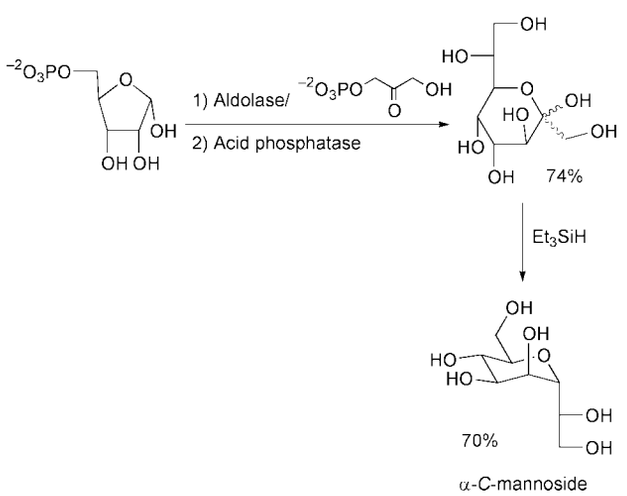


Scheme 37



Scheme 38

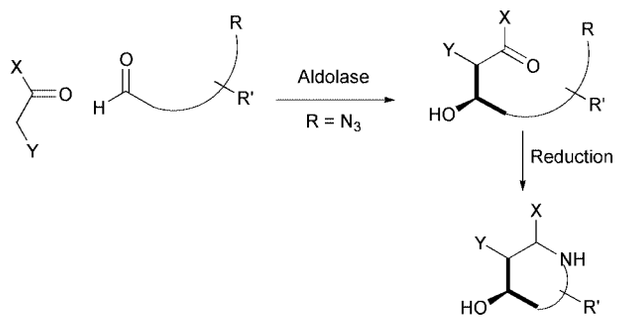
In carbohydrate chemistry, *C*-saccharides are particularly interesting because they are non-hydrolysable compounds, and so potentially valuable inhibitors of glycoprocessing enzymes. Eyrisch and Fessner described the synthesis of *C*-disaccharides by an elegant enzymatic tandem aldol addition.[380] Different disaccharides were obtained depending on the nature of the starting material used (Scheme 38). The synthesis of an α-*C*-mannoside from ribose 5-phosphate has also been described using a ready route that does not require the use of protecting groups (Scheme 39).[381]



Scheme 39

A summary of strategic routes to azasugars[382] using aldolases is shown in Scheme 40. Examples include the synthesis of five membered azasugars,[383,384] six-membered homoazasugars, such as β-1-homonojirimycin and β-1-homomannojirimycin,[385,386] and homoisofagomines (Scheme 41).[387] Seven-membered iminocyclitols were also obtained easily by this process (Scheme 41).[388] A ready approach has also been developed for the preparation of phosphorylated azasugars (Scheme 41).[389]

Aldolases are also useful for the preparation of compounds that are components of many complex natural products such as β-hydroxy-α-amino acids,[390,391] α-amino-β-hydroxy-γ-butyrolactone,[392] or heterocyclic β-hydroxy-α-amino acids.[393] Sphydrofuran has been synthesised *via* a rabbit muscle aldolase-promoted aldol condensation between two achiral precursors (Scheme 42).[394] Toone and co-workers have described the synthetic utility of 2-keto-3-deoxy-6-phospho-gluco-[395] and -galacto-nate[396] aldolases in the condensation of a wide range of aldehydes with pyruvate.



Scheme 40

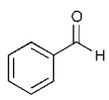
With aldolase catalytic antibodies, the kinetic resolution of racemic β-hydroxyketone *via* an enantioselective retro-aldol reaction was possible.[397] Catalytic antibody aldolases were also applied in the conversion of cyclic ketones through a Robinson annulation reaction, often used in the synthesis of steroids and terpenes.[398]

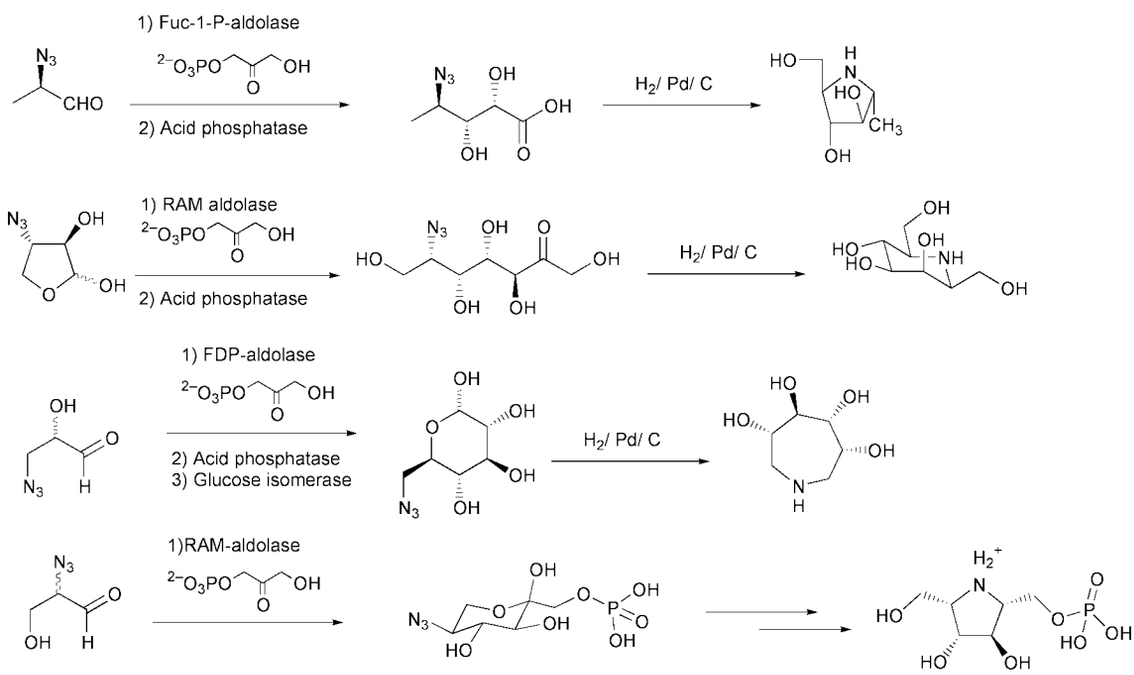
7.2 Oxynitrilases

Oxynitrilases catalyse the addition of hydrogen cyanide to aldehydes and ketones: an important methodology for the synthesis of optically active cyanohydrins[399] (Table 5).

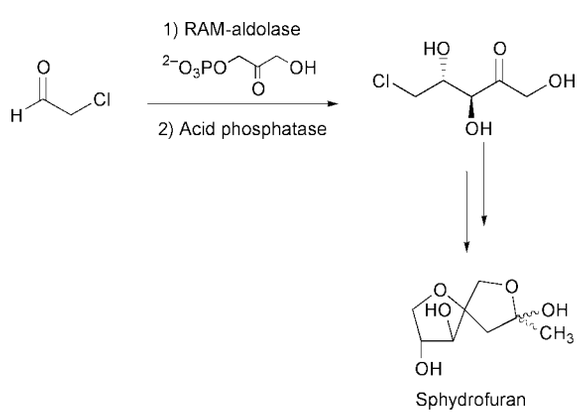
Cyanohydrins are excellent starting materials for the stereoselective synthesis of pharmacologically important compounds *e.g.*(*R*)-terbutaline or (*R*)-salbutamol (Scheme 43).[404] (*R*)-Pantolactone, the starting material for (*R*)-pantothenic acid (a constituent of coenzyme A), (*R*)-panthenol (a bactericide) and (*R*)-pantotheine (a growth factor) may also be readily prepared (Scheme 44).[405] The Williams glycine template for amino acid synthesis was prepared from benzaldehyde using an oxynitrilase.[406] The chiral cyanohydrin obtained was then converted in 8 chemical steps to the Williams glycine template (Scheme 45). The synthesis of α-hydroxy-β-amino acids from chiral cyanohydrins has also been described (Scheme 46).[407] Mitsunobu

Table 5 Enantioselective oxynitrilase-catalyzed cyanide additions to carbonyl compounds

Ketone or aldehyde	Oxynitrilase	Cyanohydrin	Reference
	(*R*)		400
	(*S*)		401,402
	(*S*)	(*S*)-Mandelonitrile	403



Scheme 41



Scheme 42

reactions on chiral cyanohydrins derived from oxynitrilase-catalyzed reactions also produce optically active amino acids.[408]

Gotor *et al.* have shown that the (*R*)-oxynitrilase also effectively catalyses an elegant tandem enantioselective decyanation of racemic ketone cyanohydrins and subsequent enantioselective addition of hydrogen cyanide to ω-bromoaldehydes in one step (Scheme 47).[409] The ω-bromocyanohydrin products can be used to readily construct tetrahydrofurans, common structural components of terpenoids, pheromones, and antibiotics.

8 Other enzyme systems

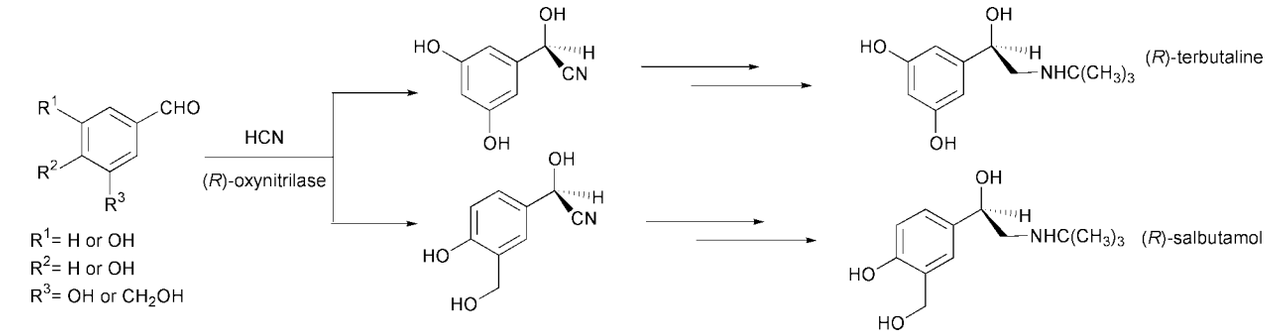
Various unnatural diols have been resolved using the glycerol kinase from *Streptomyces canus*.[410] L-Tryptophan can be chlorinated with an halogenase.[411] Wong and co-workers have described a useful multienzyme system that provides sulfotransferase-mediated regioselective sulfation of oligosaccharides as well as coenzyme regeneration.[412] The use of sulfatases in a combined chemical regioselective sulfation and enzymatic regioselective desulfation strategy has also been described for the synthesis of sulfated galacto- and lactosides.[413]

9 Novel biocatalysts

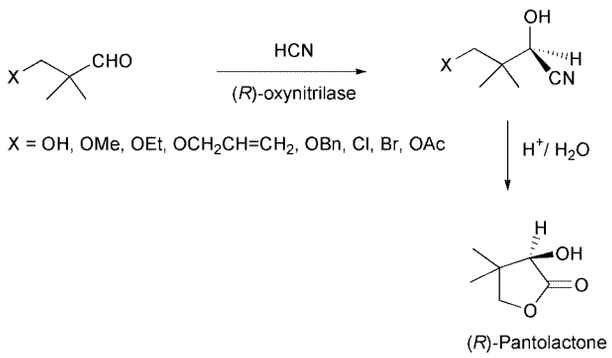
9.1 Engineered enzymes

The engineering of enzymes for use in synthesis should be viewed quite rightly in the context of the corresponding wild-type native enzymes and accordingly in this review have been discussed in the appropriate sections above. However certain selected examples stand out as of general interest to those concerned with the redesign of protein systems. An excellent review on the strategy of enzyme redesign has been published by Craik *et al.* which provides a balanced analysis of strategies and their complementarity;[414] this valuably highlights that to select just one method at the expense of others is clearly short-sighted. A review of the work of one of the pioneers in the field of redesign of synthetically useful enzymes, Bryan Jones, has also been published.[415]

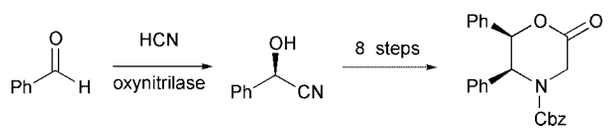
The first example of lipase redesign has been published. Using two logical point mutations to block the acyl chain binding pocket, the substrate specificity for short chains of a lipase



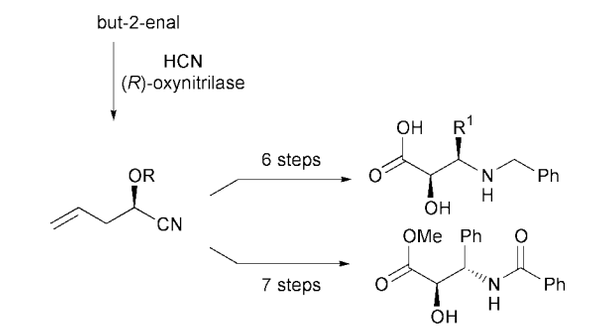
Scheme 43



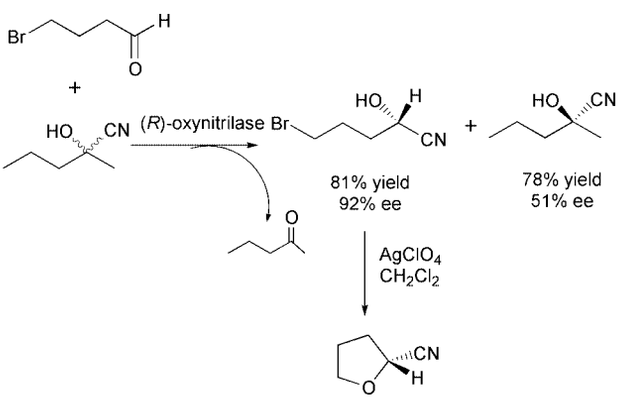
Scheme 44



Scheme 45



Scheme 46



Scheme 47

from *Rhizopus delemar* was enhanced.[416] A single point Leu → Phe mutation, predicted by modelling, in another *Rhizopus* lipase allowed enhancement of stereoselectivity.[417] Modelling allowed the successful identification of a single residue in PCL

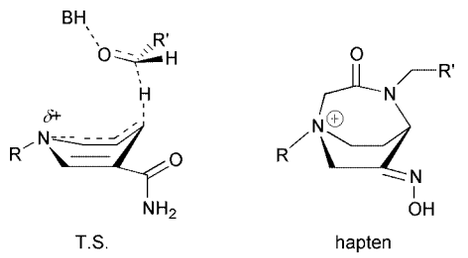


Fig. 3

as being critical to enantioselectivity; a finding that was confirmed by chemical modification.[418]

Combined site-directed mutagenesis and chemical modification gives rapid access to altered enzyme active sites beyond the limitations of the 20 most common natural amino acids. Such approaches successfully generate enhanced synthetically useful enzymes.[211] In an excellent paper describing the power of enzyme modification for greatly enhanced utility, a subtilisin-photoresponsive polymethacrylate conjugate can be used in toluene and then precipitated or resolubilized simply by UV or visible irradiation, respectively.[419]

9.2 Evolved enzymes

Two very good reviews on the directed evolution of enzymes from two of the main protagonists in this field have appeared.[420,421] Again, not all results will be selected here but a few shine out as good examples of what might be achieved in the pursuit of synthetic utility. Reetz's remarkable use of error-prone PCR to increase stereoselectivity from negligible to very good in just 4 cycles[131,132] and Arnold's creation of an otherwise non-existent deprotecting esterase activity[422] highlight the power. Clear increases in the stability of synthetically useful enzymes can be achieved too, as demonstrated by the conversion of a subtilisin into a themitase-like, more thermostable counterpart.[423]

9.3 Catalytic antibodies

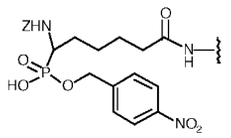
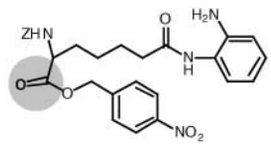
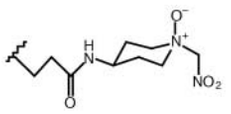
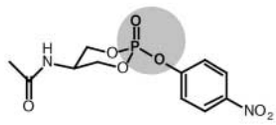
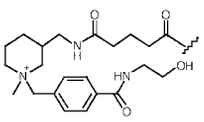
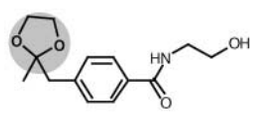
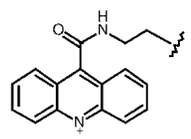
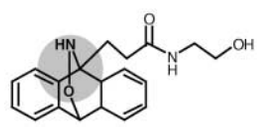
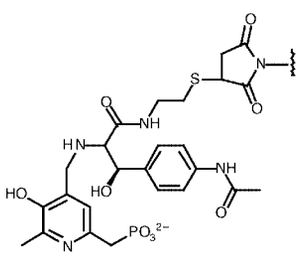
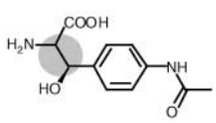
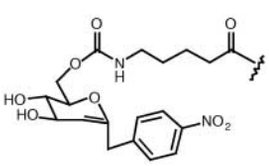
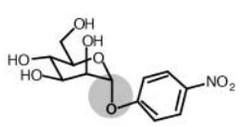
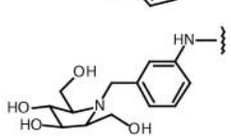
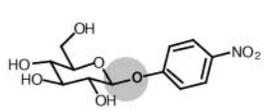
By raising antibodies to the appropriate hapten (Table 6) then a variety of catalytic activities may be generated.[424–426]

For example, the compound in Fig. 3 has been synthesized to mimic a proposed combined NADH–NAD^+ cofactor-product transition state of hydride transfer in dehydrogenase enzymes.[434] Its intended use is to act as a hapten for the generation of catalytic antibodies with the potential to act as dehydrogenase mimics.

Labile haptens have been used in a "reactive immunization" approach.[435,436] Reactive leaving groups can be used to trap only reactive antibodies that are able to catalyze desired reactions. Such a chemical selection strategy has been applied to the elucidation of a glycosidase activity.[437]

Although catalytic antibodies typically have turnovers several orders of magnitude (10^4–10^5) lower than enzymes, one of their major advantages is their catholicism with respect to

Table 6 The generation of novel activities using haptens to catalytic antibodies

Hapten	Activity created towards	Reference
		427
		428
		429
		430
		431
		432
		433

substrate as exemplified by the very broad range of substrates processed by two aldolase-like examples.[438]

9.4 Enzyme models and mimetics

Cyclodextrins are often very useful scaffolds around which to design active site mimics in that they provide a water soluble, hydrophobic binding cavity that is rich in potential points of functionalization. For example, introduction of a zinc binding motif and an imidazole creates a phosphatase mimic.[439] Similarly, small peptide motifs may display remarkable catalytic properties given their size. For example, under correct conditions coaggregates of Z-Phe-His-Leu display absolute enantioselectivity in the hydrolysis of dodecanoyl-Phe-pNP.[440]

Polyleucines can be used as asymmetric epoxidation catalysts.[441] It should not be forgotten also that polynucleic acids also display interesting catalytic properties and a review on ribozymes has appeared.[442]

Fatty-acid binding proteins provide a scaffold, in which cofactors can be introduced or repositioned, and serve as interesting enzyme mimetics.[443–445] A bicomponent lipid bilayer system consisting of a quaternized lysine-containing lipid and a quaternized pyridoxal-containing lipid as an artificial transaminase-like system can be coupled with a lactate dehydrogenase to give α-hydroxyacids from corresponding amino acids.[446]

10 Conclusions

The use of enzymes in organic synthesis is now widely accepted and as more and more synthetic research embraces the realisation that enzymes are simply an alternative source of catalysis that may be as robust as others, sometimes more so, then their use will continue to flourish. Their incorporation into current synthetic strategy will continue to require careful consideration. For example, Khmelnitsky and co-workers have discussed some of the advantages (*e.g.*, selectivity) and incompatibilities (*e.g.*, overly stringent specificity) of enzymatic combinatorial synthesis which will require both rethinking the way in which parallel synthesis can be achieved but also will allow rapid enhancement of existing library techniques.[23,447] It is clear that different strategies will be required and in this discussion paper a handful of examples are given. For example, specific iterative biocatalytic derivatizations that fail may succeed if the order of derivatizations is altered. Regioselective acylation appears a particularly powerful tool in library synthesis *e.g.*, exploiting different selectivities allows the ready preparation of varying diacylated products of polyhydroxylated compounds.[448]

Other technologies will also need to be considered carefully. A comprehensive review of supercritical biocatalysis has appeared,[449] which highlights that the advantage of being able to subtly control the physical properties of such systems may be outweighed by the need for specialist equipment. In addition, the strong relevance of biocatalysis to industrially and pharmaceutically relevant processes, something that is often forgotten, will be of continuing relevance. This is clear from an excellent review that highlights some choice examples.[24]

Finally, the redesign of enzyme active sites and the alteration of activities should over the next few years become as common place as *e.g.*, the modification of ligand structures in transition-metal complex catalysts. A primary goal of such work should be the tailoring of specificity,[415] often a challenge neglected in favour of work aimed at enhancing activity. In this regard, subtle yet important strategic aspects need to considered. For example, in many cases the ideal catalyst would maintain one aspect of specificity *e.g.*, stereospecificity, while having a breadth in *e.g.*, substrate specificity. Recombinant protein technology now means that access to synthetically useful quantities of enzyme is far less of a problem then previously and the advantages and possibilities for the creation of novel chemistries are clear.

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In memory of G. Dennis Meakins: a great man and inspiration to many.

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