Preparative biotransformations

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1 Introduction and background information

This review highlights some of the preparatively useful biotransformations that were reported in organic chemistry journals[1] in 1998. Like its predecessors,[2] this review is not intended to be comprehensive, rather to pick out important features and developing trends within the field of biocatalysis. Specifically excluded are data relating to mechanisms of enzyme function, biosynthesis (for example labelling studies) and combinatorial biosynthesis.[3]

The Synopsys Scientific Systems BioCatalysis group reports its database increasing at the rate of 2000 entries per annum. Approximately half the 1998 literature in the area of biotransformations describes work involving hydrolyses (27%) or esterification reactions (25%) employing commercially available enzymes, principally lipases. Synthetically useful reduction and oxidation reactions each account for 10% of the relevant literature, with carbon–carbon bond forming reactions being much less prevalent (4%). Biocatalytic methodology for the preparation of carbohydrates continues to be popular (8% of 1998 literature) and the search for biomimetic catalysts has experienced an upsurge (10%). Miscellaneous biotransformations account for the remaining small percentage of work carried out in the area.

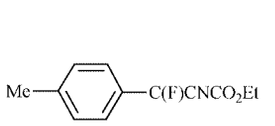
2 Hydrolysis reactions

A review on the use of lipases for hydrolyses and esterification reactions is available.[4]

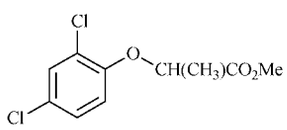
2.1 Ester hydrolysis

A simple procedure is recommended for obtaining highly active Subtilisin Carlsberg and α-chymotrypsin. This involves dissolving the commercial enzyme in aqueous buffer (2–4 mg ml^{-1}), adding silica gel (50 mg ml^{-1}), agitating, decanting off the aqueous solution, adding dry *n*-propanol, rinsing, decanting the solvent, repeating the *n*-propanol treatment six times before rinsing the enzyme-silica material with the reaction solvent and then adding the reaction mixtures.[5] This or a closely related protocol may be worth adopting for a wide variety of applications.

For example, propan-2-ol (rather than *n*-propanol), pretreatment of *Candida rugosa* lipase increased the selectivity of the hydrolysis of ester **1** ten-fold, to give the (*S*) acid ($E = 40$).[6] A similar pretreatment of the same enzyme allowed the kinetic resolution of the ester **2** to be performed efficiently in a two phase solvent system giving the (*R*)-acid and recovered (*S*)-ester (combined yield 92%; $E = 70$).[7]



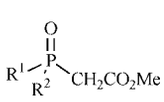
1



2

Burkholderia cepacia lipase (Chirazyme L1) is preferred for the hydrolysis of $PhCH(CH_3)CH_2CO_2Me$ giving the (*S*)-acid (not recovered quantitatively) and recovered (*R*)-ester (46%) ($E > 50$).[8] Compounds of the type **3** are hydrolysed stereoselectively using pig liver esterase in buffer over 15–144 h. Typically, for $R^1 = Ph$, $R^2 = OEt$, the (*R*)-acid was obtained in 40% yield (71% ee) and 46% of the (*S*)-ester (67% ee) was recovered.[9]

The ester **4** was formed in high optical purity ($E = 146$) on



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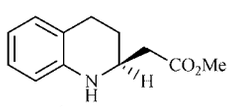

4

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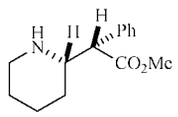
partial hydrolysis of the corresponding racemate with Novozym 435 (*Candida antarctica* lipase) in tetrahydrofuran (THF) containing 5% water over 96 h.[10]

Methyl 2-bromophenylacetate is hydrolysed to the corresponding acid (78% yield, 79% ee) using cross-linked enzyme crystals (CLECS) of *Candida rugosa* lipase in water containing a source of bromide ion (Wang phosphonium bromide). This example of dynamic kinetic resolution works because the bromine atom in the ester is more susceptible to nucleophilic substitution than the same atom in the corresponding acid.[11]



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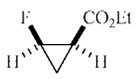
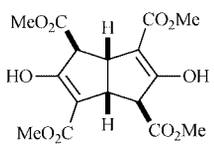


6

The stereochemically more complex ester **5** has been hydrolysed using α-chymotrypsin or *Subtilisin Carlsberg*: in both cases the (2*S*,3*R*)-ester was recovered in optically active form. The (2*R*,3*S*)-acid was rather water-soluble, militating against its isolation in high yield.[12] A *Micrococcus* organism isolated from a soil sample was found to hydrolyse the ester **6** to give the (*R*,*R*)-acid (54% yield, 76% ee) and the (*S*,*S*)-ester (41% yield, 98% ee).[13]

Lipase AY catalyses the double demethoxycarbonylation of the tetra-esters **7** to give the diester **8** (32% yield, 100% ee); the enantiomer of **8** is obtained (40% yield, 100% ee) by treatment of **7** with porcine pancreatic lipase (ppl) and lipase M under hydrolysis conditions. The diester **8** was used in a synthesis of (−)-ajmalicine while the enantiomeric diester was transformed into (+)-carbacyclin.[14]



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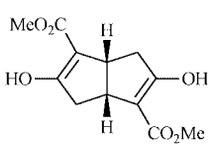
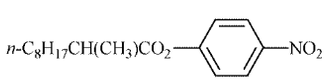


8

A truly remarkable increase in the enantioselectivity for the hydrolysis of *p*-nitrophenyl ester **9** catalysed by *Pseudomonas aeruginosa* lipase (from 2% ee to 81% ee!) is achieved by using the error-prone polymerase chain reaction, causing random mutagenesis in the 933 base-pair gene sequence. The identification of interesting mutants entailed using the (*R*)- and the (*S*)-esters in separate hydrolysis experiments and monitoring *p*-nitrophenol release. Four cycles (taking the best mutation in each case) gave incremental improvements (2%→37%→57%→75%→81%).[15] Chemically modified mutants of Subtilisin from *Bacillus lentus* catalysed transesterification reactions of $PhCH_2CH(NHAc)CO_2CH{=}CH_2$ more efficiently than the wild-type enzyme.[16]



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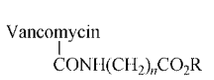
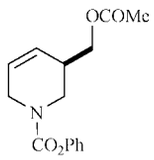


10

Vancomycin derivatives of type **10** are glycosylated molecules sensitive to harsh reaction conditions. However lipase LPL-80 (Amano) cleaves the above ester readily when R = benzyl and when the linking chain $(CH_2)_n$ keeps the reactive site distant from the hydrophilic functional groups of the macromolecule (*e.g.* $n = 10$).[17]

There continues to be large numbers of examples of enantioselective hydrolyses of chiral acetates, some involving primary esters, but mainly featuring secondary systems with the occasional appearance of a tertiary acetate.

For the acetates derived from primary alcohols the stereogenic centre is often adjacent to the reacting unit; for example the acetate **11** generates the (*S*)-alcohol ($E = 19$) on hydrolysis with lipase PS (from *Ps. cepacia*) in phosphate buffer.[18] For the five-membered heterocyclic systems (**12**; X = O) *C. antarctica* lipase in butanol is used to recover the (*S*)-acetate ($E = 11$) (the (*R*)-acetate of the compound **12** X = NBOC is obtained by esterification of the corresponding alcohol using *Pseudomonas fluorescens* lipase (PFL) in vinyl acetate ($E = 18$)).[19] The butanoate **13** was resolved by hydrolysis of the racemate in pH 7 buffer using lipase PS as catalyst, to give recovered (2*S*,4*R*)-ester ($E = 14$). The optically active ester, produced on a multigram scale, was converted into (+)-deoxymuscarine.[20]



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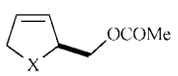

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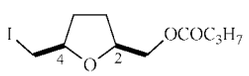
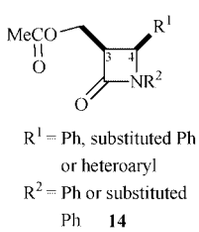


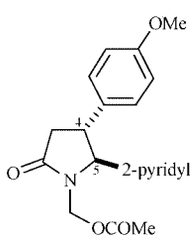
13

For the four-membered lactams **14**, a full paper reports the stereoselective hydrolyses of the ester group using ppl over 24 h in pH 7.9 buffer to give the (3*S*,4*R*)-alcohols (83–98% ee at *ca.* 40% conversion).



R^1 = Ph, substituted Ph or heteroaryl

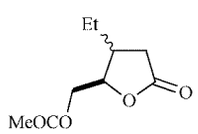
R^2 = Ph or substituted Ph **14**



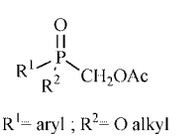
15

The hydrolysis of the corresponding 3-acetoxyethyllactams or the related *trans*-substituted lactams was not as efficient.[21]

The primary acetate unit has been more closely linked to a heteroatom in two instances. First the pyrrolidone derivative **15** has been resolved on a 10.5 kg scale to give recovered (4*R*,5*S*)-ester in 36% yield and 97.5% ee *en route* to LTB_4 inhibitor BIRZ-227.[22] Interestingly, in the series of compounds **16** the stereoisomer preferentially hydrolysed by *Ps. cepacia* lipase (lipase PS) depends on the stereochemistry of the remote ethyl group. For the *trans*-compound, the hydrolysis of the (*R*)-isomer is faster ($E = 16$), while for the *cis*-compound the (*S*)-isomer is preferred ($E = 5$). This allowed speculation on the shape of the cavities within the enzyme some way removed from the active site.[22]



16



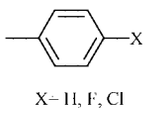
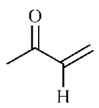
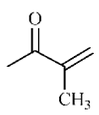
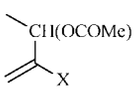
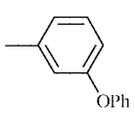
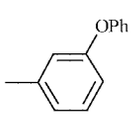
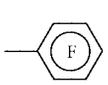
R^1 = aryl ; R^2 = O alkyl

17

In a reaction that is complementary to one described earlier (featuring compound **3**) acetates **17** have been obtained in optically active form by lipase catalysed hydrolysis of the racemate or by PFL-catalysed esterification of the corresponding alcohols.[23]

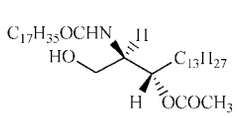
It is well-documented that a primary ester will generally be hydrolysed more rapidly than a secondary ester in enzyme-catalysed reactions. Thus in the enzymatic resolution of ceramide, the alcohol **18** is produced from the corresponding racemic diacetate ($E = 170$) using *Burkholderia cepacia* lipase (SC lipase A) in a decane–pH 7 buffer two-phase system. The

Table 1 Enantioselective hydrolysis of compounds $R^1CH(OCOMe)CH_3$ and $R^1CH(OCOMe)CN$

Entry	Reference	Compound $R^1CH(OCOMe)R^2$ R^1	R^2	Enzyme (reaction conditions)	Product(s) (enantiomeric excesses or *E* value)
1	29	 X = H, F, Cl	CH_3	*Arthrobacter sp.* lipase (3–6 h)	>98% ee[a]
2	30		CH_3	*C. antarctica* lipase	(*R*)-Alcohol *E* = 44
3	30		CH_3	*C. antarctica* lipase	(*S*)-Alcohol *E* = 100
4	31	 X = H, CH_3	CH_3	*C. antarctica* lipase	(*S*,*S*)-Diacetate and mixture of (*R*,*R*)-monoesters *E* ≥ 138
5	32		CN	Lipase *P* (hexane, butanol)	(*S*)-Cyanohydrin[b] (>99% ee) and (*R*)-ester (97% ee) *ca.* 50% conversion
6	33		CN	*Pseudonomas* lipase (Chirazyme) (BuOH, *i*-Pr_2O, 35 °C)	(*S*)-Cyanohydrin 46% (>95% ee)
7	34		CN[c]	Lipase *LIP* (buffer pH 5–6 pentan-2-one, 10.5 h)	(*S*)-Cyanohydrin *E* = 211

[a] It is unclear in the paper whether this value refers to the alcohol produced, the ester recovered or both compounds; the extent of conversion is not stated. [b] Converted into cypermethrine (an insecticide). [c] The propanoate ester was employed.

authors noted that the rate of the hydrolysis and the selectivity for the primary ester were affected by the additive Triton X-100 used in the immobilisation procedure.[24]



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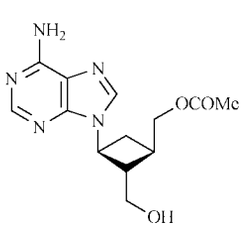


19

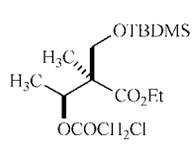
An esterase from *Rhodosporidium toruloides* has been shown to catalyse the hydrolysis of a series of peracetylated α-D-hexopyranoses and α-D-hexopyranosides selectively at the 6-position. Acid-catalysed 4→6 acetate shift then allowed access to 4-sulfates as well as the 6-sulfates of selected sugars.[25]

Even two primary acetate functions may be differentiated on some occasions. For example, the carbocyclic oxetanocin derivative **19** was obtained (90% ee) from the racemic diester by hydrolysis catalysed by lipase XIII (Sigma).[26] The elusive 8-fluoroadenosine has been obtained by hydrolysis of the triacetate using a thermostable hydrolase in methanol at 45 °C.[27]

Before leaving this section it should be noted that an NMR method for assigning the absolute configuration of compounds of the type $HOCH_2CH(R^1)R^2$, *via* formation of 9-anthrylmethoxyacetates, has been recommended recently.[28]

As intimated above, the enantioselective hydrolysis of esters derived from secondary alcohols has been researched extensively and the strategy is often used in preparative organic chemistry.

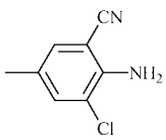
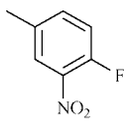
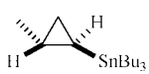
Various compounds of the structure $R^1CH(OAc)CH_3$ have been resolved using enzymes (Table 1, entries 1–4), with entries 2 and 3 serving to show that surprises can still be registered in this well-documented area. The kinetic resolution of cyanohydrin esters can be effected very efficiently as well (Table 1, entries 5–7). Occasionally esters of the type $R^1CH(OCOCH_3)CH_3$ are resolved using whole-cell systems[35] and sometimes the ester group employed is haloacetate, rather than acetate, in order to increase the reactivity: for example the chloroacetate **20** gives the (*R*)-alcohol (43%, >99% ee) and recovered (*S*)-ester (46%, 90% ee) *en route* to the side chain of furaquinocin D.[36] Note that the ethyl ester moiety is unaffected.



20

Other acyclic systems have been studied in which the terminal methyl group of the alkane chain of compounds $R^1CH(OCOCH_3)CH_3$ is replaced by halomethyl (Table 2, entries 1, 2), acetamidomethyl (Table 2, entry 3), vinyl (Table 2, entry 4) or higher alkyl (Table 2, entry 5).

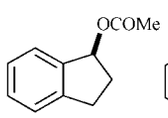
Table 2 Enantioselective hydrolysis of compounds $R^1CH(OCOMe)R^2$ where R^2 = halomethyl, amidomethyl, vinyl or higher alkyl

Entry	Reference	Compound $R^1CH(OCOMe)R^2$ R^1	R^2	Enzyme (reaction conditions)	Products (enantiomeric excesses or *E* value)
1	37		CH_2Br	*C. antarctica* lipase (*i*-Pr_2O and buffer)	(*S*)-Alcohol and (*R*)-ester $E \geq 200$[a]
2	38	CH_2OCH_2Ph	CH_2Cl	*C. antarctica* lipase (H_2O, acetone)	(*S*)-Alcohol and (*R*)-ester ($E = 180$)[b]
3	39		$CH_2NHCOCH_3$	Horse liver esterase (buffer pH 7.5, acetone 10 : 1)[c]	(*S*)-Alcohol (45%, 91% ee) and (*R*)-ester (45%, 90% ee)
4	40	CH_2OR^3 R^3 = tosyl or trialkylsilyl	$CH{=}CH_2$	Lipase *PS* (Amano) (buffer pH 7, 3–9 h)	(*R*)-Alcohol and (*S*)-ester $E > 300$[d]
5	41		C_5H_{11}	*Pseudomonas cepacia* lipase (pH 8.5, 35 °C)	(*R*)-Alcohol and (*S*)-ester $E = 34$

[a] Methodology used in a route to a new β_2-adrenergic agonist. [b] *E* value raised 50 to 180 by addition of acetone (30% v/v). [c] Other organic cosolvents, *e.g.* hexane, are less helpful. [d] The *E* value is equally high when the chloroacetate is biotransformed.

The hydrolysis of the diastereoisomers of the ester PhCH(Me)CH(OCOMe)CH_3 using *Candida rugosa* lipase as the catalyst showed that the (*R*,*R*)-diastereomer reacted fastest followed by the (*S*,*S*) and (*R*,*S*)-isomers, with the (*S*,*R*)-isomer being the isomer slowest to react.[42]

The hydrolysis of acetoxycycloalkanes has been pursued further. The selectivity for the hydrolysis of the ester **21** using ppl in wet methyl *tert*-butyl ether (to give the (*R*)-alcohol) is enhanced three-fold by using 1 mol% of 4-nitrophenyl-*N*-hexylcarbonate as a partial inhibitor. It is recommended that the inhibitor and the enzyme are mixed 10 h prior to the biotransformation; it is believed that the carbonate covalently binds to the protein, modifying the active site.[43] The closely related ketoester **22** is hydrolysed using Amano PS lipase in acetonitrile–pH 7 buffer to furnish the (*R*)-alcohol (45%, 94% ee) and recovered (*S*)-acetate (47%, 96% ee).[44]



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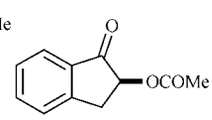

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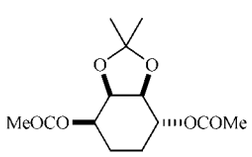

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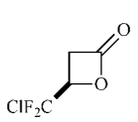

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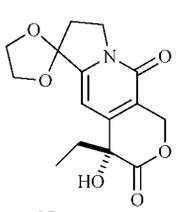


25

The diester **23** was converted into the (*R*,*R*)-diol (49% yield, >99.5% ee) using *Candida rugosa* lipase at pH 7, with the (*S*,*S*)-diester being recovered in almost the same yield and having the same very high optical purity. The compounds proved to be useful intermediates to (+)- and (−)-conduritol C.[45]

The lactone **24** is hydrolysed to hexyl (3*R*)-trihalohydroxybutanoate ($E = 46$) on treatment with hexanol in diethyl ether over 69 h. The alcohol and recovered lactone were isolated in 75% yield.[46]

In a rare case involving a tertiary alcohol, an intermediate to (20*S*)-camptothecin **25** was resolved (as the acetate) using papain in a two-phase solvent system ($E > 400$).[47] It is more common to resolve tertiary alcohols by forming an ester with a "linker" system, for example, *tert*-alkyl-$OCH_2OCOC_3H_7$, such that the attack by the hydrolase is distant from the bulky alkyl group. Franssen *et al.* resolved linanool in this way using *Candida rugosa* lipase in a mixture of *n*-butanol and toluene at 0 °C over several hours ((*R*)-alcohol obtained, $E = 9.7$).[48]

The formation of optically active acids from prochiral compounds $R^1R^2C((CH_2)_nCO_2R^3)_2$ ($n = 0$ or 1) is well known in the field of biotransformations and further examples have been recorded in the literature recently. The first two entries in Table 3 describe data for prochiral dicarboxylates of the type $R^1R^2C(CO_2R^3)_2$, while entries 3 and 4 relate to the resolution of triethyl citrate. It is noteworthy that, in contrast to the result obtained with *C. antarctica* lipase, serine proteases convert triethyl citrate into the monoacid $HO_2CC(OH)(CH_2CO_2Et)_2$ in high yields.[51]

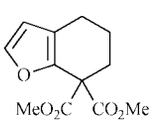
Similarly the diester **26** was hydrolysed over three days in acetone and phosphate buffer containing pig liver esterase (ple) to give the (*R*)-monoesters (100% yield, >94% ee).[52]

The corresponding *meso*-diester was hydrolysed by ple to monoester **27** (91% yield, 97% ee) (in a procedure first popularised by J. Bryan Jones), which was, in turn, converted into the β-amino acid **28**.[53]

A number of *meso*-diacetates have been converted into optically active alcohols. For example the norbornadiene

Table 3 Enzyme-catalysed hydrolysis of compounds of the type $R^1R^2C(CO_2R^3)_2$ (entries 1, 2) or $R^1R^2C(CH_2CO_2R^3)$ (entries 3, 4)

Entry	Reference	Compound			Enzyme (reaction conditions)	Product (yield and ee)
		R^1	R^2	R^3		
1	49	Ph or 3-thienyl	F	Et	Immobilised ppl	(*S*)-Monoester (60–77%, 96% ee)
2	50	Me or benzyl or allyl	NHZ	Et	Pig or rabbit liver esterase (pH 7 buffer, MeCN, 13 h–10 days)	(*R*)-Monoester (83–97%, 90–97% ee)
3	51	CO_2Et	OH	Et	*C. Antarctica* Lipase	(*R*)-Diester (90% ee)
4	51	$-OCH_2OCO-$		Me or Et	Pig liver esterase	(*R*)-Lactone (85%, 90–92% ee)



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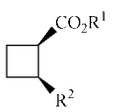
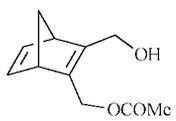
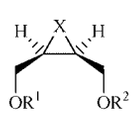


27 R^1 – Me ; R^2 – CO_2H
28 R^1 – H ; R^2 – NH_2

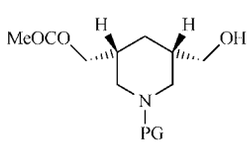


29

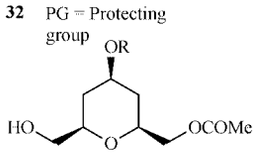
derivative **29**† is obtained from the corresponding diacetate in 82% isolated yield (>95% ee) using *Pseudomonas* lipase Amano AK in borate buffer and *n*-hexane containing butan-1-ol.[54] As expected treatment of the diol with Lipase AK in vinyl acetate afforded *ent*-**29**. The alcohol **30** is prepared by hydrolysis of the dibutyrate (73% yield, 84% ee) with ppl in aqueous solution buffered with sodium hydroxide. Other diesters afforded less selectivity.[55] On the other hand hydrolysis of the appropriate diacetate using lipase QL from *Alcaligenes* gave the monoester **31** in 81% yield and >99% ee.[56]



30 X = O ; R^1 = $OCOC_3H_7$; R^2 = H
31 X = CF_2 ; R^1 = H ; R^2 = $OCOCH_3$



32 PG = Protecting group



33 R = MOM or TBS

In six-membered ring systems the alcohols **32** are available from the appropriate diacetates using PFL in pH 7.1 buffer, optionally containing *tert*-butyl methyl ether over 5–20 h (yields 65–77%; ees >94%). The enantiomer of ester **32** is prepared by treatment of the diol with PFL in vinyl acetate optionally containing acetonitrile (yields 63–74%; >98% ees).[57] Similarly the alcohol **33** is obtained by hydrolysis of the corresponding diacetate employing *C. antarctica* lipase in pH 7 buffer containing Triton X-100 over 1–6 h (70–100% yield; 86–95% ee). Once again the enantiomer of **33** was obtained (70% yield; >95% ee) by using the same enzyme in isopropenyl acetate.[58]

Occasionally the hydrolysis of other types of esters has been reported. Thus the phenol **34** has been isolated (47% yield, 80% ee) after enzyme catalysed hydrolysis of the racemic acetate,[59] while the thiol **35** is obtained by partial hydrolysis of the triester using *C. rugosa* lipase in pH 7.5 buffer in a pathway to lipoic acid.[60]

† The IUPAC name for norbornane is bicyclo[2.2.1]heptane.

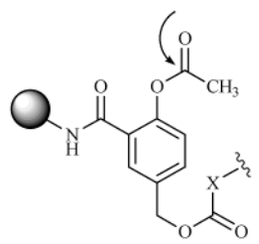
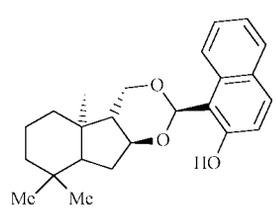


Fig. 1 Waldmann's protecting group for amines, acids and alcohols: enzyme catalysed hydrolysis releases acetic acid, quinomethane, CO_2 and the required product.



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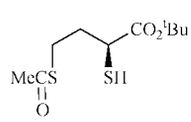
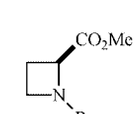


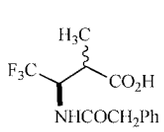
35

A very interesting protection strategy for amines, alcohols and acids is shown in Fig. 1 (X = NH, O, CR_2 respectively). The position of hydrolysis, for example by RB001-05 (from Recombinant Biocatalysts), is shown. It is noteworthy that the protecting group can be attached to a solid support *e.g.* tentagel.[61]

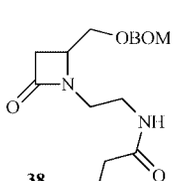
A further example of the ammoniolysis of an ester has been described. Treatment of the azetidine esters **36** with *C. antarctica* lipase in ammonia-saturated *tert*-butyl alcohol at 35 °C gave the (*S*)-amide (80–97% ee) and recovered (*R*)-ester (>99% ee).[62]



R = benzyl, allyl, benzhydryl
36



37



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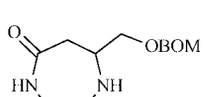
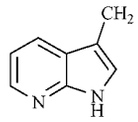
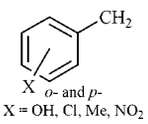
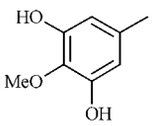


39

2.2 Amide hydrolysis

The majority of the recent work in this area has been aimed at the hydrolysis of amides derived from α-amino acids (Table 4). Usually the (*S*)-amino acid and recovered (*R*)-amide are

Table 4 Enzyme-catalysed hydrolysis of α-amino acid derivatives $R^1CH(NHCOR^2)CO_2H$

Entry	Reference	Compound R^1	Compound R^2	Enzyme (reaction conditions)	Product(s) (enantiomeric excess)
1	63		Me	*Aspergillus* acylase, immobilised (48 h)	(*S*)-Amino acid (>95% ee)
2	64	 *o*- and *p*- X = OH, Cl, Me, NO_2	Me	Acylase 1 (pH 7 buffer, 2 h)	*p*-Substituted (*S*)-amino acid (*o*-unreacted)
3	65		CF_3	Amano 3000 Acylase (pH 7 buffer, $CoCl_2$ and NaN_3 catalysts)	48% yield recovered (*R*)-amide ee ≥ 90%

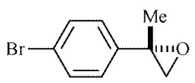
obtained. For the amides of the β-amino acids **37**, the (*R*)-enantiomer of the *erythro* and *threo*-isomer is hydrolysed preferentially ($E > 500$) using Penicillin acylase from *E. coli*.[66]

Penicillin G acylase in aqueous dimethyl sulfoxide at 40 °C catalyses amide cleavage and concurrent ring expansion of the lactam **38** to afford the diazacycloheptanone **39**.[67]

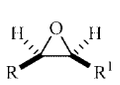
2.3 Epoxide hydrolysis

A theoretical analysis of epoxy-ring-opening reactions, catalysed by epoxide hydrolases has been published. In this paper new equations are given to allow one to work out the degree of regioselectivity of nucleophilic (*i.e.* H_2O) attack during the course of the reaction.[68]

A preparative scale hydrolysis of the epoxide **40** using an epoxy hydrolase to give (*R*)-diol (49% yield, 96% ee) and recovered (*S*)-epoxide (39% yield, 99% ee) proved to be remarkably efficient when a two-liquid-phase solvent system was employed.[69]



40



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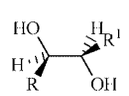


42

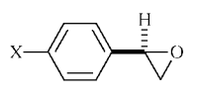
R = Me, Et, CH_2OR^2

$R^1 = (CH_2)_nCH_3$ where $n = 2 - 4$

Epoxide hydrolase-containing rabbit liver microsomes have been used for some years in the stereoselective hydrolysis of epoxides. Now a series of *cis*-substituted epoxides **41** have been shown to give the diols **42** (80 > 98% ee on 100% hydrolysis) in an enantioconvergent fashion.[70]

Increasingly, though, microorganisms containing epoxide hydrolases are being employed in preference to enzymes from mammalian sources. For example the yeast *Rhodotorula glutinis* hydrolyses epoxides derived from five alkenes, but-1-ene through oct-1-ene, to give the (*R*)-diol (47–78% yield, 29–83% ee) and recovered (*S*)-epoxide (21–48% yield, >98% ee); the best substrate was 1,2-epoxyhexane for which $E = 84$.[71] A new epoxide hydrolase activity has been found in the soluble cell extract of *Syncephalastrum racemosum*. Using this biocatalyst, it was discovered that the regioselectivity of nucleophilic attack on the epoxides **43** switched from C_1 to C_2 depending on the electronic character of X, the *para*-substituent. It was tentatively suggested that this phenomenon was due to general acid activation of the epoxide ring within the active site, an activation process absent in microsomal epoxyhydrolases.[72]

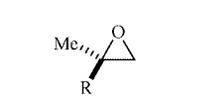
An epoxide hydrolase from *Nocardia* sp. hydrolyses the racemic epoxides **44** to the (*S*)-diol and (*R*)-epoxide. Subsequent treatment of this mixture with dioxane containing



X–Me, H, F, Cl, Br, CN, NO_2

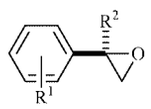
43

sulfuric acid gave a 71–98% yield of (*S*)-diol (ee 92–99%).[73] In a broader screen Furstoss and co-workers investigated the hydrolysis of the epoxides derived from oct-1-ene, 2-methylhept-1-ene, (*Z*)- and (*E*)-oct-2-ene using seven fungi. Three microorganisms were preferred, *Mortierella isabellina*, *Aspergillus niger* or *Chaetomium globosum*, yielding recovered epoxides in 8–22% yield (97–99% ee) on a preparative scale.[74]



R = C_4H_9, $(CH_2)_2CH{=}CH_2$, $(CH_2)_3Br$, Ph

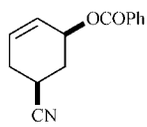
44



R^1 = H, Me, Cl

R^2 = H, Me

45



46

The epoxide hydrolase from *Agrobacterium radiobacter* has been overexpressed as described previously. It has been shown more recently that this recombinant enzyme catalyses the hydrolysis of the epoxides **45** to afford recovered epoxides (27–36% yield, >99% ee). The low yields obtained were due to non-enzymatic opening of the epoxides under the reaction conditions.[75]

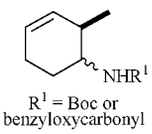
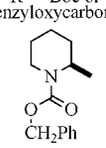
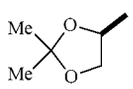
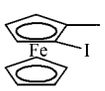
2.4 Nitrile hydrolysis

The hydrolysis of dinitriles $RCH(CN)(CH_2)_nCN$ (where R = H, Me, Et, $=CH_2$ and $n = 1,2$) occurs at the primary cyano group to give cyanoacids as their sodium salts (60–100% yield) using *Acidovorax facilis* 72W at pH 7 and 27 °C over 20 h.[76] *Rhodococcus rhodochrous*-mediated hydrolysis of a range of cyanocyclohexane derivatives has been studied. The activity of the amidase (but not the nitrile hydrolase) that is present in the microorganism is affected by the nature and position of the substituents. Typically the nitrile **46** gave the (*R*,*R*)-amide (41% yield, >99% ee) and the (*S*,*S*)-acid (49% yield, 73% ee).[77]

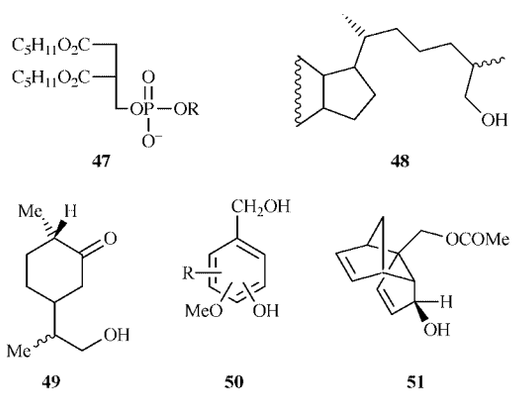
2.5 Other hydrolytic reactions

In an extension of known methodology the substrate (**47**, $R = CH_2CH_2N^+Me_3$) was converted into the phospholipid (**47**, $R = CH_2CH_2CMe_3$) in 84% yield using phospholipase D from *Streptomyces* sp. using as a solvent system 0.1 M aqueous sodium acetate, 0.5 M aqueous calcium chloride, six equivalents

Table 5 Enantioselective esterification of alcohols RCH_2OH where R is carbocyclic, heterocyclic or organometallic

Entry	Reference	R	Enzyme (reaction conditions)	Product(s) (enantiomeric excess or *E* value)
1	83	 R^1 = Boc or benzyloxycarbonyl	Lipase *PS* or lipase *M* (*i*-Pr_2O and vinyl butanoate *ca.* 3 h)	(*S*)[a]-Butanoate and (*R*)[a]-alcohol (*E* 25 to >200)
2	84		*Aspergillus* aminoacylase 1 (vinyl butyrate in wet toluene, 9 h)	(*S*)-Alcohol (49, 85% ee) and (*R*)-ester (49%)
3	85		Lipase AK (vinyl butyrate in *i*-Pr_2O, −40 °C)	(*S*)-Alcohol and (*R*)-ester (*E* = 55)
4	86		*C. antarctica* lipase (CH_2Cl_2 and vinyl acetate 6 h)	(−)-Ester and (+)-alcohol (*E* = 67)

[a] Refers to stereogenic centre adjacent to ester/alcohol group.



47 **48** **49** **50** **51**

of 3,3-dimethylbutanol and chloroform.[78] Choline esters of nucleopeptides have been hydrolysed using butyrylcholine esterase in pH 6.5 buffer at 37 °C (yields for six examples 61–96%).[79]

3 Esterification reactions

3.1 Esterification of primary alcohols

A lipid-coated lipase from *Rhitopus delemar* is soluble in supercritical $(SC)CO_2$ where it acted as an efficient catalyst for triglyceride synthesis. The activity of the catalyst was controlled by pressure and temperature.[80]

In the enantioselective esterification of primary alcohols the adjacent stereogenic centre may be composed of a simple alkyl group and a longer chain alkyl and/or more bulky unit. For example the steroid derivative **48** is acylated using *Pseudomonas cepacia* lipase in a mixture of chloroform and THF containing vinyl acetate to give the ester with the (*S*)-configuration at the adjacent centre. The diastereomer with the (*R*)-configuration next to the alcohol unit is less reactive.[81] Similarly the diastereomers **49** yield the (*S*,*S*)-acetate and recovered (*R*,*S*)-alcohol (36% yield, 98% ee) on treatment with lipase PS in isopropyl ether containing isopropenyl acetate over 17 h. The alcohol was used as a precursor to pseudopterosins.[82]

In general, kinetic resolutions have been achieved by conducting enzyme-catalysed esterifications on primary alcohols where the alcohol moiety is attached directly to a ring system. Selected examples are shown in Table 5; the adjacent ring system can be carbocyclic (entry 1) heterocyclic (entries 2, 3) or organometallic (entry 4). Polycyclic fullerene derivatives containing a primary alcohol group may also be acylated (using *Pseudomonas* lipoprotein lipase or *C. antarctica* lipase) if the alcohol group is spaced far enough away from the C_{60} unit.[87]

Equally popular has been the use of the enzyme-catalysed esterification strategy to differentiate between different types of hydroxy groups. For example, in compounds of type **50** the primary alcohol unit is acylated preferentially using lipase PS on Celite in diisopropyl ether containing vinyl acetate (96–98% yield in less than 1 h).[88] 5′-*O*-(sη-Glycero-3-phosphoryl nucleosides) are acylated with valerate and palmitate units solely on the primary alcohol unit using the trifluoroethyl ester of the fatty acid and *Rhizomucor miehei* lipase as the catalyst.[89] For (2′-deoxy)ribonucleosides *C. antarctica* lipase promotes reaction at the 5′-hydroxy group, mainly or exclusively, employing vinyl carbonate. The yield of the reaction depends on the base unit attached to the nucleoside; for example adenosine ribonucleoside gives only 20% 5′-vinylcarbonate while for uracil ribonucleoside an excellent (98%) yield of product is obtained. Notably using *Ps. cepacia* lipase the same (2′-deoxy)ribonucleosides give only 3′-carbonates in moderate to good yields.[90]

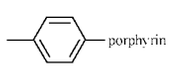
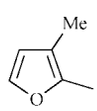
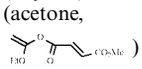
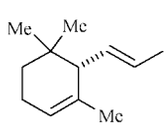
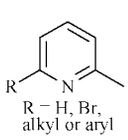
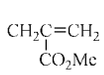
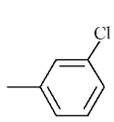
The primary alcohol group is acylated in the corresponding diol using lipase LIP (from Toyobo) in diethyl ether or *tert*-butyl methyl ether containing vinyl acetate over 3–5 h, to give the alcohol **51** (>99% ee) and the mirror image diacetate (*ca.* 95% ee) in 90% total yield giving a synthon for (−)-neplanocin.[91]

Racemic thiaheterohelicene derivatives **52** have been resolved using *Ps. cepacia* lipase to give the P-diol (45% yield, 93% ee) and by *C. antarctica* lipase to give the M-diol (40% yield, 97% ee).[92] The use of *Aspergillus niger* lipase as catalyst in toluene containing vinyl acetate is particularly useful for the selective esterification of calixarenes. While yields are low (8–19%) the enantiomeric excesses obtained (86–100%) are impressive.[93]

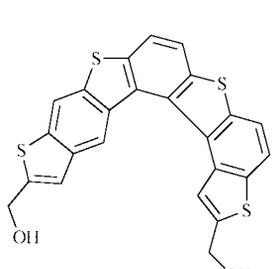
3.2 Esterification of secondary alcohols

The resolution of chiral secondary alcohols using lipase-catalysed esterification reactions is commonplace.[94] The secondary alcohol can be of the type MeCH(OH)R (Table 6, entries 1–5) or similar acycloalkanols in which the methyl group is modified slightly (Table 6, entries 6–8). Interestingly

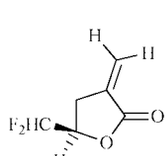
Table 6 Resolution of compounds MeCH(OH)R or closely related analogues $R^1CH(OH)R^2$

Entry	Reference	R^1	R^2	Enzyme (reaction conditions)	Products (enantiomeric excesses or *E* value)
1	95	Me or Et	$C(=CH_2)CO_2Et$	Lipase *PS* (MeCN, vinyl acetate or isopropenyl acetate, 35 °C, 7–13 days)	(*R*)-Ester and (*S*)-alcohol ($E > 300$)
2	96	Me	$(CH_2)_2CHCH_2$	*C. antarctica* (pet. ether and vinyl butyrate)	(*R*)-Ester and (*S*)-alcohol ($E \geq 300$)
3	97	Me		*Ps. cepacia* lipase (vinyl acetate in *i*-Pr_2O, 5–48 h)	(*R*)-Ester and (*S*)-alcohol ($E > 100$)
4	98	Me		*Ps. aeruginosa* lipase (Toyobo) (acetone, )	(*R*)-Ester[a] (43% yield, 84% ee)
5	99	CH_3		Lipase *PS* (vinyl acetate in *tert*-butyl methyl ether, 72 h)	(*S*,*R*)[b] and (*R*,*R*)[b] Acetates both 99% ee
6	100	Alkyl, vinyl		*C. antarctica* lipase (vinyl acetate, *i*-Pr_2O, 4 Å mol. sieves)	(*R*)-Ester and (*S*)-alcohol ($E > 500$, yields > 90%)
7	101	CHF_2		Lipase *PS* (vinyl acetate)	(*S*)-Acetate and (*R*)-alcohol[c] (34–39% yield, 93–97% ee)
8	102	CH_2Br		Lipase *QL* (Meito Sangyo Co.) (propionic anhydride in *tert*-butyl methyl ether, 35 °C, 24 h)	(*R*)-Alcohol and (*S*)-acetate ($E > 116$)

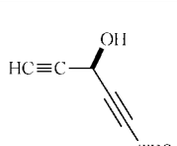
[a] The product underwent an intramolecular Diels–Alder reaction. [b] (*R*)-Stereochemistry at stereogenic centre close to newly-formed acetate unit: ratio of two diastereoisomeric esters, 1 : 1. [c] The alcohol may be lactonised using *C. antarctica* lipase (*ca.* 90% yield, 98% ee) to afford lactone **53**.



52



53



54

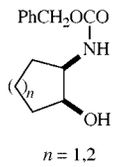
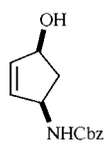
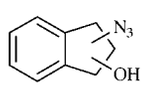
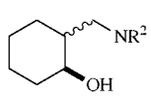
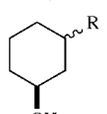
the diynol **54** is resolved using *C. rugosa* lipase and vinyl acetate to give the (*R*)-acetate (31% yield, 90% ee) and (*S*)-alcohol (57%, 71% ee) *en route* to a natural compound contained in the sponge *Cribrochalina vasculum*.[103]

The efficiency of the resolution of alcohols such as phenethyl alcohol using CLEC-subtilisin has been found to be solvent dependent. It is now believed that this is due not only to substrate desolvation phenomena but also to the presence of solvent molecules at the active site, changing the molecular recognition process by steric, electrostatic and conformational factors.[104]

Monitoring of such protease and lipase-catalysed esterification reactions can be accomplished using time-resolved thermographic IR detection, using an infra-red camera.[105]

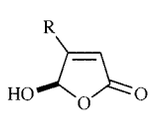
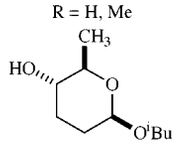
Many of the secondary alcohols that can be resolved enzymatically have the hydroxy group on a carbocyclic ring, either five-membered (Table 7, entries 1–3) or six-membered (Table 7, entries 4, 5). It has been shown moreover, that for the

Table 7 Enzyme-catalysed kinetic resolution of cycloalkanols

Entry	Reference	Substrate	Enzyme (reaction conditions)	Product(s) (enantiomeric excesses or *E* values)
1	106	 $n = 1,2$	For $n = 1$, *Ps. cepacia* lipase (vinyl acetate or isopropenyl acetate in 1,4-dioxane or *tert*-butyl methyl ether) For $n = 2$, replace *Ps. cepacia* lipase by *C. antarctica* lipase	(*R*)-Acetate and (*S*)-alcohol (*E* > 200)
2	107		*Ps. cepacia* lipase or *C. antarctica* lipase (vinyl acetate)	(*S*)-Acetate and (*R*)-alcohol[a] (*E* 16–45)
3	108		*C. antarctica* lipase (vinyl acetate in hexane–ether, 3 h at 40 °C or 10 days at room temp.)	All four isomers of 1,2-azido indanols may be obtained (yields 25–45%, ees 93–98%)
4	109	 R = alkyl, benzyl	Lipase *PS* or *Novozym 435* (vinyl acetate in diethyl ether or *i*-Pr_2O)	(*R*)-Acetate and (*S*)-alcohol in both cases (*E* > 200)
5	110	 R = Ph, CH_2Ph, SPh, SO_2Ph (*ca.* 82% diequatorial)	Lipase *PS* (vinyl acetate in benzene, 30 °C)	Diequatorial (1*R*,3*S*)-ester (35–42% yield, >97% ee)

[a] Products used in the synthesis of carbocyclic nucleosides.

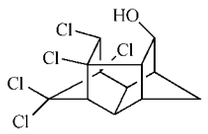
Table 8 Resolution of heterocyclic compounds possessing a secondary hydroxy group

Entry	Reference	Substrate	Enzyme (reaction conditions)	Products (enantiomeric excesses or *E* values)
1	112	 R = H, Me	Lipase *R (Amano)* immobilised on hyfloSuperCell (vinyl acetate in diethyl ether)	(*R*)-Acetates (70% yield for R = H, 52% yield for R = Me, ees > 98%)
2	113		Lipase *PS 2500 (Amano)* (vinyl acetate or vinyl butyrate)	(*S*)-Alcohol (37–40% yield, 92–99% ee)

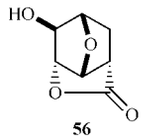
ppl-catalysed esterification of indan-1-ol and homologues the reaction rates and enantioselectivities may be increased by up to 14-fold and 9-fold respectively by subjecting the reaction to microwave irradiation.[111]

Similarly compounds having a hydroxy group attached to a saturated heterocyclic ring system may be resolved using a biotransformation. Once again the heterocycle may involve a five-membered ring system (Table 8, entry 1) or a six-membered ring (Table 8, entry 2). Even large acyclic compounds such as the polychloro compound **55**[114] and the complex heterocyclic compound **56**[115] undergo efficient enantioselective acylation employing *C. rugosa* lipase and *Ps. fragi* lipase respectively.

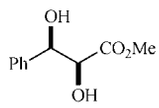
The ability of lipase enzymes regioselectively to promote esterification at one secondary hydroxy group in compounds containing more than one such unit is widely appreciated. For example racemic diol **57** is esterified using lipase PS in vinyl acetate at room temperature to give the (2*R*)-mono acetate (54% yield, 75% ee) and (2*S*)-diol (44% yield, 98% ee).[116] The same methodology is often applied to carbocyclic diols and heterocyclic diols. From the former category, the mono-ester **58** is obtained (48% yield, 85% ee) on treatment of the corresponding racemic diol with acetic anhydride in diethyl ether containing *C. rugosa* lipase.[117] In the second category sugar derivatives have featured prominently. A full paper has appeared on the regioselective acylation of glucose derivatives **59**. For the β-anomers acylation occurs predominately (for R = SPh or SePh) or exclusively (for R = SEt) at C-3. In the α-series acylation occurs with highly pronounced selectivity at C-2



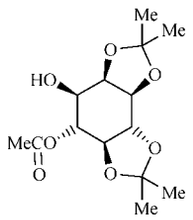
55



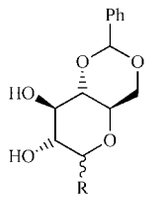
56



57

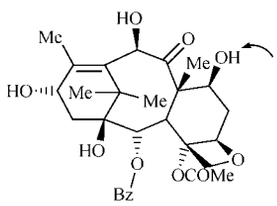


58



R = SEt, SPh, SePh, OPh

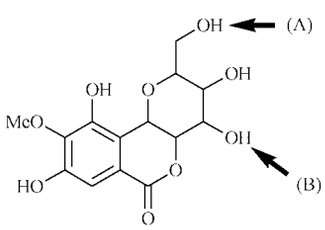
59



60

(for R = SEt, OPh). In both cases lipase P is the catalyst and vinyl acetate the acyl transfer agent.[118]

The taxol derivative **60** is acylated in the position shown (73% yield) using *Pseudomonas cepacia* lipase in trichloroacetic anhydride containing triethylamine. If this position is blocked esterification takes place at the allylic hydroxy group (84% yield).[119] The polyhydroxylated flavonoid **61** has been used to demonstrate how regioselective acylation, catalysed by different hydrolyses, may be used to create libraries of diesters. Thus the primary hydroxy group of **61** is acylated using lipase and vinyl acylate (A); this monoester when subjected to subtilisin-catalysed acylation using vinyl acylate (B) undergoes reaction at the position shown.[120]

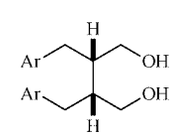


61

Note that the *non-enzymic* methods for the kinetic resolution of secondary alcohols have been reviewed.[121]

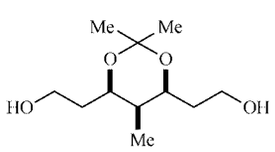
3.3 Esterification of *meso* or prochiral diols

The diol **62** is desymmetrised using *C. antarctica* lipase in benzene containing vinyl acetate to give the (2*R*,3*S*)-mono-ester.[122]



Ar = 3,4-dimethoxyphenyl

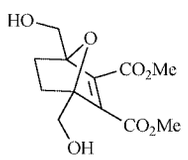
62



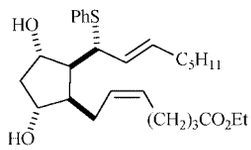
63

Similarly the 1,7-diol **63** is mono-esterified with *Ps. fluorescens* lipase in diethyl ether containing vinyl acetate to give a product of unknown configuration (*ca.* 100% ee). Interestingly, ppl-catalysed acylation gives the mono-ester of opposite configuration.[123] Likewise, enantiomers of the mono-ester derived by partial acylation of the diol **64** can be obtained using different enzymes: a *Pseudomonas* lipase in vinyl acetate affords one enantiomer (*ca.* 90% yield, 75% ee) while *C. rugosa* lipase in ethyl acetate containing vinyl acetate furnishes the other (*ca.* 85% yield, 88% ee).[124]

A *non-enzymic* acylation process, using a chiral diamine in the presence of molecular sieves, has been used to convert (1*S*,2*R*)-cyclohexane-1,2-diol into (1*R*,2*S*)-2-benzoyloxycyclohexan-1-ol.[125] The "pseudosymmetric" diol **65** is acylated with exquisite selectivity using lipase AK in neat vinyl acetate over 5 days at room temperature.[126]



64



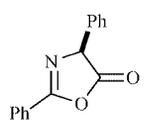
65

The desymmetrisation of prochiral 2-substituted propane-1,3-diol derivatives has been exploited quite extensively (Table 9). As can be seen by inspection of Table 9 a wide variety of 2-substituted and 2,2′-disubstituted propane-1,3-diols undergo stereocontrolled acylation.

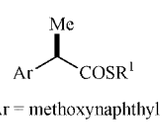
In an investigation of a substrate related to entry 1, and as a follow up to an earlier study, the desymmetrisation of 2-(3,5-dimethoxybenzyl)propane-1,3-diol using vinyl acetate and chymotrypsin in various solvents has been detailed. It has now been shown that the improvement in selectivity in changing the solvent from toluene to dibutyl ether is *not* attained at the expense of lowered enzyme reactivity; on the contrary prochiral selectivity and catalytic efficiency rise in parallel.[132]

3.4 Other esterification reactions

In the lipase-catalysed transesterification reaction of the oxazolidinone **66**, the rate and enantioselectively of the reaction is markedly improved by the addition of triethylamine. The effect is possibly due, in part at least, to the removal of traces of acid from the micro-environment of the enzyme.[133]



66



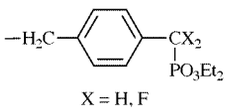
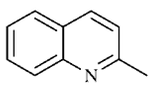
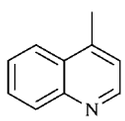
Ar = methoxynaphthyl

67

The transesterification of racemic naproxen thioesters **67** using *C. rugosa* lipase in isooctane at 37 °C over 100–200 h affords the (*S*)-ester and recovered (*R*)-thioester. The thioester moiety has to incorporate an electron withdrawing group (*e.g.* $R^1 = C_6H_5$, CH_2CF_3) for maximum efficiency.[134] In a study into the subtilisin Carlsberg-catalysed dynamic resolution of thioesters of the type $R^1CH(R^2)COSR^3$ (*e.g.* $PhCH(CH_3)COSR^3$) the choice of the group R^3 has a profound effect on the acidity of the proton attached to C-2. The trifluoroethyl thioester promotes proton exchange as does the propargylic (prop-2-ynylic) systems; the transesterification procedure using *n*-butanol as the acyl acceptor is one of the protocols of choice.[135]

Diols of the type $RCH(OH)CH_2OH$ in which R is chloromethyl, fluoromethyl or vinyl are phosphorylated on the primary alcohol group using glycerol kinases from a variety of species (including *Streptomyces canus*) employing ATP as the

Table 9 Desymmetrisation of 2-substituted propane-1,3-diols $R^1R^2C(CH_2OH)_2$ by enzyme-catalysed esterification

Entry	Reference	Substrate R^1	Substrate R^2	Enzyme (reaction conditions)	Product(s) (enantiomeric excess)
1	127	 X = H, F	H	Lipase *PS* (vinyl acetate in THF, 2 h)	(2*R*)-Monoester (82–97% yield, 99% ee)
2	128	O^{t}Bu SPh	H H	*Ps. fluorescens* lipase (vinyl acetate)	(2*R*)-Monoesters (76–97% yield, 88–91% ee)
3	129		H	Porcine pancreatic lipase (vinyl acetate, 20 h)	(2*R*)-Monoester (87% yield, 97% ee)
4	129		H	*C. antarctica* lipase (vinyl acetate, 10 h)	(2*S*)-Monoesters (62% yield, 97.5% ee)
5	130	β-galacto-pyranosyl	H	*Ps. cepacia* lipase (2,2,2-trifluoro-*n*-alkanoates)	(2*S*)-Monoesters (35%)[a]
6	130	β-galacto-pyranosyl	H	*C. antarctica* lipase (2,2,2-trifluoro-*n*-alkanoates)	(2*R*)-Monoester (52–83% of mixture—see above)
7	131	CH_2Ph or C_6H_{11}	F	Lipase *PS* (vinyl acetate, moist *i*-Pr_2O, 2,6-di-*tert*-butyl-4-methylphenol)	(2*S*)-Monoester (67–91% yield, 91–95% ee)

[a] In addition, the product from acylation of the sugar primary –OH group is obtained (*ca.* 35%) as well as the corresponding diester.

phosphate donor and recycling the triphosphate using phosphoenol pyruvate and pyruvate kinase. The (*R*)-enantiomer of the diol is phosphorylated preferentially (yields *ca.* 70%; ee 91–>99%); the specific activities of the enzymes are quite low but the reaction rate can be elevated by raising the temperature.[136]

4 Preparation of amides

A mixture of proteases from *Streptomyces griseus* (commonly available as pronase) has been studied for use in peptide synthesis. Yields up to 95% have been achieved in work that included the preparation of a hexapeptide.[137]

α-Chymotrypsin and trypsin have been used in peptide synthesis, both at room temperature and in frozen solutions, coupling *N*α-protected histidine acyl donors to lysine derivatives as the amine components.[138] The efficiency of α-chymotrypsin-catalysed coupling of inherently poor amino acid substrates (*e.g.* Z-protected alanine) is improved by the use of 2,2,2-trifluoroethyl- or carbamoylmethyl-esters as the acyl donors. Valine was also coupled efficiently using the carbamoylmethyl ester.[139] Boc-(DL)-AlaOMe and Boc-(DL)-TyrOEt have been coupled to GlyNHNHPh using α-chymotrypsin or papain in a mixed solvent system or, indeed, neat organic solvent, in good yields.[140]

Addition of crown ether can activate peptide bond formation between an amino acid amide and an *N*-acetylamino acid chloroethyl ester using cross-linked subtilisin Carlsberg. The procedures entail soaking the crystals with crown ether, then evaporation of a solution of the CLECs in acetonitrile; after drying, the activated catalyst gives a 10-fold increase in the rate of reaction in acetonitrile at 30 °C.[141]

Note that the use of the 9-fluorenylmethyloxycarbonyl (Fmoc) unit as an amine protecting group in peptide coupling reactions promoted by trypsin necessitates careful control of the reaction conditions.[142]

The C-terminal carboxy group of a partially protected peptide (for example ZGlyPheOH) may be converted into the corresponding amide using ammonium hydrogen carbonate at 40 °C and an amidase from orange peel.[143] A systematic study into the aminolysis of simpler esters shows that the main variables that control amide synthesis are temperature, hydrophobicity of the solvent, the reaction volume and the water content of the medium. Lipases from *Rhizopus niveus*, *Candida antarctica* and ppl gave the best selectivity for the resolution of racemic esters to give optically active amides such as $R^1CH(R^2)CONHCH_2Ph$ in 77–86% ee.

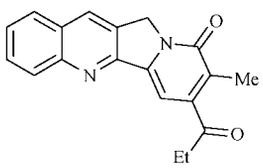
In a complementary study *C. antarctica* and *R. niveus* lipases proved to be excellent catalysts for the reaction of (±)-phenethylamine with simple esters (*e.g.* ethyl butyrate) showing a preference for incorporation of (*R*)-benzylamine into the product (95–99% ee).[144]

5 Reduction reactions

5.1 Reduction of ketones

The reduction of a selection of five-membered heterocyclic compounds, each having a CH_2COMe unit substituted at the

2-position, using bakers' yeast produces the (*S*)-alcohols (80–90% yield; >99% ee).[145] Mappicine ketone **68** has been reduced to the (*S*)-alcohol (Mappicine) in 74% yield and 86% ee using bakers' yeast in pH 7.2 buffer.[146] Ketones of the type ArylO-CH_2COCH_2OCOMe are reduced and partially hydrolysed by bakers' yeast to generate the (*S*)-acetoxy alcohol (36–84% yield; 52–>95% ee) and the (*R*)-diol (32–80% yield; 69–>95% ee).[147] $PhCO(CH_2)_nCH_2NO_2$ may be reduced to the corresponding (*R*)- and (*S*)-alcohols by choosing the appropriate microorganism. Thus *Pichia minuta* (CBS 1708) and *Pichia etchellsii* (CBS 2011) produce the (*S*)-alcohol (80–85% ee for $n = 1,2$). The optical purity can be raised using water–organic solvent mixtures. The (*R*)-alcohol is obtained (70% ee) using *Kluyveromyces marxianus* (CBS 397). All the yields were in the range of 50–80%.[148]



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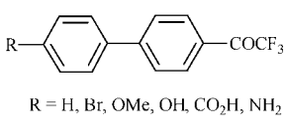


R = H, Br, OMe, OH, CO_2H, NH_2

69

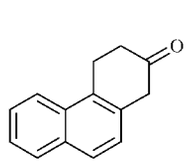
For ketones of the type $R^1COCH(X)R^2$ (where $R^1 = C_2H_5$, C_4H_9; $R^2 = C_3H_7$, C_4H_9; X = Cl, Br) the cleanest conversions are obtained using *Rhodotorula glutinis*, *Beauvaria bassiana*, or *Mortierella isabellina*; for example *R. glutinis* reduction of $H_5C_2COCH(Cl)C_4H_9$ gave a 26% yield of a 1:1 mixture of the *syn*-(3*S*,4*S*) and *anti*-(3*S*,4*R*) compounds, both showing >98% ee.[149]

A very convenient method for the preparation of (*S*)-alcohols from simple aliphatic ketones, aromatic ketones (such as compound **69**[150]) and β-keto esters, involved the use of the acetone powder of *Geotrichum candidum* (IFO 4597) in pH 7.0 buffer and propan-2-ol containing NAD^+. Isolated yields are very good to excellent, ee's often >99%.[151] Using *Geotrichum* sp38 the ketones $ArCOCH_2X$ (X = F, Cl) are reduced to the (*S*)-alcohols (80–86% ee) in good yield; the equivalent bromo- and iodo-compounds are poor substrates.[152]

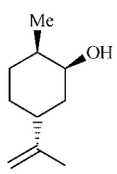
A reductase purified from bakers' yeast reduces a wide variety of ketones, diketones, and keto-esters to the corresponding alcohols employing $NADP^+$ as the cofactor and recycling this additive using glucose-6-phosphate and glucose-6-phosphate dehydrogenase. Yields are often good to very good and the stereoselectivity almost invariably excellent.[153]

The mutation of serine-39 to theonine in *Thermoanaerobacter ethanolicus* reductase completely altered the stereospecificity of the enzymes with regard to the reduction of simple ketones. For example butan-2-one gives (*R*)-butan-2-ol on reduction using the mutant enzyme as catalyst.[154]

For the reduction of some cyclic ketones bakers' yeast may still be the catalyst of choice; for example benzo-2-tetralone **70** ‡ is reduced to the (*S*)-alcohol (75% yield; 72% ee) using this biocatalyst.[155] On other occasions an alternative organism is preferred. For instance, carvone is reduced to the alcohol **71** using *Trigonopsis variablis* (ATCC 10679) in *ca*. 60% yield and >98% de.[156]



70



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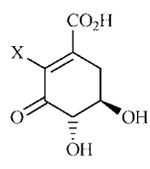


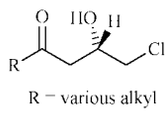
X = F, Br

72

‡ The IUPAC name for benzo-2-tetralone is 3,4-dihydrophenanthren-2(1*H*)-one.

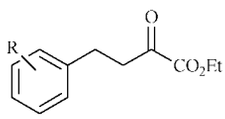
Shikimate dehydrogenase from *E. coli* has been overexpressed and used to reduce halo ketones **72** to the corresponding triols, with the (*S*)-configuration at the newly-formed stereogenic centre.[157]

Improved conditions have been found for the bakers' yeast reduction of hexane-2,5-dione to afford (*S*,*S*)-diol with >99% ee, >99% de.[158] The hydroxyketones **73** are formed (54–70% yield; 94–96% ee for short-chain alkyl) from the corresponding dione using heat-treated bakers' yeast and allyl alcohol in a two-phase solvent system comprising, for example, hexane and water containing glucose. Without the heat treatment, the allyl alcohol and the organic solvent, ee's are generally in the range 6–29%![159]



R = various alkyl

73



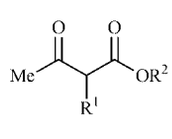
R = Cl, F, Me, OMe, NO_2

74

Chloroacetophenone has been added to bakers' yeast reductions of α-ketoesters **74** to give the (*R*)-hydroxyesters with improved ees (60–96%) and in 70–100% yields. The chloroketone acts to inhibit the (*S*)-stereoselective dehydrogenase.[160]

Bakers' yeast reductions of various β-ketoesters show improved rates and stereoselectivities on addition of a thiol such as cysteine or 2-aminoethanethiol. Yields of 75% with ee's *ca*. 90% were registered after $5\frac{1}{2}$ h bioreduction. The thiol does not act as an inhibitor, it seems, but rather as an alternative hydride source.[161] Under aerobic conditions it is well known that 3-ketoesters such as ethyl 3-oxobutanoate are reduced by bakers' yeast to the (*S*)-alcohol (ee *ca*. 90%). It has now been shown that the selectivity of the reduction can be reversed using anaerobically grown bakers' yeast giving (*R*)-alcohols in this series with ees 96–98%. The point is made, therefore, that the stereoselectivity of bakers' yeast reductions can be controlled to a large extent without the use of inhibitors, heat treatment *etc*.[162]

The reduction of the β-ketoester (**75**; $R^1 = Me$, $R^2 = C_2H_5$) has been optimised using bakers' yeast immobilised in calcium alginate. On a one-gram scale a mixture of (2*R*,3*S*)- and (2*S*,3*S*)-hydroxyesters were formed in a ratio of 9:1 respectively and in 60% combined yield.[163] Similarly employment of a yeast dehydrogenase and NADPH cofactor converts the ketoester (**75**; $R^1 = CH(Me)CH_2CO_2Me$, $R^2 = Me$) enantioselectively into one hydroxydiester (30% yield) which was cyclised to form the lactone **76** for the purpose of the confirmation of the stereochemistry.[164]



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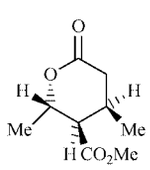
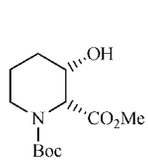


76



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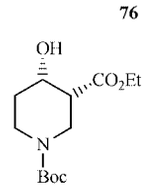

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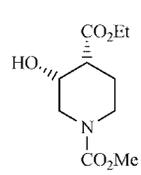


79

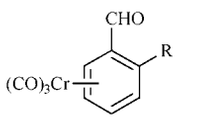
Cyclic hydroxyesters **77**–**79** are available (74–89% yield; 78–97% ee) by reduction of the corresponding ketoesters with bakers' yeast under Seebach's conditions.[165]

NADH is the cofactor necessary for the reduction of pyruvate to (L)-lactate using lactate dehydrogenase. Two new methods for the regeneration of NADH in this process have been reported, one using a platinum carbonyl cluster[166] and the other using electrochemically-recycled $FADH_2$.[167]

The reduction of $PhCH{=}CHCOCF_3$ over 27 h using *Geotrichum candidum* furnishes the corresponding (*S*)-3-en-2-ol (ee 94%) and (*S*)-1,1,1-trifluoro-4-phenylbutan-2-ol (ee 95%) in the ratio 2.5 : 1.[168]

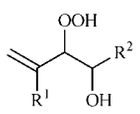
5.2 Miscellaneous reduction reactions

Chromium carbonyl complexes of type **80** may be resolved using horse liver alcohol dehydrogenase and NADH as the cofactor in pH 7 buffer containing Tween 80 at 36 °C over 8 h. The combined yield of alcohol and recovered ketone is *ca*. 80% and ees can be as high as 80–90%.[169]



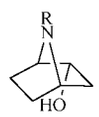
R = CH_3, F, Cl, OMe

80



R^1 = H, Me
R^2 = Me to ^{t}Bu

81



R = benzoyl or benzenesulfonyl

82

It has been shown previously that absorbing resins effectively control the concentration of the substrate in bakers' yeast reductions of β-ketobutyrate. Now such use of resins has been extended to bakers' yeast biotransformations of other substrates, *e.g.* $R^1CH{=}CH(R^2)CHO$ where R^1 = aryl, R^2 = Br, CH_3. The yield of alcohol is increased (since product recovery is made much easier): moreover the rate of reaction and the ee of the product are often improved. For example (*Z*)-2-bromo-3-phenylprop-2-en-1-ol gives (*S*)-2-bromo-3-phenylpropanol in quantitative yield and 99% ee.[170]

Similarly, bakers' yeast reduction of (*Z*)-3-bromo-4-phenylbut-3-en-2-one yields (2*S*,3*S*)-3-bromo-4-phenylbutan-2-ol in 80% isolated yield and >95% ee.[171] A novel carbon–carbon double bond reductase has been isolated from bakers' yeast which reduces enones of the type $ArylCH{=}C(CH_3)COCH_3$ to the corresponding (*S*)-ketones with optical purities >99% in many cases; yields are practically quantitative.[172]

2-Methylcyclohex-2-enone is reduced by *Yamadazyma farinosa* (aka *Pichia farinosa*) to give a mixture of 2-methylcyclohexanone and 2-methylcyclohexanols. Pyridinium chlorochromate oxidation of this mixture afforded (3*R*)-methylcyclohexanone in 67% yield and 95% ee. A similar chemoenzymatic sequence converted (*E*)-2-propylidenecyclohexan-1-one into (2*S*)-2-propylcyclohexanone in 58% yield and 74% ee.[173]

The kinetic resolution of PhCH(OOH)Me and its homologue has been accomplished for the first time. *Bacillus subtilis* was shown to give high enantioselectivity for the formation of (*R*)-hydroperoxide (>99% ee) and (*S*)-alcohol (20% ee) after 30 min and high conversion.[174] The horseradish peroxidase-catalysed reduction of hydroperoxyhomo-allylic alcohols of type **81** has been reported in full. The (*R*,*R*)/(*R*,*S*) diols and the (*S*,*S*)/(*S*,*R*) hydroperoxy alcohols are produced, often with high ees at 50% conversion.[175]

6 Oxidation reactions

6.1 Hydroxylation reactions

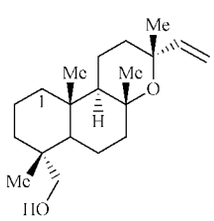
A recombinant whole cell biocatalyst *E. coli* K27 (containing cytochrome P450 from *Bacillus subtilis* and genes expressing proteins that facilitate the uptake of long-chain fatty acids) has been used in the oxidation of pentadecanoic acid to give a mixture of 12-, 13- and 14-hydroxypentadecanoic acids. The reactions are stereoselective; for example the latter product possesses predominantly the (14*S*)-configuration.[176]

The α-hydroxylation of carboxylic acids using molecular oxygen is catalysed by an α-oxidase from peas (*pisum sativum*), giving access to enantiomerically pure (*R*)-hydroxyacids RCH(OH)CO_2H where R = alkyl, alkenyl, alkynyl, $HO_2C(CH_2)_7$ or $CH_3(CH_2)_6S(CH_2)_6$. The reaction product is sometimes contaminated with the corresponding aldehyde RCHO. Note that unsaturation or a heteroatom contained in the substrate must be at least three carbon atoms distant from the reaction centre for hydroxylation to take place efficiently.[177]

The oxidation of nicotinic acid to 6-hydroxynicotinic acid (an important synthon for some pesticides) using *Pseudomonas fluorescens* has been adapted to form a continuous flow process.[178]

Hydroxylation of protected piperidine derivatives using *Beauvaria bassiana* has been studied. For example racemic *N*-protected 2-ethylpiperidine gives a 45% yield of the racemic *trans*-4-alcohol. The related 3,3-dimethylpiperidine gives a 48% yield of the 4-hydroxy compounds. These results were compared with those resulting from Johnson's earlier studies.[179] The hydroxyazanorbornanes† **82** were produced (56% yield; 22% ee) when the parent azabicycloheptanes were incubated with *B. bassiana* over a period of 5–7 days.[180]

Gibberella fujikuroi hydroxylated **83** to afford the 1α-hydroxylated compound (jhanol),[181] while *Rhizopus nigmigans* converted the diacetate **84** into three compounds corresponding to hydroxylation at positions A (15%), B (40%) and C (15%).[182] The same organism was found to oxidise shiromool acetate over 6 days to form bis-epoxide **85** in 58% yield.[183]



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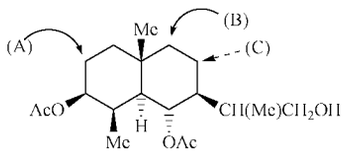

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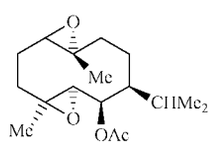

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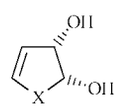


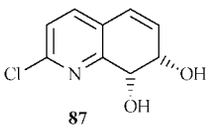
X = CH–CHCH$_2$, C–CMe$_2$, $(CH_2)_n$ where n = 1 - 4

86

6.2 Dihydroxylation

Simple aromatic compounds are oxidised to cyclohexa-3,5-diene-1,2-*cis*-diol derivatives using *Pseudomonas putida* or the mutant *E. coli* JM109. These chiral diols continue to be widely used in organic synthesis. Thus the diol derived from toluene has been used in preparation of the AB-ring of taxoids[184] the tricyclic sesquiterpene (−)-patchoulenone,[185] while the diol derived from chlorobenzene has been converted into (L)-ascorbic acid,[186] *N*-acetylneuraminic acid,[187] (+)-3-deoxy-(D)-glycero-(D)-galacto-2-nonulosonic acid[188] and all four stereoisomers of sphingosine.[189] (1*S*,2*S*)-Iodocyclohexa-3,5-diene-1,2-diol has been converted into other 3-substituted compounds using organotin chemistry.[190] The diols derived from both chloro- and bromo-benzene have been protected as a resin-linked acetal and used in the development of libraries of polycyclic compounds.[191]

Carbocyclic diols of various types **86** are available using the same biotransformation, usually in yields of around 30% and with very high ees.[192] 2-Chloroquinoline gives the diol **87** as the major product (30% yield) of a similar reaction.[193]



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6.3 Baeyer–Villiger oxidations

Baeyer–Villiger oxidations of 2-*n*-alkyl or 2-cyclohexylcyclohexanones can be effected using immobilised *Candida antarctica* and urea·hydrogen peroxide complex in diethyl ether. Yields of (*S*)-lactones were in the range 35–44% (ees 57–72%) after 3–4 days reaction at room temperature.[194]

The regioselectivity of the Baeyer–Villiger oxidation of norbornanone by *Pseudomonas putida* is affected by addition of an organic co-solvent. Extensive migration of the methylene group (giving the 3-oxabicyclooctan-2-one) is observed using toluene and water in a 1 : 1 ratio. Using toluene alone furnishes the 2-oxabicyclooctan-3-one as the major product while employment of octane as the solvent provides the 2-oxa compound as the sole product. High yields are obtained routinely.[195]

A full paper has appeared describing the use of recombinant bakers' yeast (expressing the cyclohexanone monooxygenase from *Acinetobacter calcoaceticus* NCIMB 9871) for the Baeyer–Villiger oxidation of 2,3- and 4-alkylsubstituted cyclohexanones. Lactones with good optical purities were obtained (ees >92%). One striking observation was that certain 3-substituted cyclohexanones were oxidised by the enzyme through high energy conformations.[196] The same mutant bakers' yeast has been used for the oxidation of 2- and 3-alkylsubstituted cyclopentanones. The best results were obtained for the compounds possessing long-chain alkyl groups in the 2-position; in these cases the (*S*)-lactone was formed ($E > 200$) in *ca*. 80% overall yield, taking into account recovered starting material. The authors concluded that, overall, cyclopentanone derivatives were not oxidised as efficiently as their cyclohexanone counterparts.[197]

6.4 Heteroatom oxidations

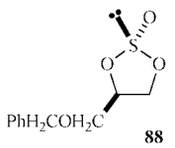
A series of phenylalkyl sulfides and a second series, comprising arylmethyl sulfides have been oxidised to the corresponding sulfoxides using whole cells expressing toluene dioxygenase (TDO) or naphthalene dioxygenase (NDO). Enantiomeric excess values over 90% were common and the two dioxygenases often showed enantiocomplementary activity. Thus oxidation of phenyl methyl sulfide with TDO gave the (*R*)-sulfoxide (90% yield; >95% ee) while oxidation with NDO afforded the (*S*)-sulfoxide (33% yield; 91% ee).[198]

The first report of the use of *Beauvaria bassiana* for a sulfoxidation reaction describes the conversion of racemic *N*-phthalidomethionine into the (*S*)-sulfoxides (60–74% de) in 85–88% yield.[199]

Detailed models of the active sites of 3,6-diketocamphane-1,2-monooxygenase and 3,6-diketocamphane-1,6-monooxygenase (enzymes from *Pseudomonas putida* NCIMB 10007) have been described. These enzymes catalyse the enantiocomplementary oxidation of a wide variety of sulfides to sulfoxides. The models allow predictions to be made of the selectivity of oxidation of new substrates. Interestingly the two enzymes have considerable sequence homology.[200] The substrate range of vanadium peroxidase from *Corallina officinalis* has been studied further; *ortho*-methylthiobenzoic acid gives the (*S*)-sulfoxides (80% yield; 97% ee) using the enzyme, peroxide and halide in water containing propan-1-ol as co-solvent. The *meta* and *para* isomers are much poorer substrates.[201]

A hydrolase (phytase) from *Aspergillus ficuum* masquerades as a peroxidase after incorporation of vanadium (as VO^{3-}) at low concentration (<15 μM). Thus thioanisole gave the (*S*)-sulfoxide in up to 66% ee using the modified enzyme at 4 °C in pH 5.1 buffer.[202]

Cyclohexanone monooxygenase from *A. calcoaceticus* NCIMB 9871 is able to resolve cyclic sulfites. For example the sulfite **88** forms optically active sulfite (94% ee) and sulfate at 62% conversion using the enzyme, cofactor (NADPH) and cofactor recycling system (glucose-6-phosphate and glucose-6-phosphate dehydrogenase) in pH 8.6 buffer.[203]



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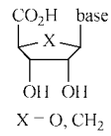

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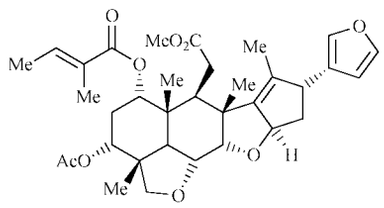


90

6.5 Miscellaneous oxidations

3,4-Dimethoxytoluene is oxidised to the corresponding aldehyde (>98% by GC) with the formation of only a trace of acid using *Trametes versicolor* laccase and 1-hydroxybenzotriazole in oxygenated pH 4.5 buffer.[204]

The oxidation of a wide range of ribonucleosides (including carbocyclic nucleosides) to the corresponding carboxylic acids **89** can be accomplished using immobilised nucleoside oxidase from *Stenotrophomonas maltophilia*. The enzyme is tolerant of a wide range of purine bases. On a preparative scale 10 g of xanthosine gave 6.6 g of the corresponding acid.[205]

Cross-linked selenosubtilisin has been used as an oxidase for the kinetic resolution of the hydroperoxide $PhCH(OOH)CH_2OH$. Thus after *ca*. 50% reaction, 43% of recovered hydroperoxide showed 97% ee.[206]

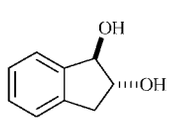
The triester **90** undergoes a three-step biotransformation with a *Nocardia* sp. over 15 h. First the Ac group is removed; then the exposed hydroxy group is oxidised to a ketone moiety before the unsaturated ester unit is eliminated to furnish the unsaturated ketone in 97% yield.[207]

Racemic *syn* diols $R^1CH(OH)CH(OH)R^2$ where $R^1 = Me$ and $R^2 = C_2H_5$ or *n*-pentyl gave recovered (*R*,*R*)-diol (48–50% yield) and (*S*)-hydroxyketone (10–18% yield), both essentially optically pure, when oxidised with diacetyl reductase from *Bacillus stearothermophilus*. The corresponding *anti*-diols behave in a very similar manner. *meso*-Cyclohexane-1,2-diol gave the (*R*)-hydroxyketone in 88% yield and >99% ee, over 72 h.[208]

Racemic 2-hydroxyacids ($RCH(OH)CO_2H$ where R = alkyl, benzyl, phenyl) are transformed almost quantitatively into the optically pure (*R*)-enantiomers using glucolate oxidase to form the 2-ketoacids which are then reduced with (D)-lactate dehydrogenase.[209]

The racemic diol **91** is transformed into the (*S*,*S*)-diol (>99% ee) cleanly when incubated with *Corynosporium cassiicola* in pH 8.0 buffer over 10 days.[210]

A study on the selectivity of the oxidation of the three secondary hydroxy groups of bile acids showed that *Xanthomonas maltophilia* oxidised the C-7 position almost exclusively, while



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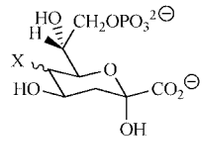

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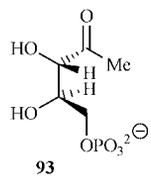


93

Acinetobacter calcoaceticus and *Pseudomonas fluorescens* were less discriminating.[211]

Incubation of *cis*-2-methylstyrene with cells of *Nicotiana tabacum* gave a low yield (7.5%) of the corresponding *cis*-epoxide.[212]

7 Carbon–carbon bond formation

7.1 Preparation of cyanohydrins

The conversion of aryl aldehydes into (*R*)-cyanohydrins using almond meal is accomplished in quantitative yield and furnishes optically pure products when the reaction medium is composed of reverse micelles. Lower yields and optical purities were recorded for cinnamaldehyde and a selection of methyl ketones.[213]

(*S*)-Cyanohydrins are formed from a wide range of alkyl and aryl aldehydes and some methyl ketones often in very high enantiomeric excess using *Hevea brasiliensis* hydroxynitrile lyase in a vigorously-stirred two-phase solvent system such as aqueous buffer and methyl *tert*-butyl ether.[214]

7.2 Aldol reactions

Since (D)-glyceraldehyde 3-phosphate is two orders of magnitude better as a substrate for KDPG-aldolase than (D)-glyceraldehyde itself, some non-natural aldehydes (*e.g.* $R^1CH(OH)CH(R^2)CHO$) were likewise phosphorylated to try to improve the extent of KDPG-aldolase-catalysed reaction with pyruvate. However no consistent trend in the reaction rate was observed as a result of the phosphorylation.[215]

3-Deoxy-(D)-arabinoheptulosonate-7-phosphate (DAH-7-P) synthase catalyses the coupling of (D)-erythrose-4-phosphate and phosphoenol pyruvate (PEP) (to give DAH-7-P). Now the enzyme has been overexpressed in *E. coli*, purified, and shown to catalyse aldol-type condensation of PEP with C_5-substrates, for example, (D)-arabinose-5-phosphate, (D)-ribose-5-phosphate and 2-deoxy-(D)-ribose-5-phosphate to give the corresponding C_8 compounds (*e.g.* **92**) in 46–94% yield.[216]

1-Deoxy-(D)-xylulose-5-phosphate synthase has been overexpressed, purified and used with rabbit muscle aldolase and triosephosphate isomerase in the synthesis of 1-deoxy-(D)-xylulose-5-phosphate **93**.[217]

7.3 Other carbon–carbon bond forming reactions

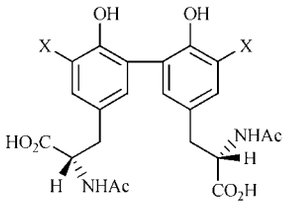
Expanding on earlier work by Itoh and Fuganti, the team of Smallridge has shown that cyanoketones of the type $ArCOCH_2CN$ (Ar = Ph, 3-tolyl, 2- or 3-anisoyl) are transformed by bakers' yeast, in a two-phase solvent system comprising petroleum ether and water, into the corresponding ethyl derivatives $ArCOCH(C_2H_5)CN$ (59–75% yield). The transformation proceeds *via* an aldol reaction, elimination of water and reduction of the enone.[218]

Two reports catalogue the first instances of enzyme-catalysed carboxylation reactions on a semi-preparative scale. Pyrrole-2-carboxylate was synthesised in 52% isolated yield from pyrrole and bicarbonate using pyrrole-2-carboxylate decarboxylase from *Bacillus megaterium* (working in the carboxylation direction). Unfortunately the enzyme is highly specific for pyrrole.[219] A supported carboxylase enzyme from *Thauera aromatica* was used to prepare *p*-hydroxybenzoic acid in 90% yield from phenyl phosphate and CO_2.[220]

Peroxidase catalysed the phenol-oxidative coupling of monohalogenated tyrosine derivatives to give compounds of type **94** (30–75% yield), which were used in the synthesis of bastadins.[221]

8 Carbohydrate chemistry

A timely review of chemoenzymatic approaches for the synthesis of saccharides and mimetics has appeared[222] and an overview of enzyme-catalysed glycosylation reactions has been published.[223]



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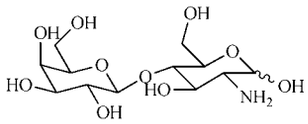


95

8.1 Use of glycosidases and glycoaminidases

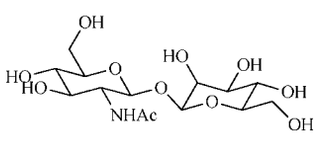
p-Nitrophenylgalactoside (Gal-pNP) can be coupled to *N*-acetylglucosamine (GlcNAc) to give β-(D)-Gal(1→4)(D)-GlcNAc in 67% yield using β-galactosidase from *Bacillus circulans* as the catalyst. The latter compound was converted into its 6-sulfate. The same concept is used to prepare the 6-sulfate of β-(D)-GlcNAc(1→4)(D)-GlcNAc except that colloidal chitinase from *Streptomyces griseus* is used to convert chitin into the required precursor.[224]

If the β-(D)-galactosidase from *B. circulans* is coated with lipid, it may be used to catalyse reactions in supercritical CO_2; for example the coupling of Gal-pNP to 5-phenylpentan-1-ol gives the β-anomer of the product in 72% yield after 3 h. It was estimated that the enzyme activity in SC–CO_2 is an order of magnitude greater than in organic solvents.[225]

Earlier studies on β-glycosidases showed that the carboxy residue in the active site could be replaced with a non-nucleophilic amino acid side chain to give a mutant protein which folded in the same way as the active enzyme but was catalytically inept. Since the active site is essentially intact it was reasoned that an activated α-glycosyl-unit and a suitable acceptor would bind to the usual pockets and subsequently react. The proposal proved to be correct; for example the new protein catalysed the coupling of α-galactosyl fluoride and β-nitrophenyl-β-cellobioside to give (D)-Gal-β(1→4)(D)-Glc-β(1→4)-(D)-Glc-p-NP in 93% yield. A number of similar reactions were observed with α-glucosyl fluoride giving, almost invariably, the 1→4 product. In some cases reaction of the first-formed product may continue, to provide higher saccharides.[226]

Transgalactosylation between lactose and $GlcNH_2$ is catalysed by a thermophilic galactosidase giving access to multigram quantities of lactosamine **95**.[227] This enzyme, nicknamed Clonezyme, also catalyses transfucosylation of xylopyranoside to give β-(D)-fucopyranosyl-β-(D)-xylopyranoxide disaccharides with 1→2 and 1→3 linkages.[228]

The β-*N*-acetylhexosaminidase from *Aspergillus oryzae* catalyses the formation of the disaccharide **96** (25% yield), together with smaller amounts of 1→3 and 1→6 coupled products, from mannose and GlcNAc-pNP.[229]



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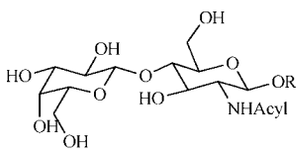


R = $(CH_2)_8CO_2Me$
Acyl = $COCH(NHAc)CH_2CH_2SMe$

97

It has been shown that *N*-glycopeptides and their mimics are good glycoside acceptors in reactions catalysed by microbial *endo*-β-*N*-acetylglucoaminidase giving complex glycopeptide products containing natural oligosaccharides. Now the strategy has been used to prepare eel calcitonin derivatives containing N-linked oligosaccharides.[230]

8.2 Use of glycosyl transferases

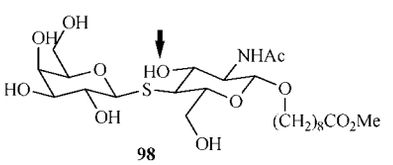
Bovine β-galactosidase has been used in the same reaction vessel as recombinant β-(1→6)-6GlcNAc transferase for the synthesis of GlcNAc(β1→6) GalNAc(α1-Bn) in over 90% yield. This is claimed to be the first example of this kind of reaction to give a practically quantitative yield of product.[231]

Bovine α(1→3) galactosyltransferase has been overexpressed in *E. coli* and used in the synthesis of carbohydrates bearing a Gal α(1→3)-Gal β-terminus,[232] while recombinant porcine α(1→4) galactosyl transferase has been employed to couple UDP-Gal to a series of non-natural acceptors (for example compound **97**) to give good yields on a multimilligram scale.[233]

Bovine β(1→4) galactosyltransferase has featured as the catalyst in the synthesis of complex branched-chain oligosaccharide mimics of fragments of the capsular polysaccharide of *Streptococcus pneumoniae* type 14.[234]

β-Glucuronides of estradiol, ethynylestradiol and several other phenols have been prepared on a preparative scale in 10–34% yield using UDP-glucuronic acid and UDP-glucuronyl transferase.[235]

Milk fucosyltransferase and GDP fucose effect the modification of the compound **98** by addition of the α-fucosyl unit at the position shown by the arrow.[236] Similarly recombinant fucosyltransferase III has been used to modify sialyl Lewis-trisaccharides **99** by the addition of fucose at the position shown. Yields of 52–100% were obtained on a 10–20 mg scale.[237] Surprisingly fucosyltransferase also effects the introduction of a fucosyl unit onto the tertiary-alcohol group in 3-*C*-methyl-*N*-acetyllactosamine to produce the corresponding trisaccharide.[238]



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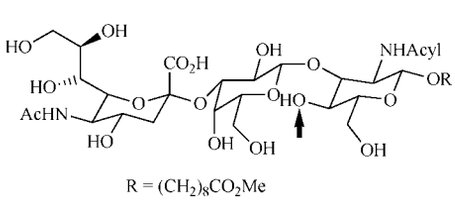


R = $(CH_2)_8CO_2Me$

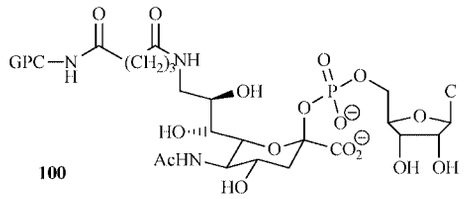
99

α-(2→3)-Sialyltransferase and α-(2→6) sialyltransferase catalyse the transfer of immobilised CMP-Neu Ac **100** to oligosaccharides and asialoglycoprotein. The rate of transfer of LacNAc was estimated to be about 3% of that observed for free CMP-NeuAc.[239]

Galactosyltransferase (with UDP-Gal), sialyltransferase (with CMP-Neu5Ac) and fucosyl transferase (with GDP-fucose) were used sequentially to prepare the sialyl Lewis X tetrasaccharide **101** on a solid support. After cleavage from the resin the yield of the product was an impressive 57%; the sequence (and particularly the second step of the sequence) has not even been optimised! Note that a new linker had to be devised to append the sepharose to Glc-NAc.[240]

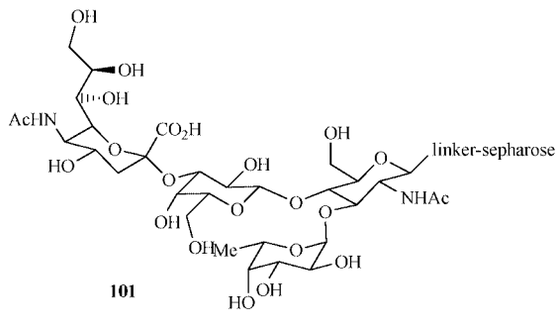
8.3 Other transformations involving carbohydrates

The synthesis of oligo- and polysaccharides catalysed by glycanases (the hydrolysis enzymes of polysaccharides) has been investigated, and the first synthesis of a cellulose–xylan polymer has been achieved by xylanase-promoted polycondensation of compound **102**. Mass spectrometry showed the formation of up to twenty-four saccharide units in 58% yield.[241]



100

CPG = long-chain alkylamino controlled-pore glass



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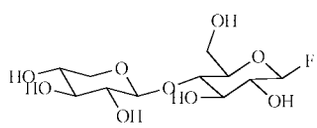

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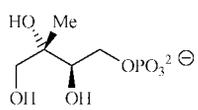


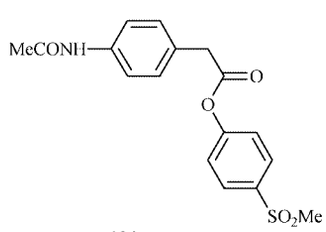
103

1-Deoxy-(D)-xylulose-5-phosphate has been converted into 2-*C*-methyl-(D)-erythritol-4-phosphate **103** on a 50 mg scale using a recombinant enzyme (designated 1-deoxy-(D)-xylulose-5-phosphate reductoisomerase) and NADPH.[242]

9 Enzyme mimetics

9.1 Catalytic antibodies

Transesterification reactions involving the activated ester **104** catalysed by a previously reported catalytic antibody SPO5OC1, can be observed for a wide selection of alcohols ranging from methanol to α-phenylethanol.[243]



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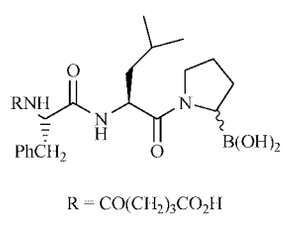
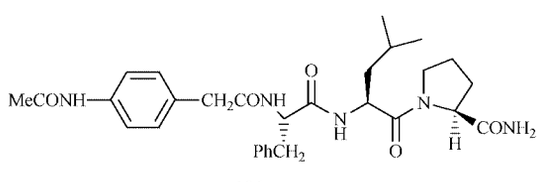


R = $CO(CH_2)_3CO_2H$

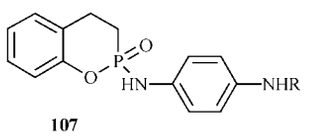
105

An antibody raised to the hapten **105** hydrolyses the $CONH_2$ bond in the compound **106** $k_{cat/uncat}$ *ca.* 3×10^4, but not a closely related ester.[244]



106

Antibodies raised to haptens **107** and **108** have been described previously. The active site(s) in these antibodies have been designed to accommodate the amide to be cleaved $RCH_2CONH–C_6H_4–para NO_2$ and the external nucleophile, phenol. A rate enhancement for amide cleavage of three orders of magnitude was measured showing, for the first time, how



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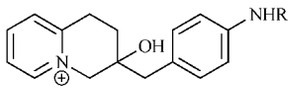

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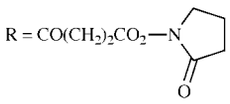
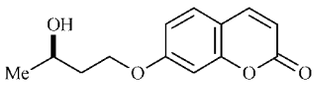


a weak nucleophile could be transformed into a powerful cofactor for amide-bond cleavage.[245]

In connection with the search for catalytic antibodies with alcohol dehydrogenase activity, Klein and Reymond have devised a fluorometric assay based on BSA-catalysed elimination of umbelliferone from ketones, derived by oxidation of the corresponding alcohols (*e.g.* compounds **109**).[246]



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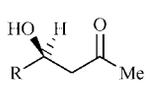


110

A similar strategy using the umbelliferone fluorophore has been used to detect aldolase activity.[247] Catalytic antibodies 38C2 and 33F12 have been shown to have the capacity to couple a wide range of substrates in aldol reactions; in the latest study twenty-three donors and sixteen acceptors were identified. Syntheses (up to 1 g in scale) involved ketone–ketone, aldehyde–ketone and aldehyde–aldehyde couplings; in some cases the resultant β-hydroxycarbonyl compounds underwent concomitant dehydration. The antibodies employ the enamine mechanism associated with natural Class 1 aldolases.[248] For the coupling of aldehydes to acetone, the hydroxyketones **110** are formed. It was found that, when R = *para*-acetamidophenethyl, the reaction was accelerated by the addition of Pd(II) salts.[249] Note that enantiomers of compounds **110** may be obtained by allowing the same antibodies to promote the retroaldol reaction on racemic substrates $RCH(OH)CH_2COMe$ and recovering unreacted starting material. On some occasions the retro-reaction works when the forward reaction does not.[250]

The substrate **111** was converted into glycine and *p*-acetamidobenzaldehyde by pyridoxal 5′-phosphate and an antibody 10HZ raised to the hapten **112**. The catalyst shows a modest rate enhancement and a moderate selectivity for the *threo* isomer of the substrate.[251]

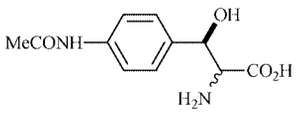
Antibody raised to hapten **113** has been shown to display good α-mannosidase activity, $K_{cat/uncat}$ *ca.* 110,000.[252]

Antibody 14D9 promoted five-membered ring acetal formation from the enol ether **114** preferentially for the (*R*)-enantiomer, casting further light on the preferred transition states in this and closely related reactions.[253]

Vanillin is made by coupling guaiacol (*o*-methoxyphenol) and glyoxylate to give vanillylmandelic acid (VMA) followed by oxidative decarboxylation. Catalytic antibodies raised to the hapten **115** showed selectivity for vanillin production from *p*-VMA but showed no reaction with *o*-VMA (a by-product in the initial reaction). While the catalyst activity is too low for industrial use at the moment, it points the way to other work in this area.[254]

Photolysis of the ketoamides **116** gives two products **117** and **118**. However irradiation in the presence of antibody MAb8C7, raised to hapten **119**, furnished the unique product **120** in 20% yield (78% ee). Obviously the substrate, located in the micro-environment of the chiral protein, undergoes a highly stereoselective reaction.[255]

Further evidence is now available which shows that an antibody can catalyse a number of different reactions if the substrates share a common recognition element (*e.g.* a nitro-



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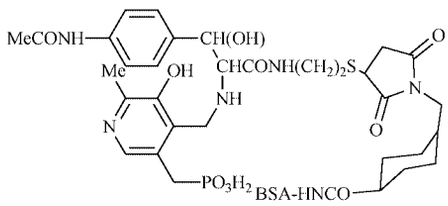

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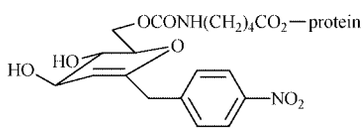

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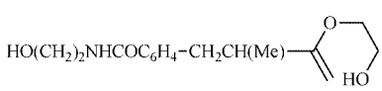

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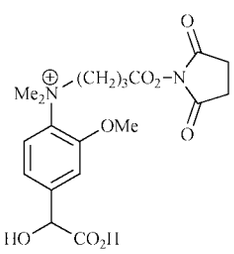

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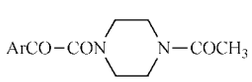

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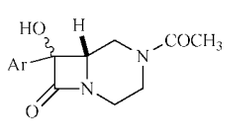

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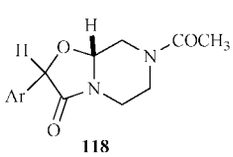

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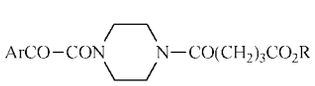

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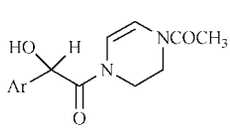


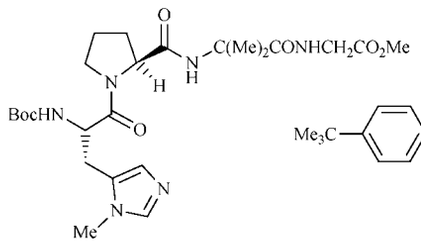
120

phenyl ring) and the reactions have overlapping mechanistic requirements (*e.g.* the need for a general base). Thus antibody Fab43D4 3D12 (which has now been cloned and over expressed) catalyses not only the elimination of HF from $p\text{-}NO_2\text{-}C_6H_4CH(F)CH_2COCH_3$ but also the elimination of HBr from $p\text{-}NO_2\text{-}C_6H_4CH_2CH_2CH(Br)CH_3$ (by abstraction of a β-proton) as well as the hydrolysis of the acetal $p\text{-}NO_2C_6H_4CH_2OCH_2OCH_3$.[256]

9.2 Other enzyme mimetics

Incorporation of non-natural imidazoylalanine derivatives into tripeptide units provided low molecular weight catalysts for enantioselective acylation reactions;[257] for example reaction of acetic anhydride with *trans*-2-acetylaminocyclohexanol in

toluene is catalysed by 2–5 mol% of compound **121** to give the (*S*,*S*)-alcohol (97% ee) and (*R*,*R*)-ester (57% ee).[258] A start has been made to design and synthesise non-peptide acylases,[259] while, at the other end of the molecular weight scale, an artificial protein, capable of cleaving α-globin heavy and light chains into polypeptides smaller than 5 kDa has been prepared by cross-linkage of a trisalicyl iron(III) complex onto poly(ethylenimine), followed by demetallation of the resultant polymer.[260]



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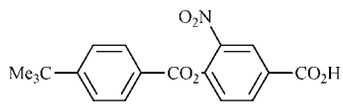


122

Cross-linking the branches of poly(ethylenimine) reduces the flexibility of the polymer and this modification improves the cyclodextrin-derivatised material with respect to its ability to catalyse the hydrolysis of the ester **122**.[261]

Cyclodextrins (Cds) have been rated as the most enduringly popular enzyme mimetics. Now it has been shown that Cds catalyse the basic hydrolysis of alkyl nitrites, the strongest catalysis by ionised β-CD being seen for nitrites with linear alkyl chains.[262] Enzyme mimetics built up from a cyclodextrin core continue to be one focus of attention of the Breslow group. Recent work has involved derivatives of β-CD with a tridentate ligand for Zn^{2+} ions and a distal imidazole group. Hydrolysis of cyclic phosphates promoted by these catalysts showed some regioselectivity.[263]

Moreover β-cyclodextrins decorated with imidazole and aminoethylthio moieties in selected positions on the periphery catalyse the aldol reaction between *p*-*tert*-butyl benzaldehyde and acetone with rate enhancements of two orders of magnitude being observed.[264]

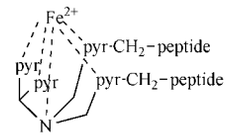
Various iron–porphyrin complexes have been found to behave like liver microsomal P450s in catalysing the dehydration of aldoximes (such as heptanaldoxime and phenylacetaldoxime) to the corresponding nitriles at 20 °C and at neutral or slightly acidic pH.[265]

A mutant form of intestinal fatty acid binding protein has been constructed and linked to pyridoxime to form a catalyst which converts α-ketoglutarate into glutamic acid. The catalytic efficiency is *ca*. 200 times greater than pyridoxamine itself; a high enantioselectivity (the product shows 94% ee) and a turnover >50 is observed.[266]

Interest in the ability of polyamino acids, such as polyleucine, to catalyse the asymmetric epoxidation of α,β-unsaturated ketones remains at a high level; a review of this area of research has appeared.[267] The substrate range continues to be broadened,[268] modifications to the reaction protocol have appeared,[269] more uses of the chiral epoxides in organic synthesis have been suggested[270] and an attempt to elucidate the mechanism of this fascinating transformation has commenced.[271] Note, as well, that a plethora of alternative methods for the conversion of enones into optically active epoxyketones have been published recently.[272]

Site-selective attachment of unprotected peptides to a non-heme catalyst **123** paves the way for the design of water-soluble peroxidase mimetics.[273]

It is well established that stereoselective addition of HCN to aldehydes using cyclic dipeptides such as [(*R*)-His-(*R*)-Phe] generates optically active (*S*)-cyanohydrins. The preferred method for activation of the catalyst has been described, namely lyophilisation of the material from aqueous solutions. It was noted



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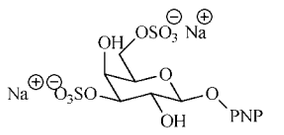


124 PNP = *para*-nitrophenyl

that the optical purity of the cyanohydrin increased in line with the extent of conversion. This was explained by suggesting that initially-formed (*S*)-cyanohydrin binds to the cyclic dipeptide to form a superior (*S*)-directing catalyst.[274]

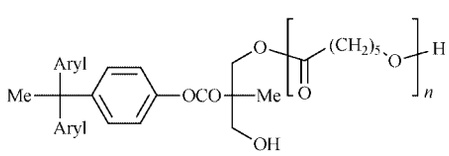
10 Miscellaneous biotransformations

A sulfatase from snail (*Helix pomatia*), abalone or limpet (*Patella vulgata*) enables selective removal of the sulfate unit from the 3-position of the galactose derivative **124** leaving the 6-sulfate unit intact. After two days of treatment with the snail sulfatase a 94% yield of the mono-sulfate was obtained.[275]

An unusual separation of alkenes has been effected using a lipase. *Aspergillus niger* lipase APE12(Amano) effects preferential esterification of (9*Z*,11*E*)-octadecadienoic acid over the (10*E*,12*Z*)-isomer using a solvent system comprising *n*-butanol and water in the ratio 3 : 1.[276]

Lipases from *Mucor miehei* or *Candida antarctica* catalyse polymer formation between divinyl adipate and glycerol or butane-1,2,4-triol, leaving pendant hydroxy groups on the polyester chain. After 28 h polymers derived from butanetriol show Mw *ca*. 3,900 while with glycerol higher average molecular weights (Mw > 10,000) are achieved.[277] The polymerisation of diols and diacids in the absence of solvent has been studied[278] and the advantage of removing the water, as formed in the condensation reaction, to force the equilibrium over to polymer formation has been demonstrated.[279]

Porcine pancreatic lipase catalyses ring-opening polymerisation of *epsilon*-caprolactone, using ethyl glucopyranoside as the initiator. The chain-length of the polymer could be varied by altering the initiator: lactone ratio. Similarly *C. antarctica* lipase-catalysed ring-opening polymerisation of trimethlene carbonate(1,3-dioxan-2-one) using the same initiator to generate a polymer with M_n = 7,200.[280] In another study, glycosides were used as initiators for ring-opening polymerisation of *epsilon*-caprolactone using *C. antarctica* as the catalyst. The enzyme effects regioselective acylation of the primary hydroxy group.[281] Specifically use of $CH_3C[C_6H_4OCOC(CH_3)(CH_2OH)_2]_3$ generates polymers of type **125** with a polydispersity calculated to be 1.3.[282]

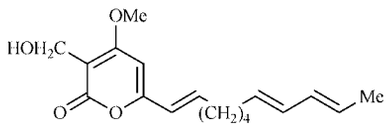


n = 1 - 48

125

The fungus *Cordyceps militaris* has been shown to convert the (*R*)-enantiomer of some common non-steroidal anti-inflammatory drugs to the more important (*S*)-enantiomer.[283] A discussion on the kinetic parameters needed for effective deracemisation of secondary alcohols or amines has been published.[284]

An enzyme preparation from *Alternaria solani* promotes a Diels–Alder reaction on the pyrone **126** by oxidising the primary alcohol group to the corresponding aldehyde. The activated system undergoes [4+2]-addition in the chiral environment of the protein to afford (−)-solanapyrone-A **127** as the major product (51% yield, 98% ee).[285] While not yet ready for use in synthetic organic chemistry, RNA species *are* capable of catalysing Diels–Alder and other reactions as described in a recent review.[286]



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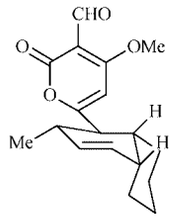

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11 Conclusions

The field of biotransformations can be regarded no longer as an orphan science but, instead, as one of the most powerful techniques for the preparation of optically active compounds using asymmetric catalysts.

The availability of a wide variety of enzymes[287] and the increased awareness that the reactions are not difficult to scale up, if necessary,[288] has encouraged most leading research groups at least to think of opportunities for using a biocatalysis step in a sequence of reactions from a simple starting material to a high value end-product.

However it is clear that advances in biotransformations are not made in isolation and there are just as many advances in areas of research utilising non-biological asymmetric catalysts. Hence there is a need to keep abreast of important developments across the wider aspect of this subject. This is most apparent when the choice of asymmetric catalyst (natural *versus* non-natural) for a particular transformation is not clear-cut. Thus while examples of enzyme-controlled epoxide hydrolysis have been monitored in this review, the recent endeavours of Jacobsen and co-workers using cobalt–salen catalysts[289] must be noted also.

Nitrile lipases append HCN to aldehydes to furnish optically active cyanohydrins. Chiral Ti(IV) complexes effect essentially the same transformation[290] (using trimethylsilyl cyanide rather than HCN) and an investigator will be offered a choice of methodology.

None more so is the competition so fierce as the area involving asymmetric oxidation of sulfides to sulfoxides. Whole cell systems (*e.g.* bakers' yeast) and isolated enzymes (haloperoxidases, monooxygenases and dioxygenases) are able to provide optically active sulfoxides but so can chiral titanium[291] and vanadium[292] systems. The advantages and disadvantages of the protocols have to be judged before the preferred method for a new substrate is chosen.

One clear-cut advantage of using biocatalysis is that more than one catalyst can operate in the same medium. While classic cases can be cited, the incorporation of unnatural genes (and hence proteins) in a micro-organism to create a new cascade of reactions in the cell (metabolic pathway engineering) is a powerful technique that is still in its infancy. As a specific recent example, *E. coli* has been modified by insertion of a cassette of genes to allow conversion of glucose into vanillin, the well-known flavour material.[293]

Whatever the pros and cons of using biocatalysis for any one particular reaction, collectively the use of biotransformations continues to be of great significance in the synthesis of enantiopure bioactive molecules[294] and other important areas of commerce and science.

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