Preparative biotransformations

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Received (in Cambridge, UK) 21st February 2001
First published as an Advance Article on the web 1st June 2001

Covering: 1999. Previous review: *J. Chem. Soc.*, *Perkin Trans. 1*, 2000, 611.

1 Introduction and background information

This review highlights some of the work which appeared in the literature in 1999, featuring the use of whole cells and isolated enzymes in synthetic organic chemistry. Fifteen journals[1] were chosen to represent a good cross-section of the activity in this area.

As in the preceding reviews of this type,[2] research work involving biosynthesis, metabolism (including microbial models) and enzyme/antibody-based sensors is excluded.

It is noteworthy that a biocatalysis database (using Synopsis software) is now available as a permanent part of the Chemical Database. The service is operated by the Central Laboratory of the Research Councils at Daresbury, UK.

A snapshot of the present state-of-the-art in biotransformations is presented in Issue 10 of the journal, *Bio-organic and Medicinal Chemistry*. Twenty review articles, collated by Dr D. C. Demirjian, describe work involving lipases, acylases, glucuronyl transferases, nitrile hydratases, and aminotransferases, as well as biomimetic poly(amino acid) catalysts. The final article in this series details some of the most important large-scale processes which employ biocatalysts.[3]

A review of the possible ways for biotransforming racemates into products in 100% yield and 100% ee using biocatalysis and *in situ* recycling of the unwanted isomer (*e.g. via* an oxidation/reduction enantioconvergent process) has appeared.[4] Another review on the designed synthesis of chiral compounds mediated by biocatalysts has been written by Ohno.[5]

2 Hydrolysis reactions

J. B. Jones has continued work on enzyme modification using a combination of site-directed mutagenesis and chemical modification of the incorporated unit (*e.g.* cysteine). Now the ability of subtilisin from *Bacillus lentus* to cleave esters without affecting amides has been improved using this technique.[6]

Four lipases were chosen to show the advantage of immobilising an enzyme onto a malleable composite. The chosen biocatalysts were adsorbed onto poly(hydroxymethyl siloxane) and then incorporated into readily vulcanizable silicones, which can be manufactured in different shapes and forms. Activity enhancement (4–7)fold was observed in hydrolysis reactions, compared with the native enzyme. The system is robust and it was shown that recycling of the biocatalyst could be effected.[7]

2.1 Ester hydrolysis

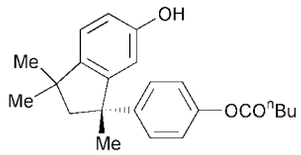
The removal of choline ester moieties from sensitive peptide derivatives using horse serum butyrylcholine esterase at pH 6.5 has been exploited by Waldmann in the preparation of palmitoylated and myristoylated lipopeptides.[8]

The same group has also employed enzyme-cleavable protecting groups in the synthesis of a glycophosphopeptide from the transactivation region of a serum response factor. Choline esters were removed as described above while a *p*-phenylacetoxybenzyloxycarbonylurethane moiety was cleaved by penicillin-G acylase.[9]

Similarly nucleobases have been constructed, using papain or *Aspergillus niger* lipase, to cleave methyl esters or methoxyethoxyethyl esters from orthogonally protected materials. The enzyme-catalysed reaction conditions were found to be sufficiently mild so as to circumvent peptide cleavage, β-elimination of nucleotide, and depurination.[10]

Compound **1** is of interest in non-linear optics; it has been prepared from the racemic diester on a 32 g scale using *Chromobacterium viscosum* lipase in *tert*-butyl methyl ether–buffer at pH 7.2 over 5 h ($E = 50$).[11] (Note that a web-based system is available to assess the selectivity in such kinetic resolutions.[12])

Williams *et al.* noted that aryl esters of the type PhCH-$(CH_3)CO_2$Ph racemise more rapidly than the corresponding

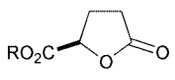


1

methyl esters and much more rapidly than the carboxylic acids. The group showed that *Candida cylindracea* lipase was able to hydrolyse such phenyl esters in the presence of DABCO (which racemised the substrate) setting the scene for the discovery of effective dynamic resolution procedures.[13]

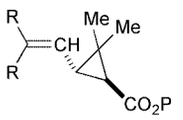
With respect to biotransformations of alkyl alkanoates, well-established protocols for the kinetic resolutions of methyl esters (Table 1) and ethyl esters (Table 2) of chiral carboxylic acids using esterases or lipases has been extended to some new substrates. Entry 2 in Table 1 describes the resolution process, conducted on a multi-kilo scale to produce the optically active renin inhibitor BILA 2157BS. The recovered (*R*)-diester was racemised using 5% DBU in toluene at 100 °C. Entry 3 describes a contribution to a major screening exercise aimed at producing synthons for nucleoside analogues. *cis*- and *trans*-isomers in this series were separated by hydrolysis of the mixture.

In the examples listed in Table 2, Entry 1 shows the methodology employed to produce an optically active acid which was used as a building block in an epothilone synthesis. Compounds of type **2** were subjected to hydrolyses in phosphate buffer at 25 °C using porcine pancreatic lipase (pp1) or horse liver acetone powder (hlap) as catalysts: (*S*)-ester was recovered (15–24% yield; 94–98% ee) on using ppl, while (*R*)-ester was obtained (10–23% yield; 53–96% ee) after exposure to hlap.[22] In connection with studies involving part-structures of the pyrethroid insecticides, it has been found that racemic resin-bound cyclopropane carboxylic acids **3** may be displaced from the solid support in enantioselective fashion using porcine pancreatic lipase at pH 7–8 over 6–16 h, releasing the (*R*)-acid (80–94% ee).[23]



R = Me, Et, ^{i}Pr, ^{n}Bu

2

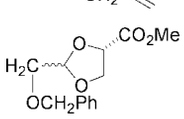
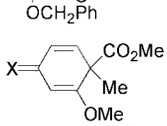


P = Merrifield, Wang or Tentagel resin
R = Cl, Br, Me

3

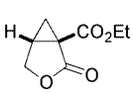
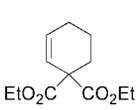
Kanerva and Liljeblad have undertaken a thorough study to determine the best enzymatic methods for the production of optically active malic and aspartate derivatives from the appropriate racemic diester **4**. *Candida antarctica* lipase B (CAL-B) is the preferred catalyst for alcoholysis of the β-ester group ($E = 55$), while acylase I is effective in the enantioselective hydrolysis of the α-ester moiety ($E > 100$). The compounds may also be resolved by acylation of the amino group ($E = 20$) or hydroxy group ($E = 40$) using *Candida antarctica* lipase A. In all instances the (*S*)-enantiomer of the racemate reacts more rapidly.[24]

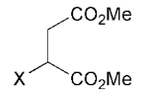
Table 1 Biotransformations involving methyl alkanoates

Entry	Substrate(s)	Enzyme	Conditions	Carboxylic acid product(s) yield (%)/ee (%) (or *E* value)	Reference
1	ArOCH(Me)CO$_2$Me	*Candida rugosa* lipase[a]	Aqueous–organic solvent	47/≤95	14
2	^{t}BuO$_2$CCH$_2$CHCO$_2$Me (CH$_2$=C(Me)–)	Alcalase	H$_2$O–acetone pH 7.5–8.5	48/98 (*S*-enantiomer)	15
3		α-Chymotrypsin or bovine pancreatic protease	pH 7 buffer 40–48 h	*E* 65–80 (*R*-enantiomer)	16
4	 X = H$_2$ or O	Horse liver esterase	pH 7 buffer 40–48 h	*E* 65–80 (*R*-enantiomer)	17
5	As above	Pig liver esterase	pH 7 buffer 43–52 h	*E* 42–96 (*S*-enantiomer)	17

[a] Previously treated with *n*-propanol.

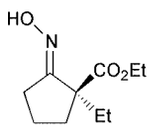
Table 2 Kinetic resolutions of ethyl alkanoates

Entry	Substrate	Enzyme	Conditions	Carboxylic acid products yield (%)/ee (%) (or *E* value)	Reference
1	CH$_2$CH(Me)CO$_2$Et, CH=CH$_2$	*Candida cylindraea* lipase	pH 7 buffer	41/90 (*S*-enantiomer)	18
2		Amano PS lipase	pH 7 buffer/iPr$_2$O, 18 h	*E* 62 (*S*-enantiomer)	19
3	Me(CH$_2$)$_n$CHCO$_2$Et, OPh	*Humicola* sp. lipase	H$_2$O	$n = 0$, *E* 13 $n = 1$, $E \approx 0$ $n \geq 3$, *E* 30–56 (*S*-enantiomer)	20
4		Porcine liver esterase	5% acetone in phosphate buffer, 20 °C	46/64	21



4

The first example of the kinetic resolution of "oxime esters" by lipase-catalysed transacylation with *n*-butanol has been recorded. This provides the oxime **5** (93% ee) and recovered starting material (98% ee). The optically active products were used to make δ-lactams *via* a Beckmann rearrangement.[25]



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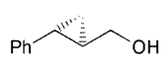
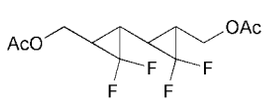


6

The alcohol **6** was formed preferentially ($E = 24$) when the corresponding racemic acetate was hydrolysed by CAL-B in methylene chloride and phosphate buffer (pH 7) at 40 °C over 22 h.[26] Modification of Tyr 29 by nitration using $C(NO_2)_4$ effected an increase in *E* value (18 to 36) for the *Pseudomonas cepacia* lipase-promoted hydrolysis of the ester $PhOCH(CH_3)CH_2OCOCH_3$.[27]

Lipase SL was employed to hydrolyse a mixture of *trans*,*trans*-diester **7** which comprised a 50 : 50 mixture of *meso* and racemic materials. After 2.5 h in pH 7.2 buffer the original *meso* form was isolated as a monoacetate. The diol **8** was formed and (−)-diacetate recovered ($E > 210$).[28]



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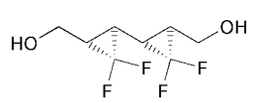

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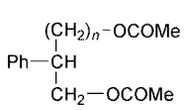
Porcine pancreatic lipase catalysed hydrolysis of the prochiral and racemic diacetates **9**; however only the prochiral compound ($n = 1$) gave a high yield of mono-acetate (ee greater than 95%).[29]



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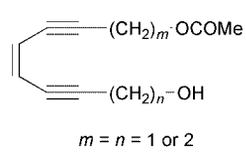


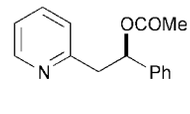
10

Access to part-protected enediynediols **10** is afforded by lipase-catalysed hydrolysis of the diester. After 24 h about 55% conversion had taken place; at this stage the monoester could be isolated in *ca*. 96% yield, based on consumed starting materials.[30]

The enzyme-catalysed hydrolysis of esters (usually acetates) derived from chiral secondary alcohols to give optically active material is common practice. The task of assessing the optical purity of the hydroxy compounds has been made more facile by a couple of new techniques,[31] and a new method for the determination of the absolute configuration of compounds ArCH(OH)R has been published.[32]

New acyclic substrates which undergo enantioselective reactions using lipases include the acetate **11** which gives the (*R*)-alcohol (40% yield, >96% ee) using porcine pancreatic lipase to catalyse the hydrolysis at pH 8.0. The process was used in a synthesis of (+)-sedamine.[33]

A series of compounds **12** were resolved using *Pseudomonas fluorescens* lipase or *Mucor miehei* lipase in *tert*-butyl methyl ether and buffer (pH 7) at 30 °C. *E* values ranged from 16 to 500; in most cases the absolute configuration of the product was not determined.[34]



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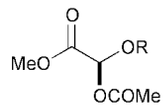
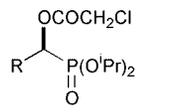


R = $(CH_2)_nCH{=}CH_2$, n = 1–3
CH_2CO_2Et; CH_2Ph;
$CH(OEt)_2$; $(CH_2)_7Me$;
$CH_2CH{=}CHPh$

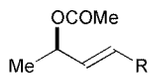
12

The chloroacetates **13** were resolved using Chirazyme P-2 protease to give the (*R*)-hydroxy phosphonates (yields 33–54%, ee *ca*. 80%) and (*S*)-esters. The esters were obtained in high ee by allowing the reactions to run to about 60% conversion.[35]



R = Et, ^{i}Pr, ^{t}Bu, $(CH_2)_2SMe$

13

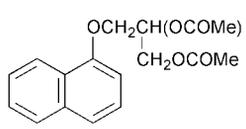


R = Ph, 1-naphthyl, 2-furyl
inter alia

14

A modification to the original Allen and Williams protocol has been reported for the dynamic kinetic resolution of acyclic allylic acetates **14**. The new procedure employs a transesterification (using *Pseudomonas cepacia* lipase or *Candida antarctica* lipase (CAL-B)) in propan-2-ol and tetrahydrofuran containing palladium tetrakis(triphenylphosphine) together with 2,1′-bis(diphenylphosphino)ferrocene. Conversions of 86% to over 99% were observed after 3–6 days, from which products were obtained in 61–78% yield and 98 to >99% ee.[36]

The diacetate **15** is hydrolysed over 72 h using CAL-B to give the (*R*)-diol (40% yield, 85% ee).[37] The regioselectivity of enzyme-catalysed hydrolyses of diesters of this type can be difficult to ascertain due to acyl group migration. The propensity of such a migration was studied using $PhCH_2OCH(OCOCD_3)CH_2OCOCH_3$ as the substrate for pig liver acetone powder-catalysed hydrolysis at pH 8. At 25% conversion the deuteriated ester $PhCH_2OCH_2CH(OH)CH_2OCOCD_3$ was present to the extent of 45% of the isolated mono-esters.[38]



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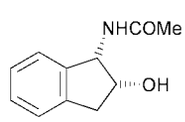
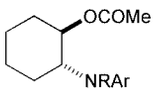
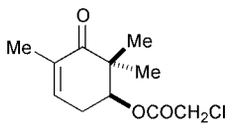


17

Esters derived from chiral cycloalkanols are often found to be good substrates for enzyme-catalysed resolution processes. The cyclopropane derivative **16** was resolved using CAL-B in tetrahydrofuran and phosphate buffer at 60 °C to afford the (*S*)-alcohol ($E = 44$)[26] The acetamidoindanol **17** was obtained from the corresponding racemic acetate using CAL-B and *n*-butanol in a range of cosolvents ($E > 500$).[39] The same high enantioselectivity was observed for the pig liver esterase-catalysed hydrolysis of the *trans*-2-aminocyclohexan-1-ol derivatives **18** furnishing the (*R*)-alcohols. A similar degree of stereocontrol is observed for the corresponding 7- and 8-membered ring systems but hydrolysis of the corresponding *trans*-*N*-phenyl-2-aminocyclopentanol ester under the same conditions gave a much poorer selectivity ($E = 13$).[40] Both enantiomers of phorenol were made available through the

enantioselective hydrolysis of the chloroacetate **19** to give the (*S*)-ester ($E = 10$) using Nagase (over 5 h at 40 °C in di-isopropyl ether and acetate buffer) and the (*R*)-ester ($E = 25$) using lipase P (Amano) at pH 7 over 24 h.[41]

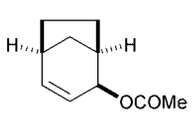


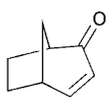


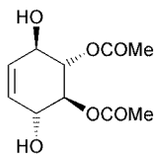
R = H; Ar = Ph; *m*-BrC_6H_4; *p*-ClC_6H_4; 2-naphthyl
R = Me; Ar = Ph

18 **19**

Resolution of the bicyclic acetate **20** (for example using lipase PS in acetone–phosphate buffer) is well documented. The Ogasawara team have used the optically active products in a synthetic route to (+)-juvabione and (+)-*epi*-juvabione.[42] The same group has used a modified procedure, employing an enzyme (*Candida antarctica* lipase) and an organometallic ($PdCl_2(CH_3CN)_2$) at room temperature in phosphate buffer over 48 h to provide the (*R*)-ester (42% yield, >99% ee) and the enone **21** (37% yield, >99% ee) *en route* to cyclopentanoid monoterpenes.[43]



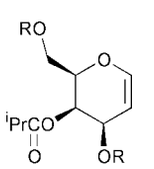


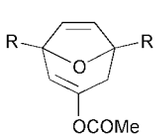


20 **21** **22**

Enzymatic resolution of (±)-conduritol B peracetate employing Lipozyme IM (*i.e. Mucor miehei* lipase on a support) in *n*-butanol and *tert*-butyl methyl ether gives mainly the diacetate **22** (44% yield, >98% ee) and recovered tetraester (47% yield, >98% ee). The products were used to make cyclitols (*e.g.* cyclophellitol) which exhibit powerful inhibition of glycosidases.[44]

The galactose derivative **23** is mono-de-esterified using *Candida cylindracea* lipase in 91% yield. On using lipase PS one of the secondary ester groups is also removed to give the diol **24**.[45] Similarly penta-*O*-acetyl α-D-glucopyranose is deacetylated regioselectively at C-1 using *Pseudomonas fluorescens* lipase (91% yield) and at C-4 using *Candida cylindracea* lipase (CCL) at pH 7 (78% yield). At lower pH values CCL hydrolyses the C-6 acetate unit preferentially to afford the 1,2,3,4-tetra-acetate (75% yield).[46]

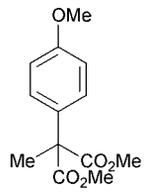


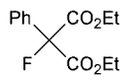


23 R = $CO_2{}^iPr$ **25** R = Me, Et, CH_2OMe
24 R = H

A series of enol acetates **25** has been resolved using silica-adsorbed *Humicola* sp. lipase in hexane and *n*-butanol over 2–48 h. The recovered optically active enol acetates have enantiomeric excesses in the range 91–94% and the prochiral ketone can be recycled.[47]

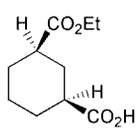
The partial hydrolysis of prochiral and *meso*-diesters to afford optically active products is a well-described domain for hydrolase enzymes. For example the 2,2-disubstituted malonate **26** gives the (*R*)-monoacid in 93% yield (97% ee). The product was used in a synthesis of sporochnol-A.[48] Similarly the diester **27** affords the (*S*)-monoacid (60% yield; >96% ee) on hydrolysis using porcine pancreatic lipase on Celite.[49]

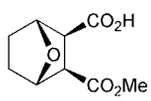


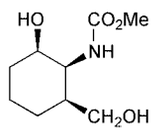


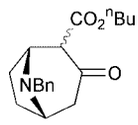
26 **27**

The carboxylic acid **28** is formed from the diester in practically quantitative yield (>95% ee) using CLECs of *Candida rugosa* lipase; the enantiomer is formed equally efficiently (>94% ee) using a *Pseudomonas* lipase from Amano.[50] It has been established previously that the acid **29** can be obtained by pig liver esterase-catalysed hydrolysis of the corresponding diester. Now it has been shown that these compounds can be transformed into aminocyclohexanol derivatives **30** which are carbasugar analogues.[51] The β-ketoester **31** is available by pig liver esterase-catalysed hydrolysis (and decarboxylation) of the *meso*-diester (51% yield; 95% ee). The material was used to make (−)-anhydroecgonine methyl ester.[52]



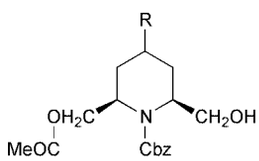


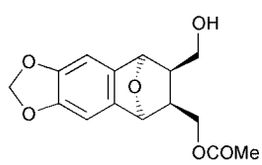




28 **29** **30** **31**

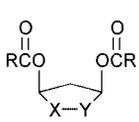
Full details for the preparation of the ester **32** from the diester have been described, together with the downstream conversion of the optically active material into pipecolic acid derivatives.[53] The selective hydrolysis of the corresponding diacetate (using porcine pancreatic lipase) to afford the alcohol **33** has been described previously. Now the transformation has been optimised using triglyme as the recommended medium, a solvent not widely used in biocatalysis. The product was used to prepare (−)-podophyllotoxin.[54]





32 **33**

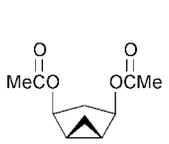
Eight cycloalkyl diesters **34** were hydrolysed by electric eel cholinesterase. All but one example gave the (*S*)-alcohol, with yields almost invariably in the range 64–90% with ee values in the range 80 to >99%. The bicyclohexanediol derivative **35** was the exception to the rule giving the *R*-alcohol under the usual reaction conditions.[55]



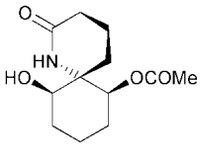
R = Me, Et, $CHMe_2$
X, Y = CH=CH, cyclopropyl

34

The hydroxyester **36** is produced from the diacetate in 87% yield (93% ee) using pig liver esterase in pH 7.5 buffer over 14 days. The compound **36** was converted into an intermediate to optically active perhydrohistrionicotoxins.[56]



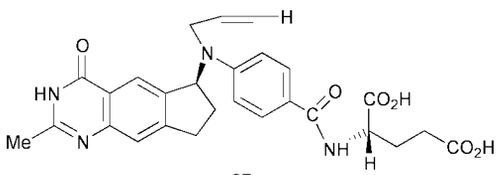
35



36

2.2 Amide hydrolysis

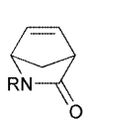
The amide **37** is obtained in 40% yield when an equimolar solution of (6*R*)- and (6*S*)-diastereoisomers in aqueous buffer containing a Zn(II) salt is hydrolysed by carboxypeptidase G2 over 10 h. (The glutamyl residue is more readily hydrolysed from the (6*R*) compound.) Prolonged exposure of amide **37** to the same enzyme gave the (6*S*)-carboxylic acid, an anti-folate.[57]



37

The amide $CH_3CH_2CH(CH_2OH)NHCOCH_2Ph$ has been resolved using penicillin-G acylase immobilised on Eupergit C. The (*S*)-amine is isolated (35% yield, >99% ee) at 40% conversion.[58]

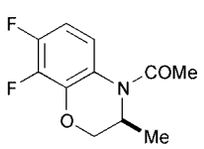
The resolution of the γ-lactam **38** has been accomplished on a scale of five metric tonnes using a lactamase from *Comamonas acidovorans*.[59] Alternative methodology for the production of these optically-active intermediates to carbocyclic nucleosides has been discovered by a GlaxoWellcome group. The lactam derivatives **39** and **40** are enantioselectively hydrolysed by savinase (a serine proteinase) in pH 8 buffer–tetrahydrofuran to give the (1*R*,4*S*)-lactam in 42–50% yield and >99% ee.[60]



38 R = H

39 R = $CO_2{}^tBu$

40 R = COMe



41

Savinase has also featured in another article which showed that, in general, glycopeptides are less susceptible to proteolytic degradation by this enzyme than are the corresponding peptides.[61]

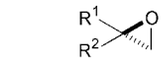
A key intermediate to levofloxacin has been prepared by enantioselective hydrolysis of the difluoro compound **41** to give the (*S*)-amine (42% yield, 99% ee) and recovered (*R*)-amide (46% yield, 99% ee).[62]

2.3 Epoxide hydrolysis

A review on the biocatalytic approaches to enantiopure epoxides has been published.[63]

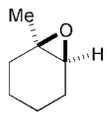
The hydrolysis of a series of epoxides **42** has been investigated using various yeasts. Strains from the genera *Rhodotorula, Rhodosporidium* and *Trichosporon* were found to be the best of those investigated, with good selectivity for production of the (*R*)-diol when $n = 5–7$ and the side chain is saturated. Only *Rhodosporidium toruloides* gave a good selectivity when the side-chain is unsaturated; the benzyloxy compound is not a good substrate, the best selectivity ($E = 8.5$) is observed with *Rhodotorula aurantiaca*.[64]

Limonene 1,2-epoxide hydrolase from *Rhodococcus erythropolis* catalyses the hydrolysis of the epoxide **43** to give the



R^1 = H, Me; $R^2 = (CH_2)_nMe$, $(CH_2)_nCH{=}CH_2$, CH_2OPh

42

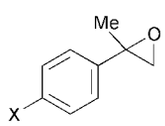


43

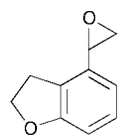
(*S*,*S*)-diol through an unusual acid-catalysed mechanism, as indicated by ^{18}O studies.[65]

A good deal of work in this area has focused on the stereocontrolled hydrolysis of styrene epoxide and simple derivatives. The epoxide hydrolase from *Agrobacterium radiobacter* AD1 has been over-expressed and used to resolve styrene oxides. Now it has been reported that replacing Tyr215 by Phe increases enantioselectivity 2–4 fold for four selected substrates.[66]

The hydrolysis of epoxides **44** was studied using ten epoxide hydrolases. Especially noteworthy was the hydrolysis of the isobutyl compound with the enzyme from *Aspergillus niger* ($E = 20$) allowing the synthesis of (*S*)-ibuprofen to be accomplished in 27% yield from racemic epoxide.[67] A paper pointing out that care should be taken in recording $[a]_D$ values in this series should be noted.[68]



44 X = ^{t}Bu, CN, NO_2, halogen



45

The epoxide **45** is stereoselectively hydrolysed by epoxide hydrolases from *A. niger* or *Rhodococcus glutinis* over 3–4 h in buffer containing 10% *tert*-butyl methyl ether, to give the (*S*)-epoxide and the (*R*)-diol (*E ca.* 25).[69]

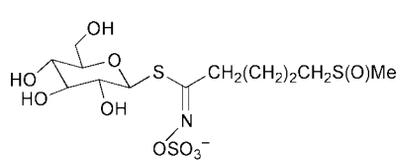
An epoxide hydrolase has been used in tandem with a recombinant halohydrin dehalogenase from *Agrobacterium radiobacter* to convert racemic 2,3-dichloropropan-1-ol into (*S*)-3-chloropropane-1,2-diol (*via* the (*S*)-epoxide) and recovered (*R*)-2,3-dichloropropan-1-ol. The halohydrin dehalogenase is highly enantioselective ($E > 100$) while the use of the epoxide hydrolase circumvents unwanted side reactions.[70]

2.4 Other enzyme-catalysed hydrolysis reactions

Alginate-immobilised cells of *Pseudomonas chlororaphis* B23 have been used to convert adiponitrile to 5-cyanovaleramide (with less than 5% over-hydrolysis to adipomide) on a large scale, 48 batches yielding an impressive 12.7 tonnes of material.[71]

A colorimetric screen was used to search for mutant dehalogenases from *Xanthobacter autotrophicus* that facilitate the conversion of 1,2-dichloroethane into 2-chloroethanol.[72]

Myrosinase-catalysed hydrolysis of glucoraphanin **46** at 37 °C, pH 6.5 yielded sulforaphane [$MeS(O)CH_2(CH_2)_2CH_2$-NCS] a potent inducer of anti-carcinogenic marker phase II enzymes *e.g.* glutathione-S-transferase and quinone reductase.[73]



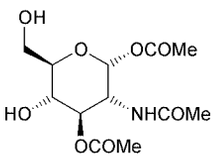
46

3 Esterification reactions

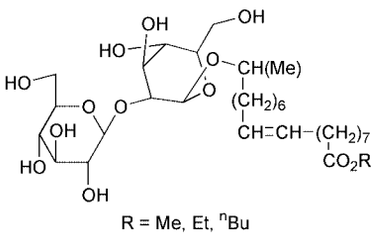
3.1 Esterifications of simple primary alcohols

There have been a number of examples published in which a

primary alcohol unit is selectively acylated in the presence of a secondary alcohol. For example the diol **47** is acetylated at the primary position (96% yield) using Novozyme (*C. antarctica* lipase) in tetrahydrofuran containing vinyl acetate.[74] In a more complex example sophorolipids **48** are acetylated at the two primary positions using, essentially, the same reaction conditions.[75]



47



48

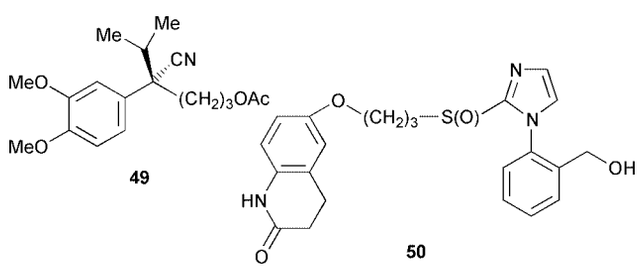
Enantioselective esterification often takes place when a stereogenic centre is located at the carbon atom adjacent to a primary alcohol moiety (Table 3). Entry 2 describes the first recorded resolution of a cyclic sulfite *via* a lipase-catalysed reaction, while entry 3 shows the resolution process employed in a route to dysidiolide and cassiol. The transformation described in entry 5 formed part of a study into the preparation of the glycosidase inhibitor (−)-1-azafagomine.

On some occasions vinyl esters more complex than vinyl acetate or vinyl butanoate can be utilised to achieve better enantioselectivity. For example enantioselective esterification of 2-phenylpropan-l-ol is better accomplished using Amano PS lipase in vinyl 3-(2-naphthyl)propanoate or vinyl 3-(*p*-tolyl)-propanoate ($E = 58$ in both cases) rather than vinyl propanoate ($E = 4$).[83]

Klibanov has demonstrated that esterification of the hydroxy acid $HOCH_2CH(Ph)CO_2H$ using cross-linked *Pseudomonas cepacia* in tetrahydrofuran containing vinyl acetate becomes more enantioselective on formation of a carboxylic acid salt (E increases from 1.0 to 7.6); the large size of the counter-ion accentuates the special requirements in the active site. Similarly transesterification of methyl phenylalanate becomes more enantioselective on formation of an ammonium salt.[84]

Note that the stereogenic centre need not be immediately adjacent to the primary hydroxy moiety; examples have been documented wherein the stereogenic centre is three carbon atoms distant from the hydroxy group (entries 6, 7): the first example has been described previously but has now been used for the synthesis of a new plant growth regulator while the second example has been employed to prepare (*S*)-γ-coronal.

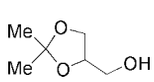
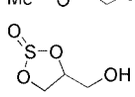
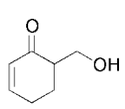
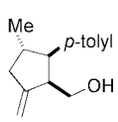
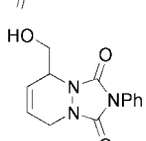
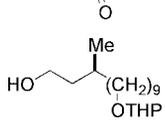
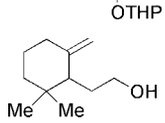
The acetate **49** is prepared from the corresponding racemic alcohol ($E = 13$) using *Pseudomonas fluorescens* lipase and vinyl acetate in hexane and ethyl acetate over 1.5 h at 32 °C. The enantiomeric ratio is increased to 17 using molecular sieves and to 49 on addition of 18-crown-6.[85] In an even more striking example the racemic sulfoxide **50** is resolved by selective acylation of the (*S*)-sulfoxide ($E = 70$) using Novozyme in dichloromethane containing vinyl acetate over 30 min. The work was aimed at the synthesis of optically active metabolites of the platelet adhesion inhibitor OPC-29030.[86]



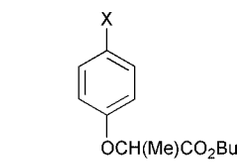
49 **50**

Esterification of acids of the type $Me_2C{=}CH(CH_2)_2$-$CH(Me)(CH_2)_nCO_2H$ using *Candida rugosa* lipase in cyclohexane containing hexadecanol gave an interesting switch in enantioselectivity. When $n = 0$ the (*S*)-ester was produced

Table 3 Esterification of primary alcohols

Entry	Substrate	Enzyme	Conditions	Product yield (%)/ee (%) (or *E* value)	Reference
1		Pig liver esterase/methoxy PEG co-lyophilisate	Vinyl propanoate, toluene–H_2O	*E* 24–29 (*R*-ester)	76
2		Ps. cepacia lipase on Celite	Vinyl butanoate, CH_2Cl_2, 6 h, sucrose	*E* 26 (*S*-ester)	77
3		Lipase AK	Vinyl acetate, benzene, 24 h	97% ee (*S*-ester) 99% ee (*R*-alcohol)	78
4		*Candida rugosa* lipase	Vinyl acetate	55/72 (*S*-ester) 42/>99 (*R*-alcohol)	79
5		Lipase R from *Penicillium roqueforti* then Novozyme 435	Vinyl acetate 42 °C, 11 d Vinyl acetate rt, 48 h	(*S*-ester)	80
6		*Candida rugosa* lipase	Vinyl acetate	(*S*-ester)	81
7		Lipase AK	Vinyl acetate, hexane, 0–4 °C, 19 h	(*S*-ester) and (*R*-alcohol)	82

($E = 41$), while for $n = 1$, the (*R*)-ester predominated ($E = 24$).[87] For transesterification reactions of esters of the acids $RCH_2CH(Ph)CO_2H$ (R = H or Me), the vinyl ester is strongly recommended, showing enhanced *E* values 26 and >100 over the corresponding ethyl esters (*E* 6.5 and 17 respectively) for reactions involving *Candida antarctica* lipase and hexan-l-ol. The (*R*)-enantiomer reacts preferentially.[88] For the esters **51**, the enantioselectivity favouring reaction of the (*R*)-enantiomers is enhanced by at least an order of magnitude by the addition of a lithium salt (*e.g.* LiCl) in a small amount of water to the substrate in diisopropyl ether containing *Candida rugosa* lipase.[89]



X = alkyl, OMe, CF_3, Cl

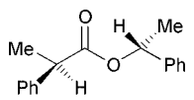
51

3.2 Esterifications of simple secondary alcohols

Secondary alcohols of the type RCH(OH)Me are often good substrates for enantioselective esterification reactions catalysed by enzymes and a selection of recent results are collated in Table 4. The *syn*- and *anti*- alcohols formed in the process summarized in entry 3 were separated by trifluoroacetylation and treatment with base. *syn*-(2*S*,3*S*)-3-Nitropentan-2-ol was obtained in >99% ee and 98.4% de and used to prepare a new anti-fungal agent. A study on the esterification of epoxy-α-ionols and epoxy-β-ionols (entry 4) showed that, whatever the stereochemistry of the substituents on the alicyclic ring, the (*R*)-configured hydroxy group was esterified preferentially. β-Lactams from the 3,4-*trans*-substituted series in entry 5 were used in the synthesis of carbapenems.

The origin of the enantioselectivity in the resolution of $PhCH(OH)CH_3$ using vinyl acetate and subtilisin Carlsberg in dry DMF has been rationalised using molecular dynamics and free-energy perturbation simulations. Steric, hydrogen-bonding and electrostatic effects were shown to be important.[95] (On a broader front a transition state model for subtilisin Carlsberg-catalysed (*S*)-selective transformation of chiral secondary alcohols has been proposed.[96]) It has been noted that addition of methylcyclodextrin enhances the rate and enantioselectivity of *Candida rugosa* or subtilisin Carlsberg-catalysed reactions involving substrates $ArCH(OH)CH_3$ and vinyl butyrate in organic solvents. The largest enhancements were observed for tetrahydrofuran (*E* 32→59) and 1,4-dioxane (*E* 31→45).[97] However, even in supposedly "dry" solvents, esterifications of substrates such as $PhCH(OH)CH_3$ are dogged by competitive hydrolysis of vinyl acetate so that reaction conditions are not held constant, acetic acid being released during the transformation.[98]

The *Candida antarctica*-catalysed coupling of $PhCH(OH)CH_3$ with the chiral vinyl ester $CH_3CH(Ph)CO_2CH{=}CH_2$ provides an addition to the limited number of examples where enzymes catalyse a doubly enantioselective process. In this case excellent selectivity is seen towards the alcohol ($E = 100$), while moderate selectivity is observed for the vinyl ester (*E ca.* 10). Thus the ester **52** is obtained in 45% yield (98% de) after recrystallization.[99]



52

Racemic α-arylethanols [$ArCH(OH)CH_3$] are converted into the corresponding (*R*)-acetates by using an organometallic Ru(II) complex (to equilibrate the alcohols) and *Pseudomonas cepacia* lipase with *p*-chlorophenylacetate (to effect esterification). For *p*-methoxyphenylethanol and *p*-bromophenylethanol yields of 82–98% (99% ee) are achieved. 1-Phenylpropan-2-ol is converted into the corresponding (*R*)-acetate in 60% yield and 97% ee using this protocol.[100] In an expansion of earlier studies, Bäckvall *et al.* have also effected dynamic kinetic resolution by coupling Ru-catalysed racemization and enzyme-catalysed acylation: the substrates were the diols $CH_3CH(OH)(CH_2)_nCH(OH)CH_3$ where $n = 1$–3. Mixtures of the (*RS*) and *meso* compounds gave the (*R*,*R*) product; for example hexane-2,5-diol gave the (*R*,*R*)-diol in 53% yield (99% ee) together with the *meso*-compound (10%). The methodology also works for *m*- and *p*-dihydroxyethylbenzenes and for benzylbis(2-hydroxypropyl)amine.[101] The variety of secondary alcohols that can be transformed in the above way extends beyond the type $R^1CH(OH)R^2$ where $R^2 = CH_3$; the substituent R^2 may be ethyl or chloromethyl while R^1 may be quite bulky *e.g.* naphthyl.[102]

Table 4 Enantioselective esterifications of alcohols RCH(OH)Me

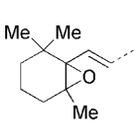
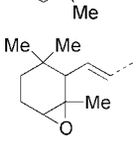
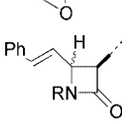
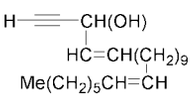
Entry	R	Enzyme	Conditions	Product(s) yield (%)/ee (%) (or *E*-value)	Reference
1	$-CH_2CH{=}CHMe$ (*Z*)-isomer	*Candida antarctica* lipase	Vinyl acetate, 3 h	(*R*-ester) $E > 200$	90
2	$-(CH_2)_2SO_2Ph$	*Ps. cepacia* lipase	Vinyl acetate, $CHCl_3$, 48–72 h	*E* 189 (*R*-ester)	91
3	$-CH(NO_2)CH_2Me$	Novozyme	Vinyl butanoate, toluene, rt, 17 h	49/>99 (*R*-esters; *syn*- and *anti*-)	92
4		Porcine pancreatic lipase	Vinyl acetate, ^{t}BuOMe	22% yield (*R*-ester)	93
		Lipase PS	As above	25% yield (*R*-ester)	93
5		Lipase PS	Vinyl acetate, 37 °C, 24–170 h	$E \geq 100$ (*R*-ester)	94

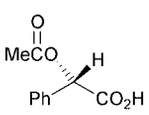
Table 5 Enzyme-catalysed acylation reactions on secondary alcohols $R^1CH(OH)R^2$ where R^1, $R^2 \neq Me$

Entry	Substrate	Enzyme	Conditions	Product(s) yield (%)/ee (%) (or *E* value)	Reference
1	RCH(OH)CH_2S(O) *p*-tolyl R = Me, Et, Pr, allyl	Porcine pancreatic lipase	Vinyl acetate	(*R*)-ester and (*S*)-alcohol	103
		Candida rugosa lipase	Vinyl acetate	(*S*)-ester and (*R*)-alcohol	
2	$ClCH_2CH(OH)CH_2CO_2R$ R = Et, CH_2Ph, ^{t}Bu, cyclohexyl	*Rhizomucor miehei* lipase	Vinyl propanoate in MeO^tBu or C_6H_6 or CCl_4	(*S*)-ester (*E* 20–>100)	104
3	$RCH(OH)CF{=}CF_2$ R = Ph or $PhCH_2CH_2$	*Pseudomonas cepacia* lipase	Vinyl chloroacetate in $^iPr_2O$, 35 °C 22–36 h	Presumed (*R*)-ester *E* ≥ 82	105
4	$PhXCH(OH)CH_2OMe$ X = OCH_2, CH_2	Pig liver esterase–PEG	Vinyl propanoate in toluene–H_2O	(*R*)-ester for X = OCH_2, (*S*)-ester for X = CH_2 (*E* 99, >200)	76
5				(*R*)-ester 41/94 for (4*E*,15*E*)-isomer (*R*)-ester 45/95 for (4*E*,15*Z*)-isomer	106

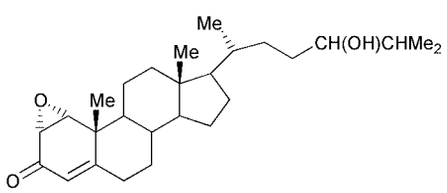
Examples of more straightforward enzyme-catalysed enantioselective acylation processes involving substrates $R^1CH(OH)R^2$, where R^1 and R^2 are not Me, are gathered together in Table 5.

By way of contrast Vedejs has produced more results concerning a non-enzymic method for the resolution of secondary alcohols $R^1CH(OH)R^2$ (where R^1 is aryl and R^2 is alkyl) using $(^iPrCO)_2O$, as the acylating agent and a 2-phosphabicyclo-[3.3.0]octane as the catalyst. Enantiomeric excesses of 76–96% are recorded for the resulting esters at 30–50% conversion.[107]

Mandelate racemase is a stable, cofactor-independent enzyme that is available in large quantities by fermentation. It has proved to be useful, in tandem with *Pseudomonas cepacia* lipase to give the (*S*)-acetate **53** in 80% yield (>98% ee) after four cycles.[108]



53



54

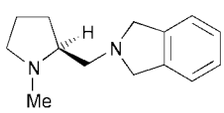
1α,24*R*-Dihydroxycholesterol has been prepared by selective esterification of the side-chain hydroxy group in compound **54** followed by a Mitsunobu reaction on the (24*S*)-compound to give the target steroid in 73% overall yield, after hydrolysis and regioselective epoxide ring opening.[109]

3.3 Esterifications of secondary cycloalkanols

Compounds having a secondary alcohol unit within a carbocyclic or saturated heterocyclic ring system often prove to be excellent substrates for enzyme-catalysed esterification reactions. Some carbocyclic examples are shown in Table 6. The resolution described in entry 4 was employed so as to obtain optically active intermediates for the synthesis of hydrindanes such as coronafacic acid. Similarly the resolution described in entry 6 provided material for the preparation of (−)-clavularin B. Parve *et al*. have shown that acetylation of prostaglandin-E_2 (PG-E_2) using *Candida antarctica* lipase in chloroform containing vinyl acetate gave 11-acetyl-PG-E_2 initially; the 11,15-diacetate is formed subsequently and then the 15-acetate of PG-A_2 is produced after prolonged treatment. Likewise PG-E_1 and PG-$F_{2\alpha}$ suffer regioselective acetylation at C-11 but in these cases the 15-position is not acetylated readily.[117]

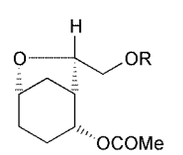
The well-known prostaglandin synthon 4-hydroxycyclopent-2-enone has been obtained in optically active form by alcoholysis of the corresponding acetate using Lipozyme in butan-2-ol over 18 h (43% conversion, *E* = 24).[118]

Two non-enzymatic methods have been reported for the resolution of cyclic secondary alcohols. *trans*-2-Phenylcyclohexan-l-ol is enantioselectively benzoylated using the diamine **55** (0.3 mol%) as the catalyst to give the (1*S*,2*R*)-benzoate (49% yield, 96% ee) and recovered alcohol (48% yield, 95% ee).[119] Alternatively Yamada has used twisted amides as agents to effect the asymmetric acylation of cyclic secondary alcohols and for the transesterification of *meso*-diols to generate optically active monoesters.[120]



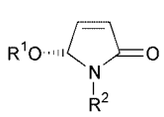
55

The esters **56** are formed (47–50% yield, >99% ee) together with recovered alcohol (47–49% yield, >99% ee) when the corresponding alcohol is reacted with vinyl acetate in THF containing lipase PS.[121] Kellogg, Feringa *et al.* have now published a full paper on the preparation of (5*R*)-acyloxypyrrolinones **57** in yields up to 100% of optically pure material using *Candida antarctica* lipase in refluxing hexane containing the requisite vinyl acylate.[122] In a related study it was shown that the alcohol **58** was converted into the (*S*)-acetate (*E* = 168) using lipase PL in vinyl acetate.[123] Acylation of swainsonine with porcine pancreatic lipase in THF containing trichloroethyl butanoate at 37 °C for 16 h gave a diester as the major product (31%). The diester was less active than the parent compound in inhibiting Jack Bean mannosidase.[124]



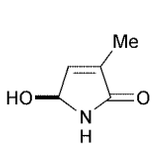
R = benzyl, ^{t}BuSiMe_2, 2-naphthylmethyl

56



R^1 = Me, Et
R^2 = Me, Ph, acyl

57

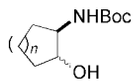
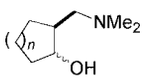
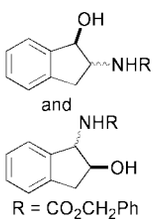
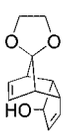
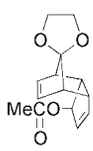
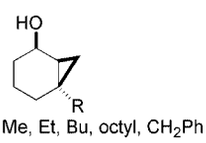
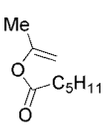
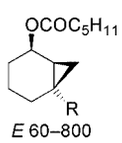
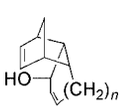
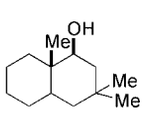


58

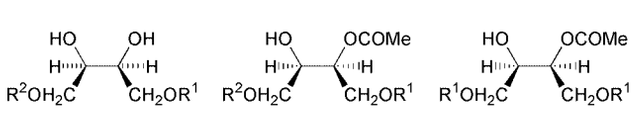
3.4 Esterifications of diols

The stereoselective esterification of diols is also an active area of study for biotransformations. For example racemic diols **59** were converted into a mixture of the monoesters **60** and **61**. Mesylation followed by treatment with potassium carbonate in

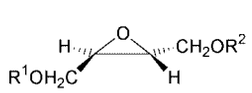
Table 6 Enzyme catalysed esterification reactions involving secondary cycloalkanols

Entry	Substrate	Enzyme	Conditions	Products yield (%)/ee (%) (or *E*-value)	Reference
1	 *n* = 1,2,*cis* and *trans*-isomers	Lipase-PS	Vinyl acetate, 35 h	(1*R*,2*S*)-ester *E* > 200 for *n* = 2, *cis*-isomer	110
2	 *n* = 1,3	Lipase-PS or Novozyme	Vinyl acetate in $^{i}Pr_2O$	(1*R*)-ester *E* 83–>200	111
3	 R = CO_2CH_2Ph	*Ps. cepacia* lipase	Vinyl acetate in dioxane or MeO^{t}Bu, 30 °C	(*R*)-ester formed. All eight isomers of *cis*- and *trans*-1,2-aminoindanols obtained	112
4		Lipase-PS	Vinyl acetate in THF, 3 h	 44%/>98% and alcohol 46%/>99% ee	113
5	 R = H, Me, Et, Bu, octyl, CH_2Ph	*Candida antarctica* lipase		 *E* 60–800	114
6	 *n* = 1,2	*Pseudomonas* sp. lipase LIP	Vinyl acetate in MeO^{t}Bu, rt	*n* = 1, 42% acetate/99% ee; 43% alcohol/95% ee *n* = 2, 36% acetate 94% ee; 43% alcohol 55% ee	115
7		*Candida rugosa* lipase	Vinyl acetate in $^{i}Pr_2O$	(*R*)-ester (*E* 72)	116

methanol gave the (*R*,*R*)-epoxides **62**. Overall yields were in the range 61–93% and the enantiomeric excess of the product was in the range 55–99%.[125] Lactone **63**, a key intermediate for the synthesis of the anti-obesity agent tetrahydrolipstatin, has been prepared (35% yield, 93% ee) from the (2*SR*,3*RS*,5*RS*)-diol $H_{23}C_{11}CH(OH)CH_2CH(OH)CH(C_6H_{13})CO_2Me$ using porcine pancreatic lipase in ether, in the presence of 4 Å molecular sieves over a period of 48 h.[126] Reaction of the diols **64** with vinyl acetate in chloroform containing *Pseudomonas* sp. lipase gave the (2*R*)-hydroxy-mono-esters and the (2*S*)-diesters (*E* 12–38).[127]



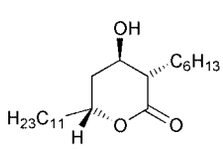
59 **60** **61**



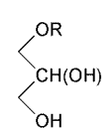
62

For **59**–**62**
R^1 = CH_2Ph or *p*-$MeOC_6H_4CH_2$
R^2 = aryl, tetrahydropyranyl, methoxymethyl

Similary the ferrocene derivative **65** gave the (2*R*)-hydroxy-monoacetate (51%, 90% ee) and the (2*S*)-diacetate (49%, 92% ee) on *Pseudomonas cepacia*-catalysed acetylation using vinyl acetate in *tert*-butyl methyl ether.[128] The monoester **66** is obtained in 84% yield when the diol is reacted with vinyl acetate in THF or pyridine over 20 h at 60 °C. The ester was used to prepare the anti-viral agent dideoxyinosine.[129]

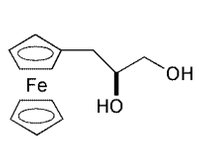


63

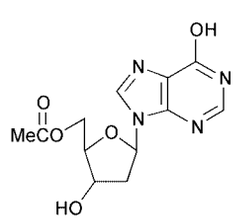


R = $C_{16}H_{33}$, $C_{18}H_{37}$
Δ^9–$C_{18}H_{13}$

64



65

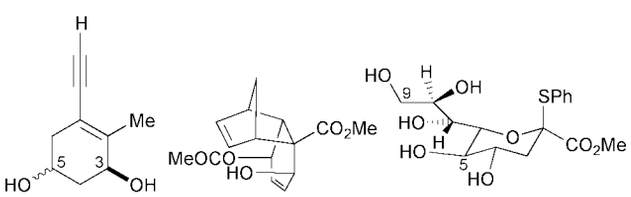


66

Table 7 Enzyme catalysed mono-esterification of compounds $R^1R^2C(CH_2OH)_2$

Entry	Substrate R^1, R^2	Enzyme	Conditions	Product(s) yield (%)/ee (%) (or *E* value)	Reference
1	H, OH	*Candida antarctica* lipase	Vinyl acetate in dioxane	94/54 increased to 94% ee by recrystallization	133
2	H, CH_2Ph	*Pseudomonas cepacia* lipase	Vinyl acetate	(*R*)-ester	134
3	H, Et	*Pseudomonas fluorescens* lipase	Vinyl acetate in CH_2Cl_2	72/46	135
4	H, OCOPh	Porcine pancreatic lipase	Vinyl acetate in THF or hexane, 1 h, rt	(*R*)-diester 63/96	136
5	H, CH_2CH_2R (R = $CH{=}CH_2$, $C{\equiv}CSiMe_2Ph$)	Amano lipase-PS or porcine pancreatic lipase	Vinyl acetate 1–2 h, 25 °C	(*R*)-ester 98/>99	137
6	F, Ph	Porcine pancreatic lipase on Celite	Vinyl acetate in $^iPr_2O$, 3 Å molecular sieve	(*S*)-mono-ester 85/≥96	49
7	CF_2CH_2	Lipase PS	Vinyl acetate in C_6H_6 and $^iPr_2O$, 1.5 h	(*R*)-acetate 96.5/91	138
8	H, O-glucosyl perchloroacetate	*Candida antarctica* lipase	CF_3CH_2OCOR (R = C_4H_9 to $C_{10}H_{21}$), THF, 45 °C, 8 h	60–65% yield	139

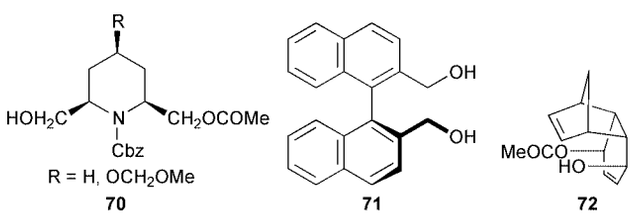
The research group of Gotor has made an extensive study of the enzyme-catalysed esterification of diols of the type **67** in connection with the synthesis of 1α,25-dihydroxyvitamin-D_2 and analogues. They have recently shown that the (3*R*,5*S*) compound is acylated regioselectively at the 5-position using *Candida antarctica* lipase and vinyl acetate in toluene and dioxane. The study was extended to cover the regioselective formation of a required carbamate.[130] The diester **68** was obtained (44%, >99% ee) from the racemic diol together with recovered starting material (47%, 98% ee).[131] When the polyol **69** was used as a substrate for lipase PS-catalysed acetylation reactions employing vinyl acetate as the acylation agent at 45 °C, it was found that esterification occurred initially at C-9 then at C-4 and then at C-8. The C-7 hydroxy group was the only unprotected position after 98 h.[132]



67 **68** **69**

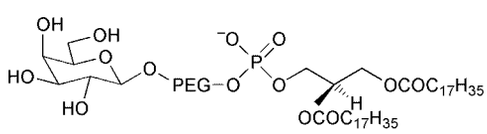
Some of the most spectacular successes involving enzyme-catalysed reactions include the transformations of *meso*- or prochiral substrates into optically active products. Compounds of the type $R^1R^2C(CH_2OH)_2$ are particularly good starting materials and some recently-recorded transformations are listed in Table 7. A noteworthy addendum to the example shown in entry 3 is that oddly (but not uniquely) hydrolysis of $H_5C_2CH(CH_2OCOCH_3)_2$ using the same *Pseudomonas* enzyme gives the same monoacetate (65%, 94% ee).

The esters **70** have been prepared in 80–83% yield (95–96% ee) using *Candida antarctica* lipase and vinyl acetate over 3–5 h at room temperature.[140] The optically active binaphthol derivative **71** is obtained by selective esterification of the racemic diol, (45% yield; >99%) using *Humicola* sp. lipase and vinyl hexanoate in *tert*-butyl methyl ether. The enantiomeric diol is available by selective esterification using *Serratia marcescens* lipase under the same conditions (41% yield; >99% ee).[141] The ester **72** has been prepared by esterification of the diol using lipase LIP (from *Pseudomonas* sp.) and vinyl acetate in THF–triethylamine over 3 h at room temperature (88% yield, >98% ee). The compound has been used to make conduritols A–F and other polyoxygenated cyclohexene derivatives.[142] The enantiomer of the ester **72** is available in 53% yield by employing lipase LIP to hydrolyse the *meso*-diacetate in 0.1 N phosphate buffer; this enantiomer has been used in the synthesis of (−)-epibatidine.[143] Monoesterification of cyclopent-2-ene-1,3-diol has been optimized to give (1*R*,3*S*)-3-acetoxycyclopent-2-en-1-ol in 64% yield (>98% ee)[144] building on earlier work by Theil.



70 **71** **72**

One example of enzyme-catalysed formation of a phosphate ester is the coupling of the C_6-OH silyl derivative of β-Gal-O-PEG-OH and distearoyl phosphatidylcholine which was accomplished in 50% yield using phospholipase-D in CCl_4–acetate buffer (pH 5.6) to give, after desilylation, the galactose derivative **73**.[145]



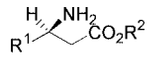
73

4 Preparation of amides

A review is available detailing the use of lipases for the synthesis of amides and carbamates.[146] Wong *et al.* have surveyed the types of acyl groups which can be used to protect amines using enzyme-catalysed reactions. The groups were chosen such that they could be removed readily to regenerate the free amine. The allyloxy carbonyl group (introduced using diallyl carbonate), the pent-4-enoyl group (introduced using allyl pent-4-enoate) and benzyloxycarbonyl (introduced using dibenzyl carbonate) were all prepared using *Candida antarctica* lipase in toluene.[147]

Primary amides such as butyramide and oleamide may be prepared by *Candida antarctica* lipase-catalysed amidation of the corresponding carboxylic acids using ammonium bicarbonate or carbamate in an organic solvent such as methyl *tert*-butyl ketone at 25 °C.[148] The dynamic kinetic resolution of phenylglycine esters has been accomplished using ammonia in *tert*-butyl alcohol containing *C. antarctica* lipase and pyridoxal (to racemise the ester *in situ*) at −20 °C. For example (*R*)-phenylglycinamide is prepared in 85% yield (88% ee) using this procedure.[149] Similarly the racemic hydroxyester $ClCH_2CH(OH)CH_2CO_2Et$ was resolved by enantioselective formation of the (*S*)-amide $ClCH_2CH(OH)CH_2CONH_2$ (*E ca.* 40) using

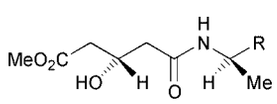
Table 8 Resolution of β-aminoesters $R^1CH(NH_2)CH_2CO_2R^2$

Entry	Substrate	Enzyme	Conditions	Product(s) yield (%)/ee (%) (or *E* value)	Reference
1	$R^1 = CH_3$; R^2 = Et, Bu	*Candida antarctica* lipase	$C_3H_7CO_2C_4H_9$	(*S*)-amino-ester (*R*)-amide (*E* ~ 78)	151
2	$R^1 = C{\equiv}CSiMe_3$; R^2 = Et	Penicillin amido-hydrolase	Phenylacetic acid, solvent	(*S*)-amine 45/96 (*R*)-amide 50 > 99	152
3	R^1 = aryl, Me, *E*-CH=CHMe; R^2 = Me, Et, ^{t}Bu	Penicillin G acylase	Phenylacetic acid, toluene or EtOCOMe with 2% H_2O 17–87 h	 + amide *E* 10 to >200	153

Candida antarctica lipase and dry ammonia in dichloromethane or THF.[150] The resolution of a variety of β-amino esters has been accomplished by enantioselective acylation of the amino group (Table 8). The reaction cited in entry 2 was used as the resolution process *en route* to an anti-platelet agent.

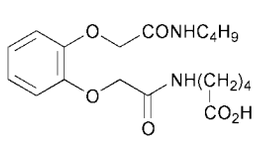
1-Arylprop-2-ynylamines ($ArCH(NH_2)C{\equiv}CH$) have been resolved using *Candida antarctica* lipase in ether containing ethyl acetate to afford, after 17–48 h at room temperature, the (*S*)-amine and the (*R*)-amide. Yields for isolated materials are 33–45% for each component. *E* values were uniformly high (310–420) except when Ar was *o*-tolyl when the *E* value dropped to 4.7.[154]

Excellent double enantioselection was observed when the racemic ester $ClCH_2CH(OH)CH_2CO_2Et$ and the racemic amines $MeCH(NH_2)R$ (R = phenyl, 2-furyl, n-C_5H_{11}) were coupled using *C. antarctica* lipase over 6–24 h. The (*S*)-enantiomer of the hydroxyester reacted preferentially with the (*R*)-enantiomer of the amine. Similarly the prochiral diester $CH(OH)(CH_2CO_2Me)_2$ reacted with the same amines to furnish the amides **74** in 96–98% de at 84–86% conversion.[155]



74

The amide **75** was obtained in 91% yield on treating the corresponding dibenzyl ester with butylamine in diisopropyl ether and toluene containing lipase P30 then switching the solvent system to buffer and acetonitrile. The oxygen atom adjacent to the ester group seems to aid the amidation reaction.[156] Racemic *trans*-1,2-diaminocyclohexane reacted with dimethyl malonate under the control of *Candida antarctica* lipase to give diamide **76** as described previously. Now the procedure has been employed to prepare hexaazamacrocycles.[157]



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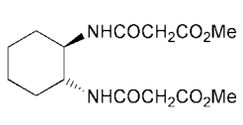
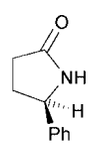


76

Several papers have focused on the use of proteases in peptide synthesis. ZTyrOEt has been coupled with GlyGlyOEt to give the protected tripeptide using chymotrypsin in mixed reverse micelles. This and similar reactions gave yields in the range 56–88% after optimisation.[158] Semicarbazones derived from four amino acid aldehydes (Phe, Ala, Leu, Val) were coupled with Z-AlaAlaOMe in 80–92% yield using subtilisin on macroporous silica in DMF–CH_3CN at 20 °C over 20 h.[159] The formation of peptides incorporating such units as H-Ala-TfmAla-AlaNH_2 (TfmAla is trifluoromethylalanine) can be catalysed by a variety of proteases such as trypsin and clostripain.[160] Subtilisin from *Bacillus lentus* has been modified by introduction of a cysteine residue and then converting the thiol group to the disulfide moiety -S-SCH_2Ar. These modified proteins have been shown to be good catalysts for peptide synthesis, accepting D-amino acid esters as acyl donors and α-branched amino acids as acyl acceptors.[161]

The lactam **77** was obtained (38% yield, >90% ee) on cyclisation of racemic ethyl 4-amino-4-phenylbutanoate with pig liver esterase at pH 9.[162]



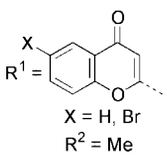
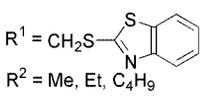
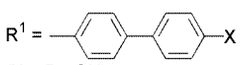
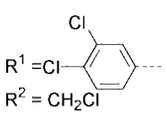
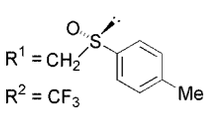
77

5 Reduction reactions

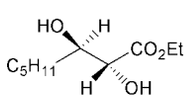
5.1 Reduction of ketones

It is well-established that ketones may be reduced to secondary alcohols by using whole cells or by employing isolated dehydrogenases (reductases) and the appropriate cofactor. In terms of whole cells, bakers' yeast is the most widely used organism mainly because of its ready availability. The variety of acyclic ketones reduced to optically active secondary alcohols using bakers' yeast (entries 1–8) and other microorganisms is depicted in Table 9. It is noteworthy that while bakers' yeast catalyses the reduction of the ketone $MeCOCH_2CH(OMe)_2$ to the (*S*)-alcohol (entry 1), the yeast *Yamadazyma farinosa* produces the (*R*)-alcohol (50% yield, >98% ee) from the same substrate. Entry 2 describes an unusual (but not unique) case wherein a substituent remote from the carbonyl moiety causes a change in the absolute configuration of the product. The authors ascribed this effect to the actions of different dehydrogenases present in the yeast. The same effect is seen for substrates of the type $R^3OCH_2COCH_2OR^4$ as reported in entry 3. Inclusion of the thiasulfone additive shown in entry 5 increases the optical purity of the alcohol produced; the effect is believed to be due to modification of thiol groups in participating enzymes by formation of disulfides. A similar enhancement of enantiomeric excess is seen on addition of allyl alcohol for the examples illustrated in entry 6. In entry 7 *Candida sorbophila* was shown to out-perform bakers' yeast in producing an optically active intermediate to a new β_3-adrenergic receptor antagonist. It should be noted that acylsilanes are good substrates for bakers' yeast reduction, yielding optically active α-hydroxysilanes (entry 8). The use of microorganisms in organic solvents continues to be explored (entry 9). Following a survey by Molinari *et al.*, lyophilised yeasts are recommended as easy-to use catalysts for the asymmetric reduction of ketones. Seven species of yeast were included in this survey and good enantioselectivity was observed even for aryl ketones. The desired enantiomer of a particular secondary alcohol can often be obtained by the appropriate choice of yeast.[174] Reduction of (racemic) ethyl 2-hydroxy-3-oxooctanoate using immobilised

Table 9 Reduction of acyclic ketones R^1COR^2 using bakers' yeast unless otherwise stated

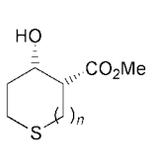
Entry	Substrate R^1, R^2	Conditions	Product(s) yield (%)/ee (%)	Reference
1	$R^1 = CH_2CH(OMe)_2$ R^2 = Me	H_2O	(*S*)-alcohol 75/≥98	163
2	 X = H, Br R^2 = Me	H_2O	X = H, (*R*)-alcohol 87/≥98 X = Br, (*S*)-alcohol 53/≥98	164
3	$R^1 = CH_2OR^3$ (R^3 = benzoyl, pivaloyl, benzyl) $R^2 = CH_2OR^4$ (R^4 = H, COMe)	H_2O, EtOH, glucose	R^4 = H, (*S*)-alcohol 50–80/55–>97 R^4 = COMe 54–60/68–>95	165
4	 R^2 = Me, Et, C_4H_9	H_2O, glucose, 37 °C, 3 h to 10 d	(*R*)-alcohol (80–91/74–>99)	166
5	 (X = Br, OH) $R^2 = CF_3$	H_2O, $MeSO_2SCH_2{}^cC_6H_{11}$	alcohol (≥90% ee)	167
6	$R^1 = CH_2CO_2Et$ R^2 = Me, Et or Cl	H_2O, CH_2=$CHCH_2OH$	(*R*)-alcohol (82–99% ee)	168
7	R^1 = 3-pyridyl $R^2 = CH_2NHCOPNB$ (PNB is *p*-nitrobenzyl)	bakers' yeast, H_2O *or* *Candida sorbophila*	(*S*)-alcohol 70–80% ee 75/>99.5	169
8	R^1 = Ph, 4-Cl-C_6H_4, 2,3 or 4-MeO-C_6H_4 $R^2 = SiMe_3$	H_2O containing 2% KCl, sucrose, Montmorillite, bakers' yeast	(*S*)-alcohol (45–70/44–88) except for R^1 = 2-MeO-C_6H_4 when (*R*)-alcohol formed (20/79)	170
9	R^1 = Ph, 2-furyl, 3-Cl-C_6H_4, 3- or 4-Me-C_6H_4, 4-MeO-C_6H_4 R^2 = Me	*Geotrichum candidum* on a water-absorbing polymer in hexane containing hexan-2-ol or cyclopentanol	(*S*)-alcohol (50–73/>95)	171
10		*Geotrichum candidum*, H_2O, resin XAD 1180	(*S*)-alcohol (95/98.5)	172
11		*Yamadazyma farinosa*, H_2O 2 d, 30 °C	(*R*)-alcohol (70/>99) 96% de	173

bakers' yeast at pH 4.0 gave a 70% yield of *syn*- and *anti*-diols with the latter predominating (70% de, 80% ee). Pure *anti*-diol **78** was obtained in 52% yield (as the acetonide).[175]



78

Whole cell reductions of cyclic ketones have been explored further. For example it has been found that yields and/or enantiomeric excesses of the hydroxy esters **79** can be improved by conducting bakers' yeast reduction of the corresponding ketones in the presence of L-cysteine or dimethyl sulfoxide. For $n = 0$, the yields increase by 15–19% while a high ee ≥ 96% is maintained; for $n = 1$, addition of DMSO improved the yield and ee by 4% and 7%, to 83% and 99% respectively.[176] Similarly the ketone **80** is transformed into the *syn*-diol in *ca.* 70% yield (>99% ee) on reduction using bakers' yeast in the presence of DMSO. The *syn*-diol was transformed into (−)-serricornin, a sex pheromone of the cigarette beetle.[177]



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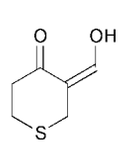
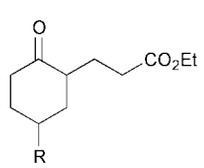


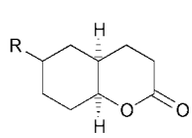
80

The ketoesters **81** have been converted into the lactones **82** (99% ee; 98% de at 50–80% conversion) using bakers' yeast preincubated at 50 °C for 30 min to denature thermolabile dehydrogenases.[178]



81

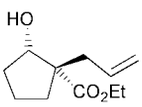
R = H or ^{t}Bu



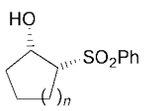
82

The products resulting from bakers' yeast reduction of ethyl 1-allyl-2-oxocyclopentane-*cis*-carboxylate in water containing copper(II) oxide have been reassigned. The alcohol **83** is formed in 40% yield while (1*R*)-ketone was recovered (39%). While enantiomeric excesses of the products were not stipulated in this paper conversion of the alcohol **83** into a known optically active compound gave a good correlation of the $[\alpha]_D$ values.[179] Dynamic kinetic resolution of the appropriate ketosulfones by bakers' yeast reduction furnished the hydroxysulfones **84** in 78–95% yield, excellent enantiomeric excesses (>95%) and high diastereoisomeric ratios. The reduction of the 7- and 8-membered ring analogues was much less efficient.[180] The stereochemistry of the major product resulting from the reduction of the ketoamides **85** with *Mortierella isabellina* depends

upon the group R. For **85**, R = H the major product is (1*S*,2*S*)-hydroxycyclopentane-1-carboxamide (51% yield; >99% ee). For **85**, R = benzyl the (2*S*,1*R*)-amide is formed (85% yield, >99% ee).[181] In studies aimed at the synthesis of the paclitaxel C-13 side chain, the hydroxylactam **86** was obtained on bakers' yeast reduction of the corresponding ketolactam (48% yield; 55% ee). Another commercially available yeast, strain INVSc1, gave the same product with an improved ee (82%) in 41% yield at about 50% conversion.[182] The hydroxyester **87** is obtained from the corresponding tricarbonyl compound (88% yield, 99% ee) using non-fermenting bakers' yeast in tap water. The enantiomer was obtained using lipase-promoted esterification of racemic substrate **87**. The optically active piperidinone derivatives were used to prepare a variety of alkaloids, for example prosafrinine.[183]



83



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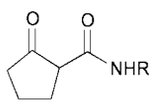

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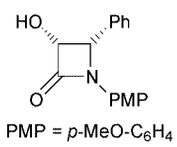
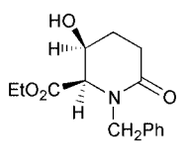


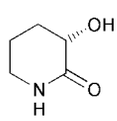
PMP = *p*-MeO-C_6H_4

86



87

A comparison has been made between two methods for the reduction of acetophenone to the (*S*)-alcohol, namely the carbonyl reductase from *Candida parapsilosis* with *Candida boidinii* formate dehydrogenase on the one hand and Corey's non-natural oxazaborolidine system on the other. The investigators concluded that the non-natural process was superior in terms of catalyst availability and space–time yield; the enzyme-based process was advantageous in terms of catalyst consumption, turnover number and enantiomeric excess of product.[184] The same reductase–dehydrogenase tandem enzyme system has been shown to reduce methyl 2-naphthyl ketone and 4-phenylbut-3-en-2-one to the corresponding (*S*)-alcohols in good yield (70–80%) and very high enantiomeric excess (>99%).[185] Know-how from earlier work on the reduction of α-ketoacids using lactate dehydrogenases ((*R*)- and (*S*)-directing) has now been used to gain access to (*R*)- and (*S*)-2-hydroxy-5-nitropentanoic acid *en route* to δ-lactams of type **88**.[186]

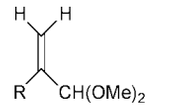


88

5.2 Miscellaneous reduction reactions

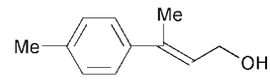
The asymmetric reduction of geminal and tri-substituted alkenes is still an under-developed area within the field of biotransformations. The recent work of the Santaniello and Fuganti teams illustrates the considerable potential. The unsaturated acetal **89** is reduced to (*R*)-2-substituted propan-l-ols (60–83% yield; >98% ee) using bakers' yeast over 14 days, during which time the acetal hydrolyses, releasing the enal slowly but surely into the fermentation broth.[187] Bakers' yeast reduction of the unsaturated alcohol **90** afforded (*S*)-3-*p*-tolylbutan-l-ol (21% yield; >95% ee) useful for the synthesis of the bisabolane sesquiterpenes curcumene, turmerone and nuciferal.[188]

A key intermediate for the synthesis of a new vasopeptidase inhibitor is (2*S*)-2-amino-6-hydroxyhexanoic acid. This compound was prepared from the corresponding 2-ketoacid using glutamate dehydrogenase from beef heart.[189]



R = Ph, CH_2Ph, CH_2OCH_2Ph

89

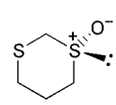


90

6 Oxidation reactions

6.1 Baeyer–Villiger and heteroatom oxidations

Detailed protocols have been published for the ring-expansion of 2-, 3-, and 4-substituted cyclohexanones (as well as 2-substituted cyclopentanones) to the corresponding lactones using "designer yeasts" incorporating a monooxygenase gene from another species.[190] Similarly recombinant strains of *Escherichia coli* and bakers' yeast expressing cyclohexanone monooxygenase from *Acinetobacter* NCIMB 9871 have been used as whole cell biocatalysts for the oxidation of selected sulfides, dithianes and dithiolanes. Interference from host cell enzymes for the thioether substrate can be problematic, nevertheless some impressive transformations were observed, furnishing, for example, the monosulfoxides **91** (73% yield; 84% ee) and **92** (46% yield; 69% ee).[191]



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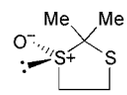
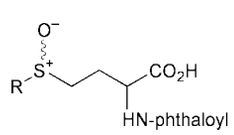


92

Given the interest in the use of chiral sulfoxides for the asymmetric synthesis of target compounds[192] it is not surprising that other whole cell systems and isolated enzymes have continued to be investigated for the stereoselective oxidation of sulfides. *Beauvaria* sp. converts the corresponding sulfides into the sulfoxides **93** (66–98% yield; 60–92% de),[193] while the vanadium bromoperoxidase from *Ascophyll nodosum* was found to oxidize a broad range of sulfides to the corresponding sulfoxides. For example, methyl 2,4-xylyl sulfide formed the (*R*)-sulfoxide in 63% ee at 98% conversion.[194] A readily available chloroperoxidase is effective in producing sulfoxides of the type **94** from the corresponding β-carbonyl sulfides, often in high yield and excellent enantiomeric excess.[195] The peroxidase from *Coprinus cinereus* will transform a mixture of a peroxide $R^1CH(OOH)R^2$ and a selected sulfide into the corresponding optically active secondary alcohol and optically active sulfoxide. For example methyl 2-naphthyl sulfide gives 89% ee sulfoxide at 53% conversion using this protocol.[196] Mutants of myoglobin (produced by application of error-prone polymerase chain reactions) improve the rate and stereoselectivity of peroxide oxidation of sulfides over the results obtained using the native protein. For example thioanisole produces the (*R*)-sulfoxide in 97% ee. The mutants can also be used in stereoselective oxidations of styrene.[197]



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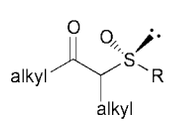
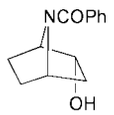


94

6.2 Hydroxylation reactions

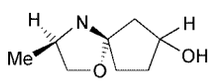
N-Benzylpyrrolidine is converted into the (3*R*)-hydroxy derivative (62% yield; 70% ee) using the three-component alkane hydroxylase system of *Pseudomonas oleovorans*. Further screening of other alkane degrading systems furnished a micro-organism that produced (*S*)-*N*-benzyl-3-hydroxypyrrolidine in 53% ee.[198]

Olivo and Hemenway have shown that *N*-benzoyl-7-azabicyclo[2.2.1]heptane is transformed by *Beauvaria bassiana* into the 2-hydroxy compound **95** (56% yield; 22% ee). The product was used to make epibatidine.[199] 7-Azabicyclo[2.2.1]heptanoylphosphonic acid diesters are transformed by the same organism with the same regioselectivity (37–43% yield; 64–77% ee) but the corresponding diphenylphosphinoyl-7-azabicycle gave a lower yield and ee.[200]

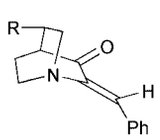


95

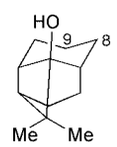
Griengl and co-workers have developed their idea of temporarily introducing a stereodirecting group into a molecule to enhance the predictability of biohydroxylations using *Beauvaria bassiana* and other microorganisms. Thus, while cyclopentanone is not readily hydroxylated using *B. bassiana*, simple derivatization of the ketone group and biohydroxylation furnishes the bicyclic compound **96** (90% de). Benzylation of **96** and deprotection of the carbonyl group afforded 3-benzyloxycyclopentanone in 30% overall yield and 84% ee.[201] On incubation with *Streptomyces griseus* the 2-substituted 1-azabicyclooctan-3-one **97** furnished the hydroxylated derivative **98** (33% yield; 84% ee). Biotransformation using *Cunninghamella echinulata* gave the same quantity of the alcohol (30% ee) and a slightly smaller quantity of a dihydroxylated derivative.[202] *Botrytis cinerea* has been shown to hydroxylate the antifungal ginsenol **99** at the 8- and 9-positions primarily.[203]



96



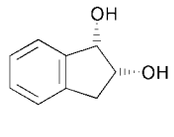
97 R = H
98 R = OH



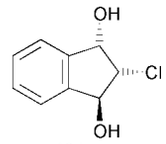
99

2-Substituted indanes may be oxidized stereoselectively in the benzylic position(s) using toluene dioxygenases. For example 2-hydroxyindane furnished the diol **100** in 35% yield (>98% ee) while 2-chloroindane afforded the halodiol **101** (38% yield; >98% ee).[204] Eugenol dehydrogenase from *Pseudomonas fluorescens* (purified and characterised previously) is also useful for stereocontrolled hydroxylation at a benzylic position. Thus after transformation in pH 7 buffer containing phenazine methosulfate, *p*-ethylphenol and *p*-propylphenol gave (*S*)-*p*-hydroxyphenyl-ethanol and -propanol respectively (63, 66% yield; 95, 97% ee).[205] Chloroperoxidase in combination with peroxide or *tert*-butyl hydroperoxide in pH 5.5 buffer over 2 h produces (*R*)-propargylic alcohols $H_3C–C{\equiv}C–CH(OH)R$ (where R = C_2H_5, CH_2OAc, CH_2Br, CH_2CH_2Br) by hydroxylation of the short, unbranched carbon chains incorporating an alkyne unit (yields 26–65%; ee's 91–95%).[206] Microbial proline 4-hydroxylase and proline 3-hydroxylase have been overexpressed in *E. coli*, and used to oxidize L-azetidine-2-carboxylic acid and L-pipecolic acid. The 3-hydroxylase introduced an hydroxy group adjacent and *cis* to the carboxy group. The 4-hydroxylase did not catalyse the oxidation of the four-membered ring system but converted L-pipecolic acid to (5*R*)-hydroxy-L-pipecolic acid. Furthermore, with 3,4-dehydro-L-proline as the substrate, the 3-hydroxylase gave the *cis*-epoxide while the 4-hydroxylase furnished the *trans*-epoxide.[207]

Cytochrome P450 from *Bacillus megaterium* has been shown to be capable of hydroxylation of long-chain hydroxy acids. Hydroxylation of (12*R*)-hydroxymyristic acid gave (12*S*,13*S*)-dihydroxymyristic acid (64%) with a smaller amount (16%) of the (12*S*,13*R*)-compound. Interestingly (12*S*)-hydroxymyristic

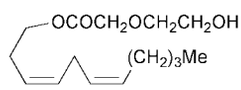


100



101

acid gave the (12*R*,13*R*)-dihydroxy compound as the major product (50%) and the (12*R*,13*S*)-compound in 13% yield. In complementary fashion (13*R*)-hydroxymyristic acid gave (12*S*,13*R*)-dihydroxymyristic acid in 72% yield together with 13% of the (12*R*,13*R*)-diastereoisomer. For these biotransformations NADP was used as the co-factor, together with glucose-6-phosphate and glucose 6-phosphate dehydrogenase in pH 8 buffer over 1 h.[208] It has been demonstrated that soyabean lipoxygenase in oxygenated borate pH 8.5 buffer at 0 °C can form peroxides from skipped dienes that do not contain a polar head-group. For example the ester **102** is converted into the peroxide **103** in 73% yield (98% ee). The peroxide moiety was reduced to the hydroxy group and the ester group was removed to allow full characterisation of the product.[209]



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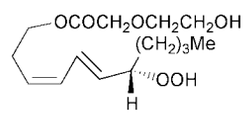
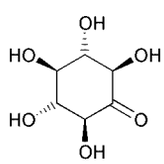


103

6.3 Oxidation of alcohols

Both whole cells and isolated enzymes have been employed to convert alcohols into carbonyl compounds. Cholic acid and chenodeoxycholic acid have been oxidized to the corresponding 7-oxo compounds in *ca*. 80% yield.[210] *myo*-Inositol was oxidized to *myo*-2-inosose **104** using *Gluconobacter oxydans* in 95% yield, as part of a two-microorganism pathway used to transform glucose into 1,2,3,4-tetrahydroxybenzene.[211] Shikimate dehydrogenase in pH 10.6 buffer containing $NADP^+$ converts shikimic acid into the ketone **105** in 85% yield over 15 min.[212] As demonstrated by Jones some years ago *meso*-1,2-bis-(hydroxymethyl) in cyclobut-3-ene is converted into optically active lactone in 53% yield (>99% ee) using horse liver alcohol dehydrogenase, NAD^+ and FMN in pH 9 buffer at 25 °C over 4 days.[213] Interesting enantioselective oxidations of racemic glycidol and *cis*-2,3-epoxyhexanol have been accomplished using chloroperoxidase in organic solvent–buffer mixtures. The reactions were conducted at 5 °C and at pHs between 5.0 and 6.5 whereupon residual alcohols with ee values >95% were obtained after 48–54 h and 50–54% conversion.[214]



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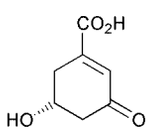


105

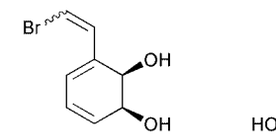
Galactose oxidase from *Fusarium* sp. is known to convert a wide range of primary alcohols to the corresponding aldehydes. Now an electrochemical method for regeneration of the oxidase has been invented.[215]

The conversion of racemic 2-phenylpropanol into (2*S*)-phenylpropanoic acid using *Gluconobacter oxydans* has been optimised so as to give the product in 37% yield and >98% ee.[216]

6.4 Dihydroxylation

A review on the enzymatic dihydroxylation of aromatic compounds and applications to organic synthesis has appeared,[217] and the methodology of the fermentation processes has been detailed.[218] Novel biotransformations of this type include the

preparation of the diol **106**,[219] the diols **107** and the tetraols **108**.[220] The latter transformations were accomplished using *Sphingomonas yanoikuyae*. Meanwhile the chiral diols continue to be excellent sources of starting materials for synthetic endeavours. The (1*S*,2*S*)-3-halo-1,2-dihydroxycyclohexa-3,5-dienes have been used to prepare D-aldopentoses,[221] steroid analogues[222] and have been employed in solid phase combinatorial work.[223] (1*R*,2*S*)-3-Iodo-6-bromo-1,2-dihydroxycyclohexa-3,5-diene has been used to make *ent*-7-deoxypancrastistatin (which retains some of the anti-cancer activity of its mirror image),[224] while (1*S*,2*S*)-3,5-dibromo-1,2-dihydroxycyclohexa-3,5-diene was employed in a short synthesis of narciclasine, a synthesis which also included a catalogue of named reactions (Suzuki, Mitsunobu, Diels–Alder, Bischler–Napieralski).[225] The product from toluene dihydroxylation has been acetylated and subjected to osmylation to gain access to the cycloalkene **109** in 70% overall yield.[226] d_6-Cyclohexane-3,5-diene-1,2-diol has been converted into the fully deuteriated *anti-ortho*,*ortho'*-benzene dimer,[227] while the diol available from naphthalene in one step has been transformed into conduramine and conduritol analogues.[228]



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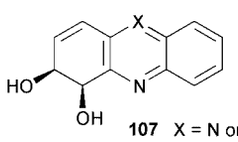
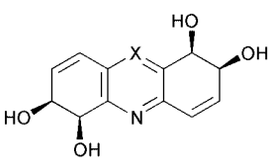
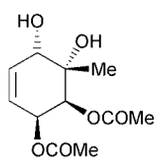


107 X = N or CH



108 X = N or CH



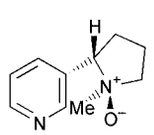
109

6.5 Miscellaneous oxidations

A review on the biocatalytic preparation of optically active α-oxy-functionalised carbonyl compounds has been compiled by Adam and co-workers.[229] Palmitic acid was converted into (2*R*)-hydroperoxypalmitic acid (99% ee, yield not stated) using a crude enzyme from the marine green alga *Ultra pertusa*. C_{15} and C_{14} fatty acids were also substrates for the enzyme-catalysed oxidation.[230]

Chloroperoxidase with *tert*-butyl hydroperoxide in pH 5.5 buffer catalyses the epoxidation of *Z*-alkenes of the type $MeCH{=}CH\text{-}(CH_2)_n\text{–}X$ when n = 1–3 and X = OAc,Br or CO_2Et. Yields were mainly in the range 50–95% with good enantiomeric excesses for the products (90–96%) except for the compound where n = 2, X = CO_2Et for which a poor conversion and low ee of product was observed.[231] The enzymatic preparation of epoxides has been reviewed.[232]

The first asymmetric oxidations of tertiary amines using cyclohexanone monooxygenase from *Acinetobacter calcoaceticus* have been recorded. The enzyme, in combination with NADPH, glucose-6-phosphate and glucose-6-phosphate dehydrogenase catalysed the transformation of *N*-methyl *N*-substituted benzylamines into *N*-oxides possessing about 30% ee at 60–90% conversion. The *N*-oxide **110** was formed from the corresponding *N*-methylpyrrolidine in >99% de.[233] The same enzyme and cofactor have been shown to oxidize cyclic sulfites to sulfates in enantioselective fashion.[234]



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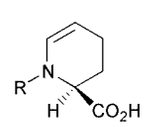


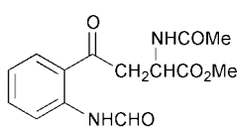
R = Boc or Cbz

111

The apoprotein from "old yellow enzyme" isolated from yeast has been obtained as described previously and reconstituted with 8-cyano-8-demethylriboflavin. This change transformed the enzyme from a reductase to a desaturase. The new catalyst was able to convert dihydrocinnamaldehyde, cyclopentanone and other saturated carbonyl compounds into the corresponding enones.[235] The compounds **111** are produced from the corresponding lysine on treatment with *Rhodotorula graminis*, followed by removal of the cells, and treatment with acid to effect ring closure and dehydration. The yield is approximately 40% in both cases.[236] The ability of mushroom (*Agaricus bisporus*) tyrosinase to oxidize various hydrazides RCONHNHPh to labile acyldiazenes RCON=NPh means the technique may be useful for the construction of acid- and base-labile peptide conjugates such as lipo-, glyco-, phospho-, and nucleo-peptides: for example the yield of acid from BocVal-AlaCONHNHPh was 90% after 16 h.[237]

Oxidative coupling of furulic acid derivatives containing a chiral sultam or oxazolidinone unit has been catalysed by horseradish peroxidase in acetone or dioxane–pH 3.5 buffer mixtures containing hydrogen peroxide. However yields and de's showed little difference to those obtained from silver(I) oxide-promoted reactions.[238] Horseradish peroxidase and hydrogen peroxide in the presence of sulfonated polystyrene was used to form polyaniline with minimal branching, most suitable for its role as a conducting polymer.[239] A mixed culture of *Klebsiella* and *Rhodococcus* species, isolated from soil by the enrichment culture technique (employing caffeine as the carbon source), efficiently promoted C-8 oxidation of adenine and various 1-, 3- and 7-substituted xanthines. After 12 h at 30 °C on a rotary shaker the uric acid derivatives were isolated in very high yield.[240]

Catalase I from *Bacillus stearothermophilus* has been shown to behave as a dioxygenase in cleaving the heterocyclic ring of methyl L-*N*-acetyltryptophanate to give the ketone **112** in 34% yield.[241] *Crotalus adamanteus* (rattlesnake) L-amino acid oxidase has been used to purify (2*S*,3*R*)-2-amino-3-methylpent-4-enoic acid from a mixture containing the (2*S*,3*S*)-diastereomer. The latter isomer is oxidized preferentially.[242] Coupling glucose dehydrogenase and the hydrogenase from *Alcaligenes eutrophus* (through NAD^+/NADH) gives a system for the production of hydrogen. The optimum pH for the dual enzyme system was 6.5 whereupon approximately 8 μmol of hydrogen was liberated over a 5 h period.[243]



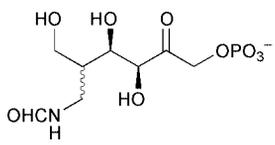
112

7 Carbon–carbon bond formation

7.1 Aldol reactions

The aldehyde $HOCH_2CH(CHO)CH_2NHCHO$ has been coupled to dihydroxyacetone phosphate (DHAP) using rabbit muscle aldolase (RAMA) to form the triol **113** which was converted over several steps into homoisofagomins, potent inhibitors of β-glucosidase from almonds.[244] Note that a new method for the preparation of dihydroxyacetone phosphate dimethyl acetal (a stable, storable precursor of DHAP) has been reported.[245] Mannose derivatives have been coupled to DHAP using DHAP-dependent aldolases in yields of about 30%, to provide selectin inhibitors.[246] DHAP reacts with butanal under catalysis by fructose-1,6-bisphosphate aldolase from *Staphylococcus carnosus* to form (after dephosphorylation using wheat-germ phosphatase and acetylation) the triester **114** in approximately 30% overall yield.[247] This aldolase from *S. carnosus* has been over-expressed in *E. coli* and is mooted to

be competitive with the better known (but less readily available) RAMA. The microbial enzyme is better able to transform glutardialdehyde and DHAP into novel bicyclic sugars.[248] The same enzyme has been used to prepare 4-deoxy KDO and analogues by catalysing the coupling of DHAP and aldehydes such as $HO_2CC(OMe)_2(CH_2)_2CHO$. The enzyme-catalysed reaction shows good stereocontrol at the new stereogenic centres (C-5 and C-6) such that, after dephosphorylation, the triol **115** was isolated in 15% yield.[249] Furthermore fructose-1,6-bisphosphate aldolase has been used as the C–C bond-forming catalyst in a four-enzyme, one-pot procedure to convert glycerol (*via* glycerol-3-phosphate and DHAP) and butanal into 5-deoxy-5-ethylxylulose in 57% yield from L-glycerol-3-phosphate.[250]



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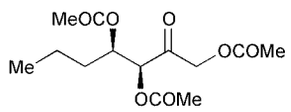

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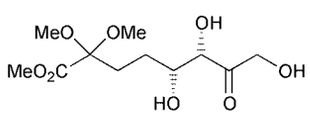


115

2-Deoxyribose-5-phosphate aldolase catalysed the coupling of ethanal and pyruvaldehyde to give D-2,5-dideoxyribose,[251] while transketolase-A has been used as a key enzyme in a one-pot procedure for converting glyceraldehyde-3-phosphate and hydroxypyruvate into D-xylulose-5-phosphate in 82% yield on a 1 g scale.[252]

7.2 Preparation of cyanohydrins

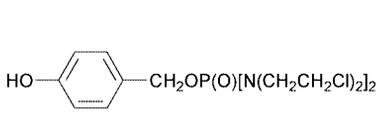
Two reviews have been published on the use of oxynitrilases in asymmetric synthesis.[253] Protected hydroxyaldehydes of the type RCH_2OCH_2CHO have been converted into the (*R*)-cyanohydrins using the oxynitrilase from almonds. A good enantiomeric excess is obtained when R is allyl (but not when R is benzyl[254]). The (*S*)-cyanohydrin is available, albeit in lower optical yield, using the oxynitrilase from *Manihot esculenta*. Alkyl substituted compounds CH_2=$CHCH_2OCH(alkyl)CHO$ behave similarly.[255] Almond oxynitrilase converts the bromoaldehydes $BrCH_2(CH_2)_nCH_2CHO$ (n = 3–5) into the corresponding (*R*)-cyanohydrins (40–65% yield; 90–97% ee) *en route* to optically active azacycloalkanols, for example (*R*)-piperidin-3-ol.[256] Bicyclo[3.2.0]hept-2-en-6-one underwent enantioselective addition of HCN to give the (+)-cyanohydrin, providing one of the first examples of a complex ketone giving optically active product using almond oxynitrilase as the catalyst.[257] As an alternative to enzyme-based methodology, a bimetallic chiral (salen) titanium complex has been shown to catalyse addition of Me_3SiCN to acetophenone, ethyl phenyl ketone and analogues giving products in yields 32–100% and ee's 32–72%.[258]

7.3 Other carbon–carbon bond forming reactions

An asymmetric acyloin reaction is catalysed using partially purified phenylpyruvate decarboxylase from *Achromobacter eurydine*. For example, phenyl pyruvate and ethanal react together to form (3*R*)-hydroxy-1-phenylbutan-2-one in 45% yield (91% ee).[259] Benzoyl formate decarboxylase from *Pseudomonas putida* has been cloned in *E. coli* and used in the dimerization of aromatic aldehydes. Benzaldehyde and pyridine-2-carbaldehyde afford the corresponding (*R*)-hydroxyketones ArCOCH(OH)Ar in 70% yield and 94–99% ee. Other aryl aldehydes give lower yields of products.[260] Selenolo[3,2-*b*]pyrrole and furo[3,2-*b*]pyrrole have been coupled with L-serine through the 3-position of the pyrrole ring to give tryptophan analogues in 35–50% yield using tryptophan synthase from *Salmonella typhimurium* in pH 7.8 buffer at 37 °C.[261]

8 Carbohydrate chemistry

E. coli β-galactosidase hydrolyses galactose from 4-(β-D-galactopyranosyl)benzyl *N*,*N*,*N*′,*N*′-tetrakis(2-chloroethyl)-phosphorodiamidate to give the unstable phenol **116** which spontaneously collapses to produce the alkylating antitumour agent tetrakis(2-chloroethyl)phosphorodiamidic acid, in studies concerned with gene-directed enzyme prodrug therapy.[262] Generally, though, glycosidases are employed to couple carbohydrates to other units *via* the anomeric position. For example a range of β-glucosides **117** have been prepared (22–36% yield) from the corresponding alcohol (ROH) and *p*-nitrophenyl (PNB)-β-glucopyranoside using β-glucosidase from almonds in pH 5 buffer at 30 °C over 3–26 h.[263] PNB-glycosides are often used as glycosyl donors but it is noteworthy that nitropyridyl glycosides have been recommended as effective alternatives for trans-glycosylation reactions catalysed by β-glucosidase, β-galactosidase and *N*-acetyl-β-hexosaminidase from *A. oryzae*.[264] Ten natural and non-natural β-PNP-galactose derivatives **118** were coupled to α-D-galactopyranoside using snail acetone powder to give 1–4 coupled products (15–37% yield) and using β-amylase from barley to give 1–6 coupled products (25–44% yields, mainly).[265] α-D-Galactosidase from *Penicillium multicolor* has been used in an easy, inexpensive method for the preparation of α-galactosyl epitopes **119** (yields 43–46%) which are important for studies connected with xenotransplantation and toxin binding.[266] Improvements can sometimes be made in the preparations of α- and β-disaccharides using α- or β-galactosidase by using *in situ* NMR spectroscopy to follow the progress of the reaction.[267] The α-galactosidase from *Talaromyces flavus* has been used to prepare trisaccharides **120** (yields 20–30%) by coupling galactose on to the acylated disaccharide.[268] β-Mannosidases from *Helix pomatia* (the edible snail) and *Aspergillus oryzae* (as well as the *N*-acetylhexosaminidase from the same organism) have been isolated and used to couple PNP-β-D-mannopyranoside and *N*,*N*′-diacetylchitobiose to give the core trisaccharide of *N*-linked glycoproteins **121** in 3% and 26% yield respectively.[269] A library of thermophilic glycosidases has been screened to find an enzyme (coded Gly001-09) which catalysed the coupling of glucosamine and lactose to give lactosamine in 23% yield after reaction in pH 6.0 buffer at 85 °C over 3 days. The same enzyme coupled galactose to the appropriate trisaccharide to produce the tetrasaccharide **122** regioselectively in 11% yield.[270] The CLONENZYME thermophilic glycosidase library has been used to select enzymes capable of catalysing efficient trans-glycosylations to prepare β(1→4), β(1→6) and α(1→6) galactobiosides with high regioselectivity and in moderate to high yields.[271]



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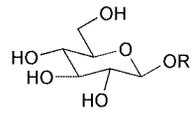
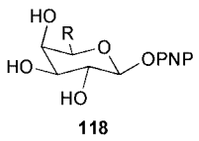


117

R = $CH_2CH{=}CMe_2$, CH_2Ar
$CH_2CH{=}CHPh$, $CH_2CH(OBn)CH_2OH$

A review of glycosyl transferase-catalysed synthesis of non-natural oligosaccharides has been published.[272] *n*-Butyl-2-amino- 2-deoxy-β-D-glucopyranoside has been prepared by direct transglycosylation of chitosan and butanol using resting cells of *Penicillium funiculosum* as the source of *exo*-β-D-glucosaminidase. The product was obtained to the extent of 210 mg per gram of chitosan.[273] The hexapeptide derivative **123**



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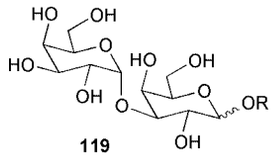
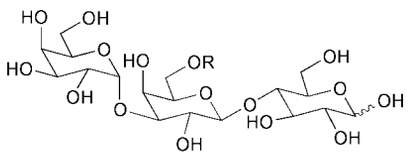


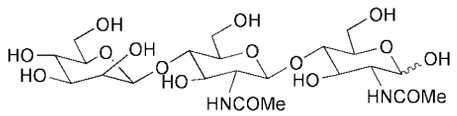
119

R = *inter alia*, Me, CH_2F, Et, C≡CH, CH=CH_2 CH(OH)Me



120

R = COMe, COEt, CO^nPr



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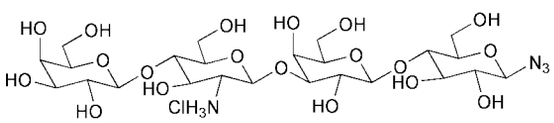
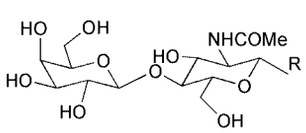


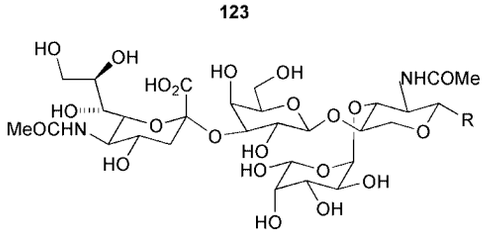
122

provides an excellent substrate for sequential coupling with sialylic acid (catalysed by α(2[1]→3) sialyltransferase) and fucose (catalysed by α(1→3) fucosyl transferase) to afford a hetero-bifunctional glycopeptide **124** in over 40% yield. The glycopeptide was shown to bind P-selectin and human integrin β1 concurrently.[274] A review on the synthesis of complex *N*-glycopeptides, including enzymatic methodology, is available.[275]



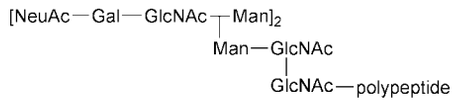
R = $O(CH_2)_3CONH$–HKGRGDS–OH

123



124

Natural oligosaccharides have been appended to glycoprotein containing *N*-acetylglucosamine by a transglycosylation reaction using *endo*-β-*N*-acetylglucosaminidase from *Mucor hiemalis* to yield eel calcitonin glycopeptide analogues **125**.[276] A mercury-containing sialic acid derivative has been used as a substrate for α-2,3-sialyltransferase to give oligosaccharide and glycoprotein species, for example a sialyl-Lewis-x derivative with a covalently bound mercury atom in the sialyl residue **126**.[277] Xylanase from *Aureobasidium pullulans* with octanol in supercritical CO_2 converts xylan into *n*-octyl-β-D-xylotrioside and xylobioside in 15% and 52% yield respectively.[278] Davis and Jones have reviewed site-selective



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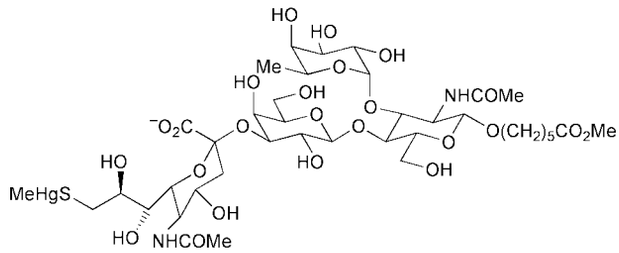


126

glycosylation of proteins, tailoring enzymes to develop novel catalysts of potential use in organic synthesis.[279]

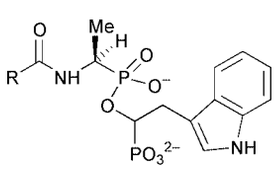
9 Enzyme mimetics

9.1 Catalytic antibodies

Two useful critical reviews on organic synthesis supported by antibody catalysis have been published.[280]

9.1.1 Antibodies catalysing hydrolysis reactions

Antibody 12F12 raised to the hapten **127** catalysed the hydrolysis of (*S*,*R*)-diastereoisomer in the mixture **128**. Note that the analogous compounds containing an (*R*)-alanine residue were not hydrolysed.[281] Similarly a catalytic antibody raised to the hapten **129** hydrolysed the C_2-ester of the phospholipid **130** with a two-fold preference for the (*R*)- over the (*S*)-enantiomer.[282] A comparison has been made for the catalysis of substrate **131** between five antibodies classically raised to a transition state analogue and five antibodies produced by the newer technique of reactive immunisation. The transition state analogue approach gave better catalysts in terms of turnover number and enantiomer discrimination. Reactive immunisation elicited biocatalysts which are more proficient because they have improved substrate recognition and did not suffer from product inhibition.[283]



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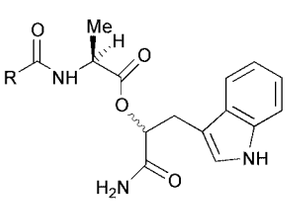

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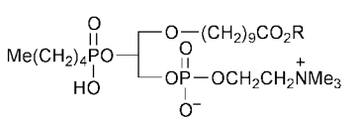

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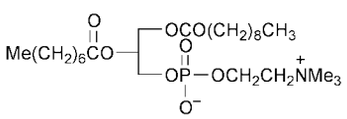

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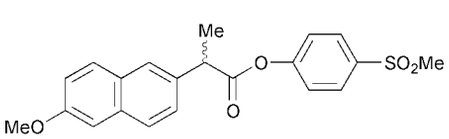
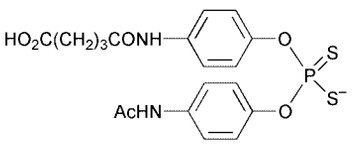


131

A transition state analogue (TSA) mimicking cholesterol ester hydrolysis has been synthesised and used to pick out 4 mg of a 131-mer from a DNA library. 1 mg of this oligonucleotide was used to perform a polymerase chain reaction to produce the initial RNA library. After six cycles of selection the TSA-binding RNA was cloned. Such a clone enhanced the rate of hydrolysis of *p*-nitrophenol carbonate ester of cholesterol by 110 fold.[284] The novel hapten **132** elicited antibodies that enhanced the rate of hydrolysis of aryl carbonates of the type **133** by over four orders of magnitude. This is amongst the highest enhancement ever noted for any carbonate-hydrolysing antibody.[285]



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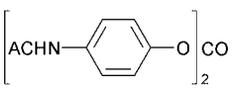
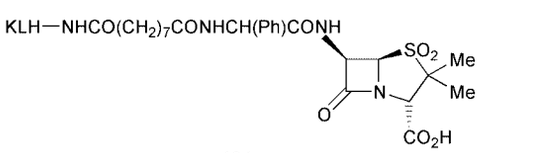


133

A single chain catalytic antibody has been isolated that possesses β-lactamase activity, hydrolysing ampicillin such that K_{cat}/K_{uncat} was 5200. The antigen used was the penicillin sulfone **134**.[286]

Work has continued to identify catalytic antibodies for the hydrolysis of the phosphotriester paraoxon **135**. Previously, conformationally constrained haptens had been employed to raise the hydrolases. Now more flexible systems, such as the *N*-oxide **136** have been used. However the latter haptens gave antibodies which were an order of magnitude less effective in catalytic activity.[287]



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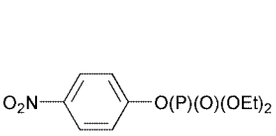

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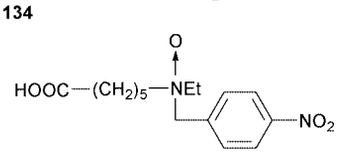
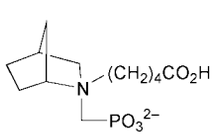


136

An antibody (15M3) raised to the hapten **137** catalysed the enantioselective hydrolysis of (±)-*endo*-2-norbornyl mesylate to give (1*R*,2*R*,4*S*)-*exo*-norbornan-2-ol in 96% ee. Measurement of the kinetic isotope effect showed that the antibody-catalysed reaction was consistent with an SN1 mechanism, probably through enhanced stabilisation of the classical cation.[288]



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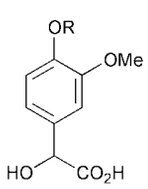
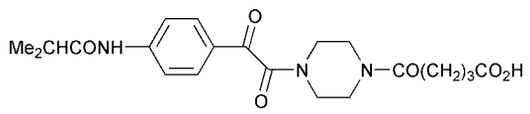


138

R = COEt, PO_3H_2, PO_3Et_2, $CH_2CH{=}CH_2$

Identification of catalytic antibodies capable of hydrolysing compounds of the type **138** has been accomplished by detection of the corresponding phenol by immunoassay. The technique was also used to detect catalytic antibodies that could promote the formation of the same phenol from *o*-hydroxyanisole and glyoxylic acid.[289]

The ketoamide **139** has been introduced as a new motif for raising catalytic antibodies for acyl transfer reactions. One antibody mAb 3H11 was selected for detailed study as a hydrolase, using substrates such as the esters **140** and **141**.[290] Note that careful analyses of the transition states in the hydrolysis of phenyl acetate and *p*-nitrophenyl acetate has indicated that aryl phosphonates (commonly used as haptens to raise catalytic antibodies for such transformations) are *not* good mimetics of the transition states, particularly with regard to their electrostatic properties.[291]



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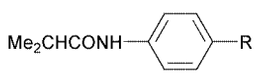
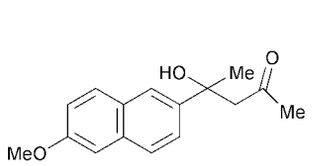


140 R = OCOMe
141 R = CO_2-C_6H_4-NO_2

9.1.2 Antibodies catalysing carbon–carbon bond formation and cleavage

The aldol and retro-aldol reactions continue to be the most studied transformations in this area. Thus the commercially available antibody 38C2 has been used to couple benzyloxyethanal and hydroxyacetone to produce (after removal of the benzyl group) 1-deoxy-L-xylulose (26% overall yield) in a very efficient procedure.[292] The same antibody catalyses the enantioselective retroaldol reaction of *tert*-methodol **142** to give the recovered (*R*)-compound (>99% ee). Many other compounds of the type $R^1C(OH)(Me)CH_2COR^2$ gave similar results with ee's often ≥94%. Products from the latter reactions were used in syntheses of frontalin as well as (+)- and (−)-mevalonolactone.[293] The mechanism by which antibody 38C2 catalyses the retro-aldol reaction depends on the substrate. For electron-deficient substrates deprotonation precedes C–C bond cleavage; for electron-rich species, C–C bond cleavage takes place first, leading to a positively charged intermediate. It was concluded that the catalytic apparatus of 38C2 comprises both "acid" and "base" tools operating in concert.[294] Antibody 38C2 has been shown to produce enones **143** (>95% yields; 42–62% ee) from the corresponding heptane-2,6-diones.[295] The haptens **144** gave rise to two catalytic antibodies that acted as stereoselective catalysts for aldol and retro-aldol reactions, exhibiting the highest catalytic activity of such antibodies to date and showing a stereoselectivity opposite to the archetypal catalytic antibodies such as 38C2. Thus *p*-nitrocinnamaldehyde and acetone were coupled to give (4*R*)-6-(*p*-nitrophenyl)-4-hydroxyhex-5-en-2-one.[296] Antibodies 38C2 and 33F12 react with lactams of the type **145** to leave a thiazolium residue in the heart of the active site of the antibody. This strategy links a cofactor to the antibody surface. Such a chemically modified protein showed the ability to decarbonylate $PhCOCO_2H$ to produce benzoic acid, whereas the starting aldolase does not show any such activity.[297]



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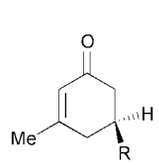

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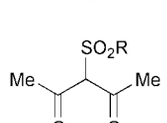

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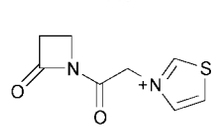
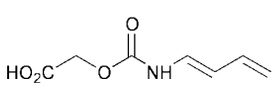


145

Antibody 39-A11 is known to catalyse a Diels–Alder reaction between the diene **146** and the dienophile **147**. Now, by site-directed mutagenesis, mutants have been raised which show ten times greater catalytic activity, thus helping to develop an important insight between such activity and antibody binding.[298]



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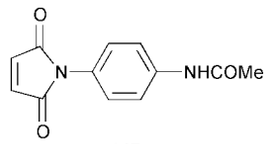
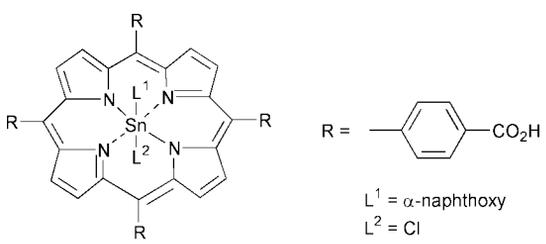


147

There has been further analysis of the ability of antibody HA5-19A4 to catalyse the tandem cationic cyclisation of a polyene, based on X-ray data on the Fab–hapten complex. The antibody caused an extremely large enhancement in rate over the non-catalysed reaction and resembles a terpenoid cyclase[299] in its action.[300]

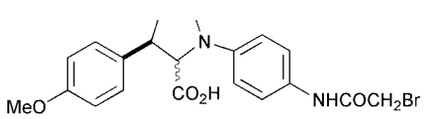
9.1.3 Other types of catalytic antibody

A good model of natural haem-dependent oxidation enzymes has been prepared by raising antibodies to hapten **148** conjugated to bovine serum albumin (BSA). One protein (SN37.4) was isolated and complexed with a Ru(II) analogue of porphyrin **148** to give a catalyst for the oxidation of aryl alkyl sulfides to the corresponding sulfoxides using PhIO as the source of the oxygen atom. For example PhSMe gave the (*S*)-sulfoxide in 43% ee.[301]



148

The proline derivatives **149** have been coupled to BSA and thyroglobulin and antibodies were raised. These proteins were assessed for their ability to catalyse the sulfoxide **150** to sulfenate **151** rearrangements, trapping the latter species with dithiothreitol (DTT). Up to 800-fold accelerations were monitored. The antibody could recognise the chirality of the sulfoxide and, as a result, the allylic alcohol (produced on formation of the ArS-DTT disulfide) was optically active (up to 40% ee).[302]



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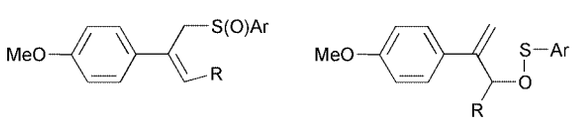
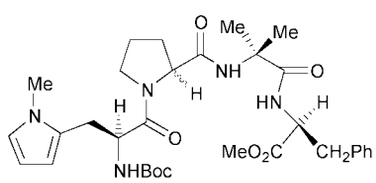


150 R = H, Me; Ar = *p*-NO_2-C_6H_4 **151**

9.2 Polyamino acids and polypeptides

A review of the use of polyamino acids for the asymmetric oxidation of α,β-unsaturated ketones is available,[303] and the technique have been put in the "spotlight" by Gielen.[304] Further developments of the methodology have included the use of sodium percarbonate as a very cheap source of oxidant (and the requisite base). In addition, adsorbing the polyamino acid on silica has been found to generate a very efficient, immobilised catalyst which is extremely easy to recover and recycle.[305] The catalyst has been shown to be able to overcome intrinsic diastereoselection in studies involving enones having a pre-existing stereogenic centre.[306] Furthermore the resultant optically active chalcone epoxides have been used in novel syntheses of anti-inflammatory α-arylpropanoic acids[307] and a portfolio of naturally-occurring styryl lactones.[308] (Note an alternative route to chalcone epoxide (49% yield; 71% ee) involves the use of chiral crown ethers derived from D-galactose as catalysts in stereocontrolled Darzens reactions.[309]) The mechanism by which the polyamino acids exert their catalytic action is obscure though the revelation that poly-L-leucine on porous graphite binds the enantiomers of chalcone epoxide to different degrees may be relevant.[310]

Miller *et al.* have published a full paper that details the use of small peptides as stereoselective acyl transfer agents. The authors emphasise the peculiarities of the systems inasmuch as the tetrapeptide **152** incorporating L-proline acylates the (*S*,*S*)-enantiomer of *trans*-2-aminocyclohexanol preferentially while the diastereomer containing D-proline acylates the (*R*,*R*)-enantiomer of the amino-alcohol more readily.[311]



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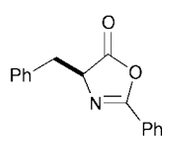


153

The tetrapeptides have been supported on Wang resin together with a fluorescent marker to assist in catalyst optimisation.[312] ZPheHisLeu catalyses the stereoselective hydrolysis of the L-enantiomer of a phenylalanine derivative when the temperature of the reaction and the ionic strength of solution are optimised for co-aggregate systems.[313]

Together with L-isopropyl tartrate as an auxiliary and ethanol as the nucleophile, the cyclic dipeptide [(*S*)-His-(*S*)-Phe] catalyses the alcoholysis of the oxazolone **153** to give (*S*)-*N*-benzoylphenylalanine (39% ee).[314]

9.3 Modified proteins

Subtilisin has been converted into selenosubtilisin by substituting serine-221 by selenocysteine. This semi-synthetic enzyme catalyses the enantioselective reduction of chiral hydroperoxides; for example $PhCH(OOH)CH_2OH$ produces the (*R*)-diol (82–99% ee) and residual (*S*)-hydroperoxide (86–99% ee) (the ee's vary with the extent of conversion). The selenosubtilisin gave the opposite sense of enantioselectivity compared with that shown by horseradish peroxidase or chloroperoxidase.[315]

The peroxidase activity of myoglobin has been enhanced by chemical modification of the prosthetic group to allow access of neutral aromatic substrates to the active site.[316]

The first, efficient bovine serum albumin-catalysed oxidation of tertiary amines to the corresponding amine oxides (100% yields; up to 66% ee) has been achieved using $PhCH_2N(Me)C_5H_{11}$ as the substrate and hydrogen peroxide at the oxidant in pH 9 borate buffer.[317]

Palladium and pre-reduced wool fibres catalyse the hydrogenation of 3-methylbutanone to the (*R*)-alcohol.[318]

Intestinal fatty acid-binding protein linked to a phenanthroline ligand and Cu(II) salt gave an artificial metalloenzyme which catalysed the hydrolysis of α-amino acid esters.

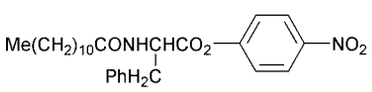
Enantiomeric excesses up to 94% (for alanine isopropyl ester) were recorded.[319]

9.4 Other biomimetic systems

Goto *et al.* have used a "surface molecular imprinting technique" to prepare a polymer that exhibits hydrolase activity. The polymer is manufactured using an oleyl imidazole as one component, cobalt ion, a cross-linking agent and *N*-Boc-L-histidine as the imprinted guest. After washing out the guest and the cobalt ion, the polymer hydrolysed *N*-Boc-L-alanine *p*-nitrophenyl ester at a rate that was greater than that observed for the corresponding non-imprinted polymer.[320]

β-Cyclodextrin modified to possess an oxime unit as part of a Zn(II) binding site has been shown to cleave *p*-nitrophenyl acetate with K_{cat}/K_{uncat} >10^4.[321] β-Cyclodextrin itself assists the opening of racemic epoxides with trimethylsilyl azide to furnish azido alcohols ArylO-$CH_2CH(OH)CH_2N_3$ with yields in the range 38–47% and ee's 64 to >99%.[322]

The enantioselective hydrolysis of *p*-nitrophenyl esters *e.g.* **154** using chiral micelles as catalysts has been observed previously. Heretofore, other reactions, for example asymmetric reductions, had not been documented, probably because the chiral interface was too mobile. Now it has been reported that sodium borohydride reduction of alkyl phenyl ketones generates the (*S*)-alcohol (95–100% ee) using an amphiphilic third-generation dendrimer, abbreviated G(3)G.[323]

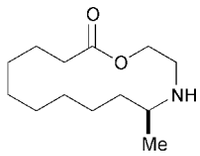


154

10 Miscellaneous biotransformations

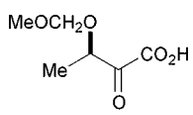
A full paper has been published on the coupling of diacids and diols to yield polyesters using *Candida antarctica* lipase as the catalyst. The mechanism of the stepwise build up of the low-dispersity polymer is discussed.[324] Glycols $HO(CH_2)_nOH$ have been inserted into polyanhydrides $[CO(CH_2)_mCOO]_p$ to give aliphatic polyesters $[CO(CH_2)_mCOO(CH_2)_nO]_q$ using the same enzyme in toluene at 60 °C for 4 h. For octane-1,8-diol and polyazelaic anhydride a polymer with M_n 5800 and M_w/M_n 1.6 was obtained.[325] Ring-opening polymerisation of the cyclic diesters ethylene dodecanedioate and ethylene tridecanedioate is also catalysed by *Candida antarctica* lipase to give 51–60% conversion after 1 h to furnish polymer with M_n 2.4–3.6 × 10^3 and M_w/M_n 1.8–1.9 *Pseudomonas cepacia* lipase performed in a similar fashion though reaction times were longer.[326] Copolymerization of benzyl β-malolactonate and β-propiolactone using *Candida cylindracea* lipase as the catalyst gave a significantly increased M_w for the polymer without a substantial decrease in the masked carboxy content.[327] A *Chromobacterium* species from soil gave a polyester blend of poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) and poly(α-3-hydroxyalkanoate) consisting of both saturated 3-hydroxyalkanoic units of even carbon number (C_4 to C14) and unsaturated 3-hydroxyalkanoic acid units of carbon number C_{12} and C_{14} when 4-hydroxybutanoic acid was used as the sole source of carbon.[328]

New galactose-containing polymers of the type $[CH_2(CHOH)_4CH_2NH(CH_2)_nNH]_m$ have been prepared by use of galactose oxidase to give galactose 6-aldehyde, addition of a diamine (for example 1,7-diaminoheptane) and reduction of the imine bonds using sodium cyanoborohydride.[329] Epoxidation of alkene bonds in the backbone of synthetic polymers such as polybutadiene has been achieved using *Candida antarctica* lipase as the catalyst in organic solvents containing hydrogen peroxide and acetic acid. Pendant vinyl groups are untouched.[330] The same enzyme has been used to form the lactone **155** (74% yield) from the corresponding hydroxy methyl ester using isooctane containing molecular sieves at 65 °C. For the corresponding hydroxyester where the nitrogen is protected as the Boc-derivative the dilactone is formed as the major product in 32% yield.[331]



155

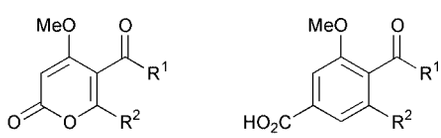
Leucine dehydrogenase, with formate dehydrogenase, ammonium formate and NADH in aqueous solution effects kinetic resolution and reductive amination of the keto acid **156** to furnish (after deprotection of the OH-group) (2*S*,3*S*)-2-amino-3-hydroxybutanoic acid, together with the small amount of the (2*S*,3*R*)-diastereomer, in 47% combined yield.[332] In the presence of 33 mol% cysteine sulfinic acid (and pyridoxal phosphate in pH 7.2 buffer over 24 h at room temperature) racemic 2-oxo-4-propylglutaric acid is converted into (2*S*,4*R*)-2-amino-4-propylglutaric acid (31% yield, 98% pure) using glutamic oxalacetic transaminase as the catalyst. When a full equivalent of the sulfinic acid is used the glutamate derivative is produced as the (2*S*,4*R*)- and the (2*S*,4*S*)-diastereomers in the ratio 3 : 1 and in 68% yield.[333]



156

Fusarium oxysporum lipase was shown to catalyse bromination of monochlorodimedone by using the catalyst in the presence of hexanoic acid, hydrogen peroxide and bromide ion.[334]

Macrophomate synthase has been purified and used to convert various 2-pyrones **157** into the corresponding benzoates **158**.[335]



157 R^1 = Me, OMe, CH=CHMe, $CH_2CH(OH)Me$; R^2 = H, Me **158**

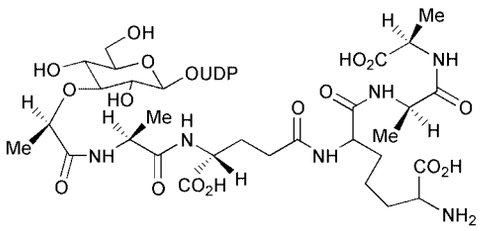
The sulfate group donor 3′-phosphoadenosine 5′-phosphosulfate has been generated from adenosine-31,51-diphosphate using a recombinant aryl-sulfotransferase. The methodology simplifies the enzymatic sulfation of oligosaccharides.[336]

A full paper has been published presenting the evidence of the functioning of a natural Diels–Alderase in the biosynthesis of solanapyrones.[337]

Immobilised myrosinase has been employed to convert natural glucosinolates from Brassicaceae into various 1,3-oxazolidine-2-thiones.[338]

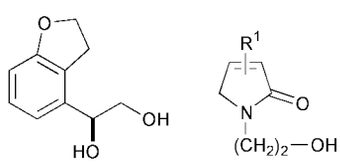
Intermediates on the murein biosynthesis pathway, for example polypeptide **159** have been prepared in high yield on a preparative scale using six purified enzymes from the biosynthetic route (labelled Mur A-Mur F).[339]

Candida boidinii and *Pichia methanolica* have been used to deracemise the alcohol **160** to provide 62–71% yields of the (*S*)-diol (90–100% ee) after 3–4 days.[340] Various cinnamic acid derivatives have been decarboxylated to form the corresponding styrene using cell cultures from plants such as *Catharanthus roseus*, *Nicotiana tabacum*, *Daucus carota* and *Camellia sinensis*.



159

Thus 4′-hydroxycinnamic acid furnished the corresponding hydroxystyrene in 32% yield.[341] *Cunninghamella* sp. NRRL 5695 was found to convert the furan ring of the natural product palinurin into an *N*-hydroxyethyldehydropyrrolidin-2(5*H*)-one derivative **161**. It was suggested that the same transformation may be possible with other furan derivatives.[342]



160 161

L-2-Aminobutyrate has been prepared from L-threonine and L-aspartate using recombinant *E. coli* cells expressing cloned genes for threonine deaminase, aromatic aminotransferase and acetolactate synthase.[343] Labelled chorismic acids have been prepared from shikimate esters in *ca*. 50% yield on a 500 mg scale using engineered, chorismate mutase-deficient *E. coli.*[344] Frost has undertaken metabolic pathway engineering in *E. coli* to produce shikimic acid (14% yield) and quinic acid (23% yield) from glucose. This methodology could provide a better supply of these materials than the conventional plant sources.[345]

11 Conclusion

Judging from the content of this review it is quite clear that many research groups employ lipase and esterase enzymes in synthetic organic chemistry. This volume of work accounts for just under 50% of work published in journals featuring preparative organic chemistry. Oxidation and reduction reactions, mainly using bakers' yeast and other whole cells, account for another 25% of the published work. However, it is still likely that some chemists will be put off from attempting such whole cell biotransformations if an organism other than a commonly available yeast is required. Carbon–carbon bond forming biocatalysts and catalytic antibodies continue to be used mainly in laboratories with expertise in the relevant area. Surprisingly the use of enzymes for the preparation of carbohydrate targets seems to be slightly less popular than in previous years, at least in terms of publications in the learned journals covered by this review.

This will be the last annual Perkin Review on Preparative Biotransformations; in its stead an expert Edinburgh team is producing Graphical Abstracts of key papers in the area of biocatalysis (year 2000 onwards); these are appearing at regular intervals in this Journal.

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