Preparative biotransformations: the employment of enzymes and whole-cells in synthetic organic chemistry

Stanley M. Roberts

Department of Chemistry, Liverpool University, Liverpool, UK L69 7ZD

REVIEW PERKIN 1

Covering: 1996

1 Introduction and background information

This Review takes highlights from the 1996 literature to illustrate some of the recent advances in the use of biocatalysis in synthetic organic chemistry. Since the emphasis in the Review is on useful preparative methods, most of the information has been abstracted from those learned Journals dealing with synthetic organic chemistry.[1] Items from some specialist Journals[2] are included when the transformations have actual or potential synthetic utility.

The majority of enzyme-catalysed reactions carried out by non-specialist laboratories involve the hydrolysis of esters or the reverse process, *i.e.* esterification, using commercially available lipases. (A new book on the latter area of research is now available.[3]) Not surprisingly, therefore, about 40% of the biocatalysis literature features one or other of these transformations. Other hydrolytic procedures (*e.g.* hydrolysis of nitriles, epoxides *etc.*) make up less than 5% of the modern literature.

The use of enzymes to prepare and modify complex polysaccharides has increased markedly (to *ca.* 5% of the biotransformations literature), not least because exquisite regio- and stereo-control is achieved using enzyme-catalysed reactions.

The reduction of carbonyl compounds, often using dehydrogenases, bakers' yeast or other microorganisms is popular (10% of the literature) while the reduction of carbon–carbon double bonds and other functionality is still scarcely explored. Oxidative biotransformations also make up 10% of the current literature; one aspect, namely the preparation of *cis*-cyclohexa-3,5-diene-1,2-diols using dioxygenases and the employment of these compounds in synthetic organic chemistry continues to be of widespread interest.

Carbon–carbon bond-forming reactions such as cyanohydrin formation using oxynitrile ligases and the synthesis of polyhydroxylated compounds using aldolases and transketolases make up a small percentage of the literature (*ca.* 2%) but are of considerable and significant potential.

The employment of catalytic antibodies to promote selected transformations is still restricted to specialist laboratories and there is no evidence, as yet, that the technique will become of routine use. Other proteins such as bovine serum albumin, which have no overt catalytic activity *in vivo*, and polypeptides such as polyleucine give opportunities for the construction of optically active compounds, such as sulfoxides and epoxides, often through obscure mechanisms. The design and preparation of enzyme mimetics is, though, still in its infancy and awaits better integration of binding and catalytic processes, *i.e.* the molecular recognition of transition states.[4]

This Review specifically excludes reports involving the elucidation of enzyme mechanisms and also the design and synthesis of compounds leading to enzyme inhibition. In addition the Review is not meant to be comprehensive even in its coverage of preparative biotransformations. For a more thorough coverage of the literature the Warwick Biotransformation Club *Biotransformation Abstracts*[5] is recommended.

For conducting full surveys of all biotransformations two excellent databases are available. The BioCatalysis database[6] contains material selected by Professors Bryan Jones (Toronto) and Bert Holland (Brock University) and covers the whole biotransformation field in a critically comprehensive manner.[6] Professors David Crout, Howard Dalton (Warwick) and Manfred Schneider (Wuppertal) have compiled a biotransformations database covering 40 000 reactions which is available on CD-ROM.[7] Detailed recipes for the use of isolated, partially purified enzymes and simple-to-use whole-cell systems are featured in a continually updated laboratory manual.[8]

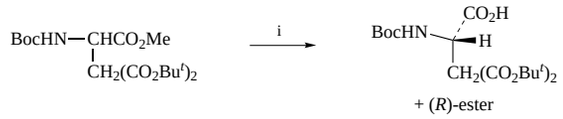
While biotransformations are now accepted as a method of producing useful compounds efficiently and often in optically active form, it is obvious that the techniques serve only to complement other modes of catalysis using non-natural systems. The latter can compete with enzymes most favourably on some occasions. Thus while chorismate mutase accelerates the rearrangement of chorismate by 10^6, catalysis by trivalent aluminium compounds matches or, in some cases, exceeds these rate enhancements![9] It should be noted, therefore, that this survey discusses advances in only one sector within the wider field of catalysis.

2 Hydrolysis and aminolysis of esters and hydrolysis of amides

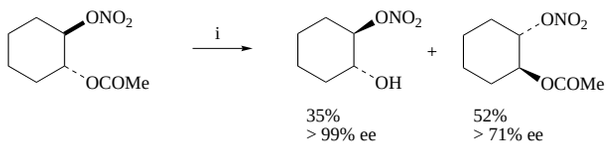
The chemoselectivity of enzyme-catalysed hydrolysis is important in some instances. Thus butylcholine esterase from horse serum and acetylcholine esterase from the electric eel have been used to cleave choline from esters of the type Boc-Phe-$OCH_2CH_2N(CH_3)_3^+$ (50–95% yields). The choline moiety serves to solubilise the compound in water as well as to act as a protecting group.[10] The synthesis of complex and sensitive phosphoglycohexapeptides has utilised a *p*-phenylacetoxybenzyloxycarbonyl protecting group for amino-functionality. The protecting group is removed by penicillin G acylase (yields 66–78%).[11]

A remarkably simple procedure for the synthesis of monoprotected diols of the type $AcO(CH_2)_nOH$ by hydrolysis of the diacetate using porcine pancreatic lipase (ppl) at pH 6.9 has been published (yields 79–95% for $n = 3$–6).[12]

Esterases and lipases have gained widespread use as resolving agents for racemic esters giving either optically active carboxylic acids (*e.g.* **Scheme 1**)[13] or alcohols (*e.g.* **Scheme 2**).[14] A detailed study of the hydrolysis of water-insoluble acetylsulcatol to the natural optically active alcohol generated information that may be of general utility. Thus the enantioselectivity of the process increased with increase in concen-



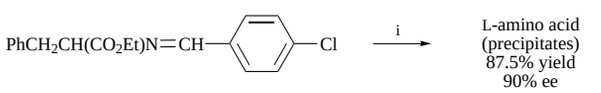
Scheme 1 *Reagents:* i, papain, 20 h, yield of acid >85% of theoretical on 30 g scale



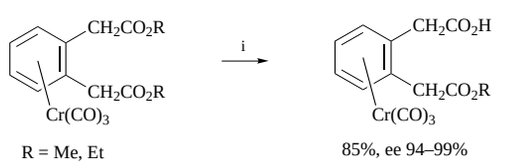
Scheme 2 *Reagents:* i, pig liver acetone powder, 3 d, Et_2O, buffer

tration of the substrate. Addition of hexane, cyclopentane, benzene or diisopropyl ether improved enantioselectivity while cyclohexane had a detrimental effect. The solvent effects may be due to interactions with the substrate binding pocket and the lid of the enzyme.[15]

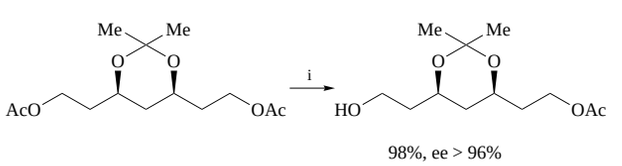
The efficiency of enzyme-catalysed generation of optically active materials may be enhanced by effecting a dynamic resolution (**Scheme 3**)[16] or by employing a *meso*-substrate (*e.g.* **Schemes 4**[17] and **5**[18]). The product described in **Scheme 5** was



Scheme 3 *Reagents:* i, chymotrypsin, DABCO, H_2O, MeCN, 96 h

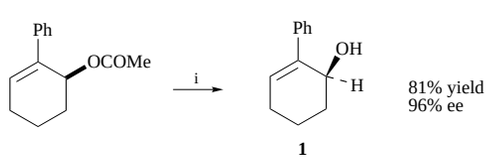


Scheme 4 *Reagents:* i, pig liver esterase, H_2O



Scheme 5 *Reagents:* i, *Pseudomonas fluorescens* lipase, H_2O

converted into the C_1–C_{10} fragment of nystatin-A_1. The combination of palladium-catalysed isomerisation and lipase-catalysed hydrolysis has been used to provide the alcohol **1** in high yield and excellent enantiomeric excess (**Scheme 6**).[19]



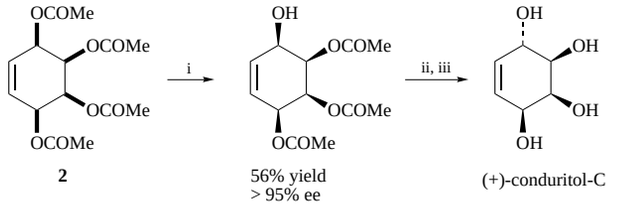
Scheme 6 *Reagents:* i, PFL, 40 °C, pH 7, 0.1 M phosphate buffer, 5 mol% $PdCl_2(MeCN)_2$, 19 d

Regio- and stereo-selective transesterification of the tetra-acetate **2** gave a triester which was readily converted into (+)-conduritol-C (**Scheme 7**).[20]

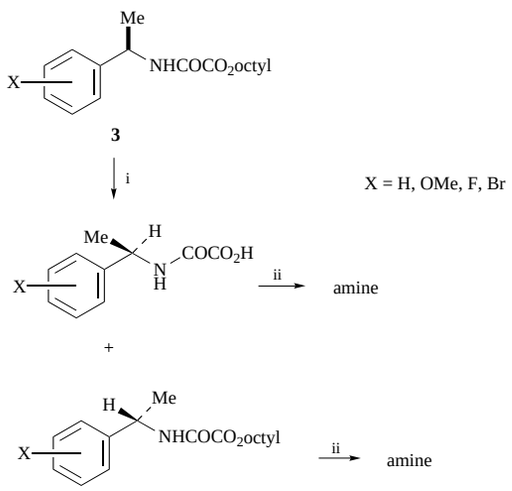
A full report (and an excellent reference list) on enantioselective synthesis involving enzymatic asymmetrisation has appeared.[21]

Chiral amines may be obtained in optically active form by hydrolysis of compounds of type **3** (**Scheme 8**).[22]

The formation of di-, tri- and poly-peptides using enzyme-catalysed coupling reactions is well established. The employment of trifluoromethyl esters as coupling partners for amino



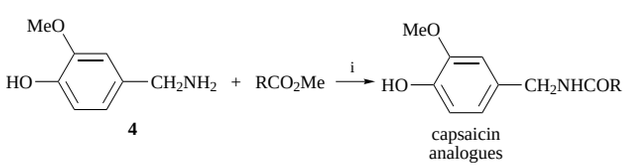
Scheme 7 *Reagents:* i, porcine pancreatic lipase, Bu″OH; ii, Mitsunobu reaction; iii, chemical hydrolysis



Scheme 8 *Reagents:* i, *Candida antarctica* lipase, buffer; ii, OH^-

acids (such as L-leucine) has been recommended for those cases where the ester is derived from a non-natural amino acid.[23] Penicillin amidase-catalysed coupling of esters such as ethyl phenylacetate with ethyl *p*-hydroxyphenylglycine has been optimised (98% yield using equimolar amounts of substrates) by effectively controlling water activity in the solvent system (benzene : water, 97 : 3) through the addition of salts *e.g.* Na_2HPO_4.[24]

Coupling of the amine **4** with methyl alkanoates using chicken liver acetone powder (CLAP) gives capsaicin analogues, albeit in low to modest yield (**Scheme 9**).[25] Better yields (51–

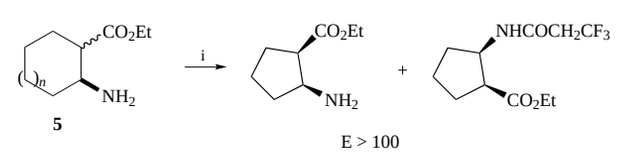


R = C_7H_{15}; $C_{15}H_{31}$; $(CH_2)_7CHCH(CH_2)_7CH_3$

Scheme 9 *Reagents:* i, CLAP, 37 °C, borate buffer, 48 h

>99%) were obtained using *Pseudomonas cepacia* lipase (Amano PS 30) to couple a variety of benzyl esters [*e.g.* $CH_2(CO_2CH_2Ph)_2$] and amines (*e.g.* tyramine) over a period of *ca.* 7 days.[26]

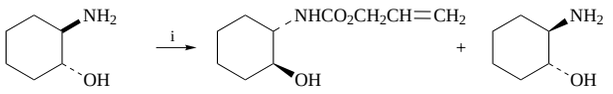
Ethyl esters of ten alicyclic 2-amino carboxylic acids of type **5** have been resolved using lipase PS or lipase SP526 and trifluoroethyl esters $RCO_2CH_2CF_3$. E values range from 33 to >100 (**Scheme 10**).[27] Similarly a serine acylase such as subtilisin



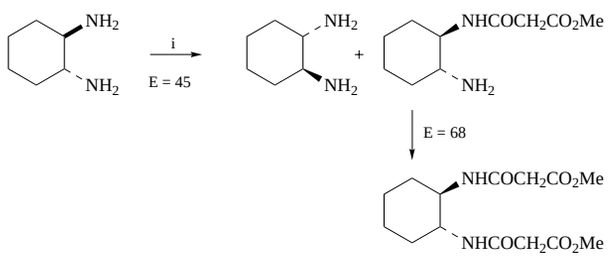
Scheme 10 *Reagents:* i, for $n = 0$, *cis*-amino ester, lipase PS, $RCO_2CH_2CF_3$

Carlsberg has been employed to resolve amines using cyanomethyl pent-4-enoate as the acylating agent in 3-methylpentan-

3-ol. The solvent was chosen so as to suppress background reactions.[28] Stereoselective protection of amines as allyl carbamate derivatives can be accomplished by employing diallyl carbonate and subtilisin in phosphate buffer or organic solvents (**Scheme 11**).[29] Likewise diamines may be acylated selectively (**Scheme 12**). In preparative runs the reaction was allowed to



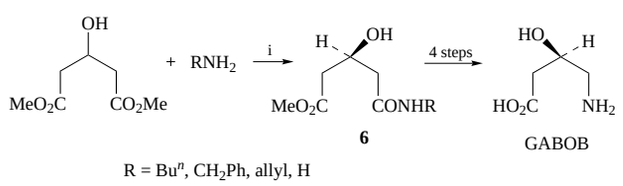
Scheme 11 *Reagents:* i, diallyl carbonate, subtilisin, phosphate buffer, 69 h



Scheme 12 *Reagents:* i, *Candida antarctica* lipase, diethyl malonate, 1,4-dioxane

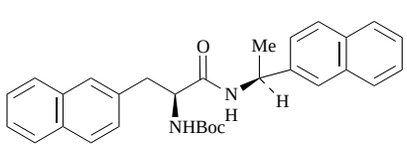
proceed to give highly enantiopure diamine and bis-amide.[30]

Chiral and prochiral esters may be converted into optically active materials in the same way. For example dimethyl 3-hydroxyglutarate is converted into the monoester monoamide **6** in high yield and enantiomeric excess (both 95–98%) *en route* to GABOB (**Scheme 13**).[31]



Scheme 13 *Reagents:* i, *Candida antarctica* lipase

The involvement of a chiral amine and a chiral ester can lead to a double enantioselection as observed for the CLEC†-subtilisin-catalysed coupling of β-naphthyl-$CH_2CH(NHBoc)$-CO_2Me and α-naphthyl-$CH(NH_2)CH_3$ in acetonitrile which gave, after 8 h at 40 °C, the amide **7** in >49% yield and >98% ee.[32] Similarly (±)-ethyl 2-methyloctanoate and (±)-phenethylamine couple at 70 °C under catalysis by Novozyme to give the



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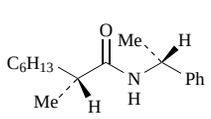

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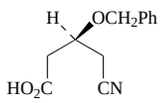
amide **8** in 45% de at 99% conversion of the ester. The racemisation of the ester seems to be enzyme-catalysed.[33]

The deprotection of phthalyl protected amines may be effected by a two-step one-pot sequence using pH 8 buffer to give a phthalyl amide which is hydrolysed using a phthalyl amidase from *Xanthobacter agilis* (cloned and over-expressed in *Streptomyces lividans*) to give the free amine.[34]

3 Hydrolysis of nitriles and oxiranes

While interesting results involving enzyme-catalysed hydrolyses of oxiranes and nitriles have been obtained, as illustrated below, widespread application of the methodologies must await the commercial availability of the relevant hydrolases.

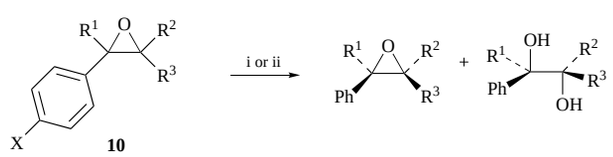
Turner and co-workers[35] have shown that the cyanocarb-



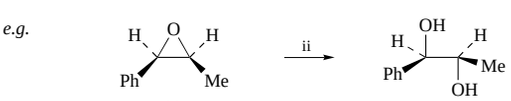
9

oxylic acid **9** is available from the corresponding prochiral dinitrile in good yield (65%) and high enantiomeric excess (88–99% ee) (on a 7 g scale) over a period of 24 h using *Rhodococcus* sp. SP361 or *Brevibacterium* sp. R312. The acid **9** was readily converted into the lactone moiety of mevinic acids.

A review of the field of enzyme-catalysed epoxide hydrolysis has been published.[36] Much of this early work featured the employment of liver microsomal epoxide hydrolases and, indeed, work continues in this area.[37] However the very nature and origin of the catalyst means that work will be limited to a relatively small scale. The identification and use of more readily available microbial whole-cell systems containing epoxide hydrolases has been a noteworthy trend. The employment of the fungus *Beauvaria sulfurescens* has been popular. Thus an epoxide hydrolase in resting cells of *B. sulfurescens* gives (*R*)-epoxide (34% yield; 98% ee) and (*R*)-diol (45% yield; 83% ee) from styrene oxide. Indene oxide gives (1*R*,2*R*)-diol (48% yield; 69% ee).[38] Two full papers by the same research group report such ring-opening reactions for compounds of type **10** in more detail, comparing *B. sulfurescens* with *Aspergillus niger* as useful biocatalysts.[39] A particularly interesting example is the stereoconvergent ring-opening of the epoxide **11** which gives the diol **12** in 85% yield and 98% ee (**Scheme 14**) (one enantiomer of the



$R^1 = R^2 = R^3$ = H or Me; X = H
X = Me, Hal, CN, NO_2; $R^1 = R^2 = R^3$ = H



85%; 98% ee

Scheme 14 *Reagents:* i, *A. niger*, H_2O; ii, *B. sulfurescens*, H_2O

epoxide suffers attack by water adjacent to the phenyl group, the other enantiomer is attacked by water at the carbon atom bearing the methyl group).

Similarly Faber and co-workers have shown that the epoxide **13** is hydrolysed using lyophilised cells of *Nocardia* EH1 to give the corresponding (2*R*,3*R*)-diol in 79% yield (91% ee) over twenty days (**Scheme 15**).[40]

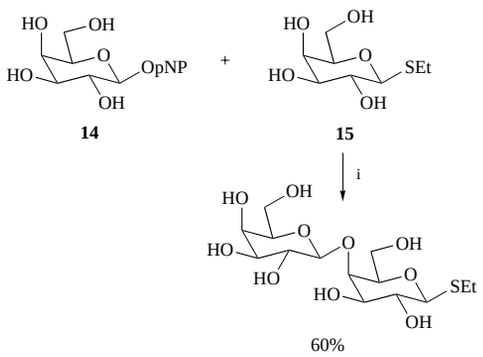
Scheme 15 *Reagents:* i, *Nocardia* EH1, H_2O

† CLEC: cross-linked enzyme crystals.

In a closely related strategy 1-methyl-1,2-epoxycyclohexane gives (1*S*,2*S*)-1-methylcyclohexane-1,2-diol in a two-step, one-pot procedure in 80% yield and 95% ee. Thus the racemic epoxide is incubated with resting cells of *Corynebacterium* C12 to give optically active epoxide and diol. *In situ* treatment of the mixture with perchloric acid transforms the remaining epoxide into the same (*S*,*S*) enantiomer of the diol.[41]

4 Preparation of carbohydrates, nucleosides and nucleotides

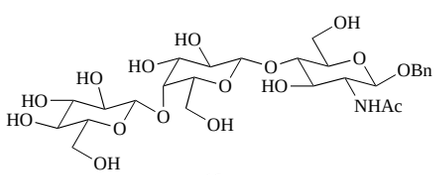
There has been a noteworthy increase in the amount of work aimed at the enzyme-catalysed synthesis of complex carbohydrates, with glycosidases and glycosyl transferases being used most extensively. For example, β-galactosidase from *Bacillus circulans* has been used to couple a variety of galactosyl donors (*e.g.* **14**) to selected glycosyl acceptors (*e.g.* **15**) (**Scheme 16**).[42]



14 **15**

Scheme 16 *Reagents:* i, β-galactosidase

The same enzyme has been useful in the syntheses of lactosamine derivatives **16**[43] and can be used in tandem with



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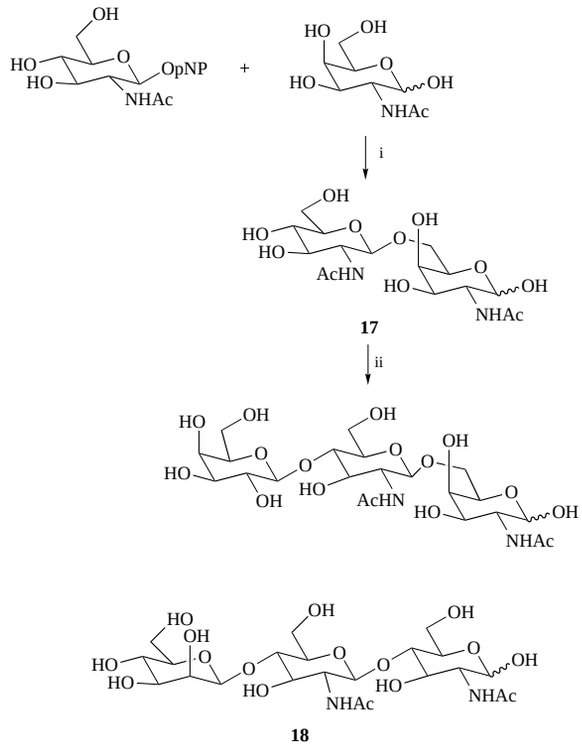
galactose oxidase to provide *N*-acetyl-D-lactosamine derivatives which (as hydrated aldehydes at the C-6 position) have enhanced stability to β-galactosidase-catalysed hydrolysis.[44] β-*N*-Acetylhexosaminidase from *Aspergillus oryzae* has been shown to accept *N*-acetylgalactosamine as a substrate, affording compound **17**, which can be further derivatised using β-galactosidase (**Scheme 17**).[45] Similarly a two-step synthesis of the core trisaccharide of N-linked glycoproteins **18** may be accomplished using β-*N*-acetylhexosaminidase and β-mannosidase from *Aspergillus oryzae*.[46]

α-Galactosidases from *Coffea arabica* and *Aspergillus oryzae* have been used to prepare trisaccharides having an α-D-Gal-(1→3)-D-Gal terminus, a motif which is responsible for hyperacute rejection response in cross-species transplant rejection (*e.g.* from pig to man).[47]

Transgalactosylation in organic solvents (such as diisopropyl

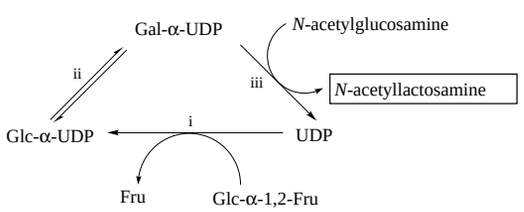


17 **18**

Scheme 17 *Reagents:* i, β-*N*-acetylhexosaminidase from *A. niger*; ii, β-galactosidase from *B. circulans*

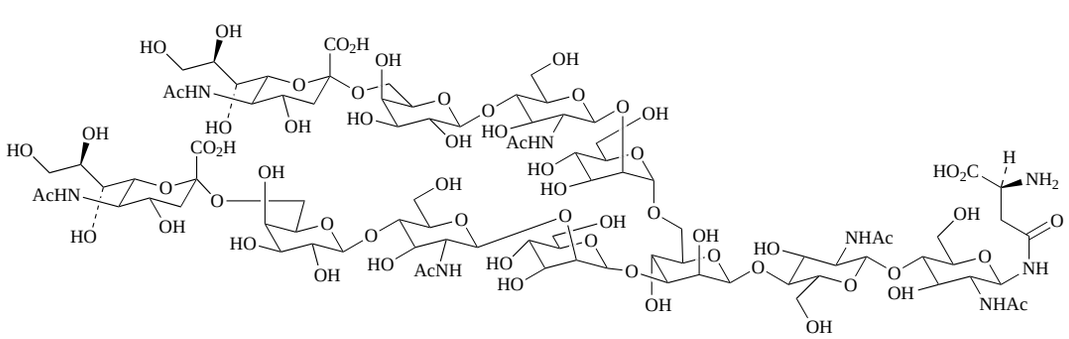
ether or benzene) promoted by β-D-galactosidase from *Escherichia coli* or *B. circulans*, using *p*-nitrophenyl-β-D-galactopyranoside as the acceptor and 5-phenylpentan-1-ol or nonan-2-ol as the donor, is aided by the enzyme having a suitable coat (*e.g.* *N*-D-gluconyl-L-glutamate) so that hydrolysis is prevented.[48]

An efficient, three-enzyme reaction cycle, featuring β-1,4-galactosyl transferase, has been employed for the synthesis of *N*-acetyllactosamine (**Scheme 18**).[49]



Scheme 18 *Reagents:* i, sucrose synthetase; ii, UDP-Glc-4′-epimerase; iii, β-1,4-galactosyl transferase

An impressive 86% yield has been recorded for double incorporation of galactose into a heptasaccharide asparagine conjugate, using galactosyl transferase, followed by double incorporation of a sialyl residue, using α(2→6)-sialyl transferase and CMP-*N*-acetylneuranimic acid, to give compound **19** (CMP = cytidine 5′-monophosphate).[50]



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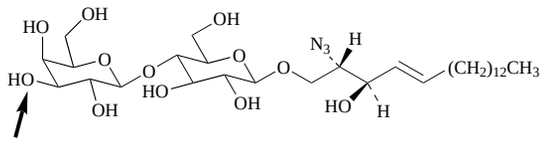
α-2,3-Sialyl transferase has been employed to couple CMP-Neu-5-acetate to the acceptor **20** at the position arrowed *en route* to ganglioside GM_3.[51]



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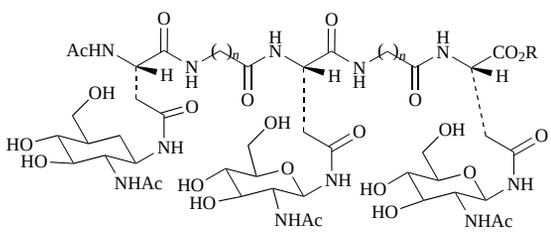

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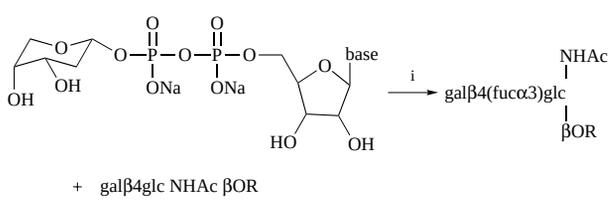
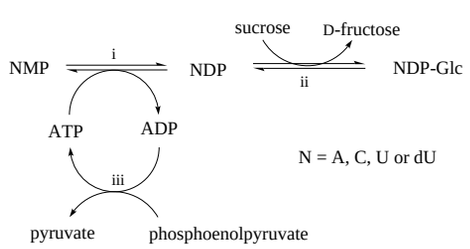
Compound **21** has been modified using UDP-Gal and galactosyl transferase (yield 75–88%) then CMP-sialic acid/α(2→3)-sialyl transferase (85–99%) and GDP-fuc/fucosyl transferase VI (68–77%) to give complex sialyl Lewis^x glycopeptide clusters (in 6–20 mg quantities) for monitoring the affinity of such species to E-selectin (UDP = uridine 5′-diphosphate; GDP = guanosine 5′-diphosphate).[52] (For the synthesis of a trivalent sialyl Lewis^x conjugate on a cyclopeptide template see Sprengard *et al.*[53])

Non-natural GDP-fucose analogues (with guanosine replaced by other bases) have been incubated with cloned fucosyl transferase III or VI to generate Lewis^a or Lewis^x trisaccharides (**Scheme 19**).[54]



Scheme 19 *Reagents:* i, catalyst fucosyl transferase VI

Syntheses of nucleotide diphosphate (deoxy) sugars have been achieved on a 100 mg scale using the sequence shown in **Scheme 20**.[55]



Scheme 20 *Reagents:* i, kinase from pig, chicken, rabbit or cow; ii, sucrose synthetase; iii, pyruvate kinase

5 Esterification reactions

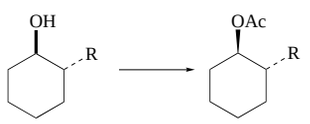
The availability of lipases, proteases and acylases and the relative simplicity of the operating procedures has allowed synthetic organic chemists to explore enzyme-catalysed esterification reactions extensively. The reactions are carried out in organic solvents, often with just a trace of water present.

New enzymes have been added to the list of those able to catalyse reactions in non-aqueous media. For example acylase 1 from *Aspergillus niger* has been used to resolve *N*-acylated 2-hydroxymethylpiperidines using vinyl acetate (the acyl group donor) in toluene.[56] While vinyl acetate or propenyl acetate are widely used as the acyl donors in this methodology the use of 1-ethoxyvinyl acetate has been recommended as an alternative possibility, since ethyl acetate is released as the by-product rather than the more reactive acetaldehyde or acetone.[57] Mixed carboxylic–carbonic anhydrides have also been shown to be good acyl-transfer reagents for lipase-catalysed esterification reactions.[58]

The regioselective esterification of polyhydroxy compounds using enzyme-catalysed reactions is well documented. For example, using subtilisin BDN′ in dimethylformamide containing vinyl acetate and a small amount of buffer and triethylamine, *N*-acetylneuranimic acid is acetylated on the primary hydroxy group in 76% yield.[59]

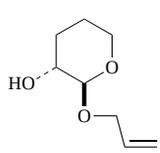
Mainly, though, the method has been used for the kinetic resolution of secondary alcohols, either acyclic systems such as $PhCH(OH)CH_2CH{=}CH_2$ (*en route* to homochiral fluoxetrine)[60] and (3-pyridyl)ethanol [*en route* to (−)-tubifoline] or cyclic systems such as the cyclohexanol derivatives **22–26**.[61–65] The kinetic resolution of *trans*-2-methoxycyclohexanol has been scaled up to provide a key intermediate to the trinems, potent new antibacterial agents.[66] The stereoselection takes place such that the (*S*)-alcohol and the (*R*)-acetate are produced in all five cases (**Table 1**). Heterocyclic alcohols, for example oxygen-containing

Table 1



Compd.	R	Ester yield (%)	Ee (%)
22	I	47	97
23	Ph	'high'	'high'
24	CH_2COPh	—	99
25		45	>99
26	OMe	47	>95

molecules **27**[67] and nitrogen-containing ring systems **28**[68] can be resolved in a similar manner; the (2*R*)-alcohol derived from the latter process was converted into crooksidine. Note that the addition of thiacrown ether has been shown to improve the enantioselectivity of some kinetic resolutions of this type.[69]



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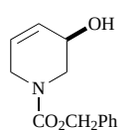
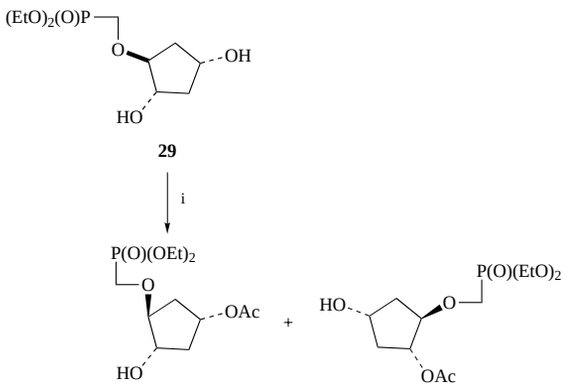


28

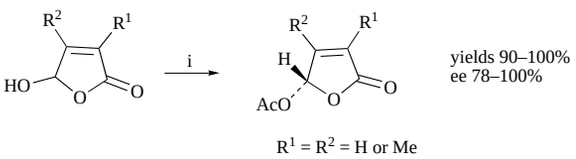
Acetylation of the diol **29** gives two optically active monoacetates, useful for the preparation of carbocyclic nucleotides (**Scheme 21**).[70] Monoacetylation of the diol **30** using Amano lipase AK gives optically pure monoacetate in 18% yield.[71]

Obviously in all the above-described resolution processes the maximum yield of one component that can be attained is 50%. More efficient strategies employ a dynamic kinetic resolution [as used to access optically active 5-acetoxyfuran-2(5*H*)-ones (**Scheme 22**)][72] or *meso* and prochiral substrates such as the diols **31**,[73] **32**[74] and the triol **33**.[75] The position of lipase-catalysed acylation using *Pseudomonas fluorescens* lipase and vinyl acetate is indicated in the latter cases: yields and enantiomeric excesses in all cases were greater than 90%.

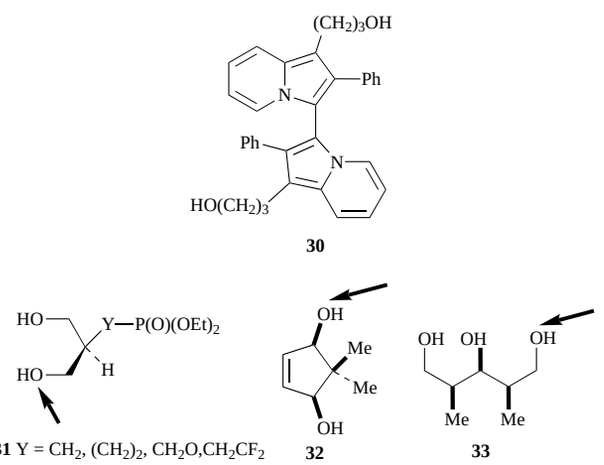
The importance of stereoselective esterification and transesterification reactions in synthetic organic chemistry has prompted much work on optimising the nature of the catalyst. Highly efficient heterogeneous biocatalysts can be obtained by



Scheme 21 *Reagents:* i, lipase PS (Amano), vinyl acetate, 30 °C, 30 h



Scheme 22 *Reagents:* i, lipase PS, vinyl acetate, CH_2Cl_2, 7 d

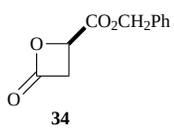


fixing lipase-containing hydrophobic sol–gel materials on to sintered glass. The process involves mixing tetramethoxysilane and polydimethylsiloxane with an aqueous solution of the lipase (*e.g.* lipase SP 523), polyvinyl alcohol as an additive, catalytic sodium fluoride and finally sintered glass. Chemically and mechanically stable catalysts are obtained with enhanced activity (compared with the traditional suspension method) in a model esterification reaction.[76] There has been much interest in cross-linked enzyme crystals (CLECs) as robust and efficient biocatalysts. Thus the CLEC from lipase S (1.3 mg), prepared in its active state by washing with organic solvent containing surfactant, catalyses the conversion of 1-phenylethanol into the (*R*)-acetate (98% ee) in 16 h on a 5 g scale.[77] Note that by coupling *Ps. fluorescens*-catalysed acylation with racemisation of the slower reacting component by hydrogen transfer (σ-phenanthroline, acetophenone, KOH) (±)-phenethyl alcohol is converted into the (*R*)-acetate in 60% yield and 98% ee.[78]

The behaviour of CLEC-subtilisin in acetonitrile depends on the hydration history of the enzyme; highest rates of reaction were obtained with crystals dried by washing with organic solvent. Transesterification activity of the solvent-washed enzyme was profoundly affected by any water in the system.[79] In a complementary study it was shown that lyophilised or crystalline proteins do not lose α-helix content in pure solvents such as tetrahydrofuran or acetonitrile. The α-helix content declined markedly in mixed solvent or aqueous systems. The more rapid denaturation of the enzymes in mixed solvents is believed to be due to increased conformational mobility.[80] Undoubtedly CLECs in organic solvents offer performance and mechanical advantage over lyophilised counterparts. The catalytic activity of CLEC-subtilisin can be increased 100-fold by adding to the solvent a 'buffer' consisting of a mixture of a suitable acid and its conjugate base.[81]

CLEC-α-chymotrypsin catalyses the transesterification of $PhCH(CH_2OH)CO_2CH_3$ with propanol. The configuration of the product depends on the solvent; for example running the reaction in cyclohexane gives the (*S*)-propyl ester (85% ee) while the use of acetone gives the enantiomer (21% ee). The solvent exerts its effect on selectivity through the differential Gibbs free energy of desolvation of the transition states of the reaction. The conclusion drawn was that, in choosing a biocatalyst for the resolution of a chiral compound, one should seek an enzyme with an active centre that maximises the difference in desolvation between the two enantiomers.[82]

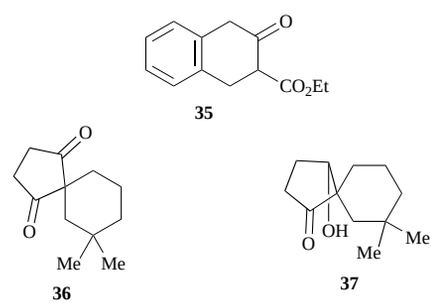
The application of lipases to esterification/polymerisation reactions has continued. *Pseudomonas fluorescens* lipase immobilised on Celite catalysed ring-opening–polymerisation of 12-dodecanolide: the addition of sucrose as a 'lyoprotectant' was beneficial.[83] Benzyl β-malolactonate **34** undergoes ring-



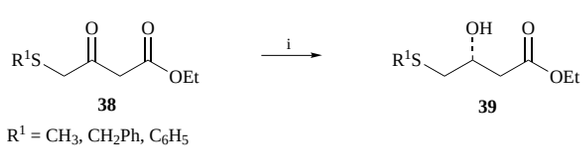
opening–polymerisation using porcine pancreatic lipase or Novozyme 435 at 60 °C to yield poly(benzyl β-malate) (MW >7000).[84]

6 Reduction reactions

Bakers' yeast continues to be a popular catalyst for the asymmetric reduction of carbonyl compounds. Thus a model for predicting the result of bakers' yeast reduction of β-keto esters of the type **35** has been proposed,[85] while the spirodiketone **36** is reduced to the keto alcohol **37** with high selectivity.[86] Reduction

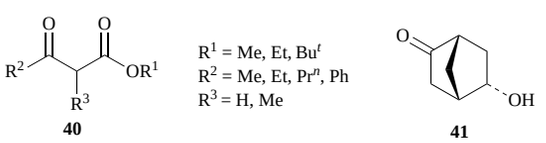


of acyclic β-keto esters **38** afforded the alcohols **39** in 26–99% ee and 47–85% yield (**Scheme 23**).[87]



Scheme 23 *Reagents:* i, bakers' yeast, EtOH, H_2O

Extending the work of Smallridge *et al.*, North has now shown that bakers' yeast reduction of a variety of β-keto esters **40** in petrol gives good to excellent conversions and, generally, very high ees of the (*S*)-alcohols.[88] Similarly the alcohol **41** was



obtained (*ca.* 25% yield; *ca*. 75% ee) from the corresponding dione using bakers' yeast in hexane containing 10% water. The product was converted over 11 steps into the right-hand moiety of azadirachtin.[89]

As an alternative to bakers' yeast, an acetone powder of *Geotrichum candidum* (with added $NADP^+$ and propan-2-ol) has been recommended for the reduction of ketones such as CH_3COR^1 [$R^1 = CH_2CO_2R^2$; aryl; $CH_2CH_2CHC(CH_3)_2$] to the corresponding (*S*)-alcohols; ease of preparation, extended shelf-life and ready manipulation have been cited as reasons for consideration of the use of this biocatalyst.[90]

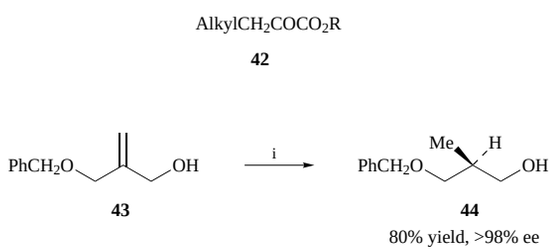
On the other hand *Yarrowia lipolytica* reduces ketones of the type CH_3COR [R = Ph, $CH_2CH_2C(CH_3)CH_2$] to the (*R*)-alcohol (66–100% ee).[91] Likewise the yeast *Pichia farinosa* also reduces ketones of the type CH_3COR (*e.g.* R = CH_2CH_2SPh) to the (*R*)-alcohol (90% yield; 91% ee). In this case enhancement of the enantiomeric excess was achieved by sequential oxidation with *Rhodococcus rhodochrous* which selectively removes the (*S*)-alcohol.[92] *Pichia farinosa* reduces acyclic diones to diols with similar anti-Prelog selectivity.[93]

An NADPH-dependent formate dehydrogenase has been engineered from an NAD-dependent formate dehydrogenase (*ex Pseudomonas* sp. 101) to work with NADPH-dependent alcohol dehydrogenase from *Lactobacillus* sp. so as to produce (*R*)-1-phenylethanol from acetophenone.[94]

A two-step, one-pot procedure for the conversion of keto esters **42** into the corresponding (*S*)-amino acids employs *Candida cylindracea* lipase (to effect a racemisation-free hydrolysis of the ester) and leucine dehydrogenase, formate dehydrogenase and NAD^+ together with ammonium formate; the yields were in the range 70–80%.[95]

An artificial amino transferase has been coupled with a natural lactate dehydrogenase to convert an α-amino acid [*e.g.* $CH_3(CH_2)_3CH(NH_2)CO_2H$] into the corresponding α-hydroxy acid.[96]

Bakers' yeast reduction of the alkene **43** affords the optically active alcohol **44** (**Scheme 24**) probably *via* the intermediacy of



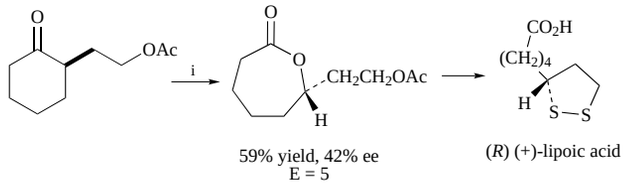
Scheme 24 *Reagents:* i, bakers' yeast, 30 °C, 14 d

the unsaturated aldehyde.[97] Bakers' yeast reduction of compounds of the type ArCH=C(Me)COMe gives the saturated ketone with the (*S*)-configuration. Substitution in the *meta*- and *ortho*-positions of the aryl ring dramatically improves the stereoselectivity of this reaction; for example *meta*-chloro and *meta*-nitro derivatives give products having ⩾96% ee (91% yield and 56% yield respectively).[98]

The reduction of aryl azides into aniline derivatives, by using bakers' yeast in aqueous methanol over three hours, has been reported.[99]

7 Oxidative biotransformations

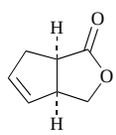
Stereoselective Baeyer–Villiger oxidations of 2-substituted cyclohexanones using *Acinetobacter* TD63 and a NADPH-dependent monooxygenase from *Pseudomonas* NCIMB 9872 have been compared. Both systems give optically active lactones and recovered, optically active starting materials in 43–95% yields and, in the majority of cases, good enantiomeric excess.[100] A similar enzyme-based methodology has been used as a key step in the preparation of (*R*)-(+)-lipoic acid (**Scheme 25**).[101] A modified bakers' yeast, expressing cyclohexanone



Scheme 25 *Reagents:* i, cyclopentenone monooxygenase from *Pseudomonas* sp. NCIMB 9872

monooxygenase from *Acinetobacter calcoaceticus* NCIMB 9871 (CMO ex 9871), gives excellent optical purities of (*S*)-lactones from 2-alkyl- and 4-alkyl-cyclohexanones. Other transformations typical of wild-type bakers' yeast (*e.g.* reduction of the carbonyl group) were minimised by appropriate choice of growth conditions. Added β-cyclodextrin was effective in promoting solubility of the substrate and reducing toxicity.[102] Note that an active site model of CMO ex 9871, based on cubic space descriptors, is documented.[103]

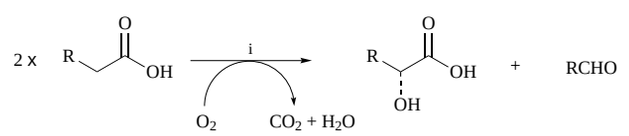
(±)-Bicyclo[3.2.0]hept-2-en-6-one is oxidised enantioselectively using *Cunninghamella echinulata* NRRL 3655 to furnish the γ-lactone **45** (30% yield, >98% ee) which was readily converted into (+)-multifidene in 40% yield.[104]



45

The complementarity of the enantioselectivities of some of the more popular Baeyer–Villiger monooxygenases has been illustrated using monocyclic, bicyclic as well as tricyclic ketones as substrates[105] and a proposal regarding the origin of the stereoselectivity has been put forward.[106]

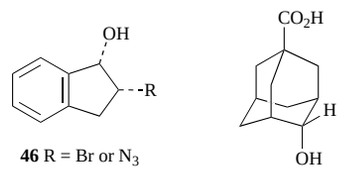
Enantioselective α-hydroxylation of carboxylic acids can be accomplished with molecular oxygen when catalysed by enzymes from young pea leaves (**Scheme 26**). As well as pea



Scheme 26 *Reagents:* i, oxidase(s) from *Pisum sativum*

leaves, enzymes in germinating peanuts, cucumber, potatoes and some marine algae catalyse the same transformation.[107]

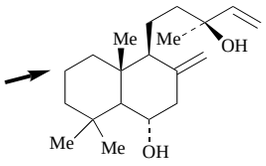
The regioselectivity of hydroxylation of phenylcyclohexane by cytochrome P450 can be altered by site-directed mutagenesis. One mutant gave the *cis*-3-alcohol (97% yield, 42% ee) while a second gave the *trans*-4-alcohol (83% yield).[108] Oxidation of 3-bromo- and 3-azido-indane afforded the *cis*-substituted compounds **46** in 50–70% yield and >98% ee.[109] 1-Substituted adamantanes are hydroxylated with high selectivity using *Absidia* sp. For example adamantane-1-carboxylic acid affords the hydroxy acid **47** in 40% yield on incubation with *A. cylindrospora* IMI 342950.[110]



46 R = Br or N_3

47

The natural product larixol **48** and the taxane **49** are hydroxylated at the arrowed positions in 60% yield and 30% yield



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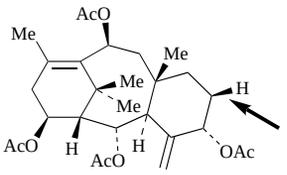
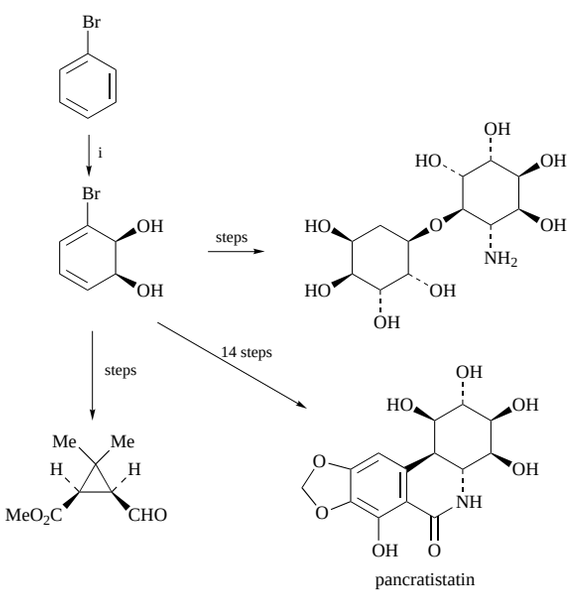


49

respectively using *Mucor plumbeus*[111] and *Cunninghamella echinulata*.[112]

The major problem concerning enzyme-catalysed hydroxylation reactions, the unpredictability of the site of hydroxylation, is being addressed by Griengl and co-workers. In a series of papers[113] the Austrian group report their first results aimed at finding readily introduced and easily removed groups that will act to direct hydroxylation to a specific position within the target molecule, at a set distance from the auxiliary.

The conversion of benzene (and derivatives) into (3-substituted) *cis*-cyclohexadiene-1,2-diols is a well-documented and well-cited biotransformation. The dienediols are extremely useful synthetic building blocks and some of the early successes have been reviewed.[114] More recently the 3-chloro compound has been used to prepare polydeuteriated mannose[115] and cyclophellitol derivatives;[116] the bromodiol has been used to make 1,2-L-*chiro*-inositol conjugates and oligomers[117] as well as pancratistatin[118] and *cis*-chrysanthemic acid[119] (**Scheme 27**).

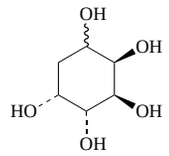


Scheme 27 *Reagents:* i, *Pseudomonas putida*, O_2, H_2O

The tetrols **50** were prepared from (1*S*,2*R*)-3-cyanocyclohexa-3,5-diene-1,2-diol by a route that was slightly longer than that previously recorded utilising toluene-*cis*-diol [(1*S*,2*R*)-3-methylcyclohexa-3,5-diene-1,2-diol] as the starting material.[120] The cyano compound has also been employed by two research groups, independently, to make (6*R*)-6-hydroxyshikimic acid.[121]

Newly reported syntheses using dioxygenases include the preparation of the diols **51**,[122] **52**[123] and **53**[124] in good yields and enantiomeric excesses.

A review is available describing the synthesis of chiral sulfoxides in optically active form using bacterial flavin monooxygenases *e.g.* cyclohexanonemonooxygenase from *Acinetobacter calcoacetius* NCIMB 9871 (CMO ex 9871).[125] The range of substrates tested using this monooxygenase has been extended to include compounds of type **54**. For X = NH_2 the (*R*)-sulfoxide is obtained (65% ee) while for X = CF_3 the (*S*)-sulfoxide is isolated (56% ee). Such results have allowed the model of the active site of this enzyme to be refined.[126] Mono-



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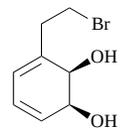

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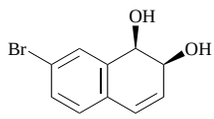

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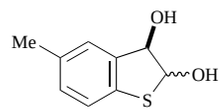

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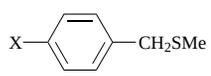

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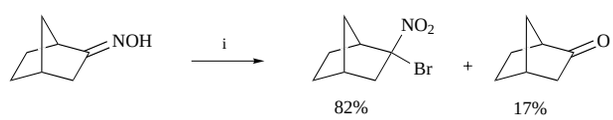
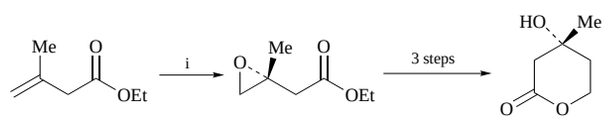
oxygenases from *Pseudomonas* sp. NCIMB 9872 and *Xanthobacter* sp. NCIMB 10811 have been compared to CMO ex 9871: in some cases, *e.g.* for $C_6H_5SCH_3$, the monooxygenase from *Pseudomonas* sp. NCIMB 9872 provides the sulfoxide of opposite configuration.[127] The same is true for cyclic disulfides; thus while 1,3-dithiolane gives the (*R*)-(+)-monosulfoxide with *A. calcoaceticus* (76% yield >98% ee) the (*S*)-(−)-monosulfoxide is prepared using *Pseudomonas* sp. NCIMB 9872.[128] The oxidations using CMO ex 9871 have been extended to 2-substituted dithiolanes and dithianes giving mainly or exclusively *trans*-monosulfoxides in high isomeric purity.[129]

Stereoselective S-oxidation may also be accomplished using the chloroperoxidase from *Caldariomyces fumago*. For instance dihydrobenzothiophene is oxidised to the (*R*)-sulfoxide in quantitative yield and 99% ee.[130] The same enzyme has been shown to convert oximes into α-halonitro compounds, in good yields in some cases (**Scheme 28**).[131] The first multi-step syn-



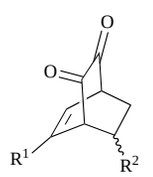
Scheme 28 *Reagents:* i, *C. fumago* chloroperoxidase, H_2O_2, H_2O, Br^-, pH 5.0

thesis featuring an enantioselective epoxidation moderated by haloperoxidase has been reported (**Scheme 29**).[132]



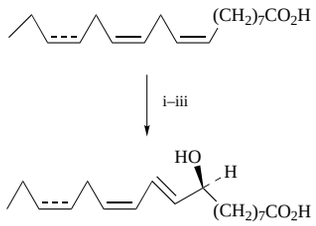
Scheme 29 *Reagents:* i, chloroperoxidase (Sigma) Bu^{t}OOH, citrate buffer, 1 h

Immobilised tyrosinase from mushrooms (*Agaricus bisporus*) has been used to oxidise *para*-substituted phenols to 4-substituted cyclohexa-3,5-diene-1,2-diones which were subsequently trapped by dienophiles to give adducts **55** in 55–82% yield.[133]



55

High substrate concentrations (up to 14 g l^{-1}) have been employed in the conversion of linoleic and α-linolenic acids into the corresponding hydroperoxides which, on reduction and

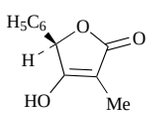


Scheme 30 *Reagents:* i, barley seeds lipoxygenase, O_2, H_2O; ii, PPh_3; iii, CH_2N_2

methylation (**Scheme 30**), yielded methyl (9*S*)-hydroxyoctadecadienoic acid (HODE) and methyl (9*S*)-hydroxyoctadecatrienoic acid (HOTE), respectively, in 39–44% yield and ⩾98% ee.[134]

8 Carbon–carbon bond-forming reactions

One of the simplest enzyme-catalysed carbon–carbon bond-forming reactions involves the addition of HCN to aldehydes and ketones. There has been a good deal of activity in this area recently, employing enzymes to afford (*R*)- or (*S*)-cyanohydrins. Synthetic opportunities using these chiral materials have been reviewed,[135] and new conversions of cyanohydrins include the transformation of PhCH(OH)CN into the tetronic acid **56** (util-

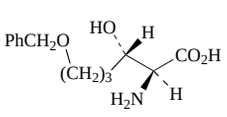


56

ising the Blaise reaction)[136] and the transfer of chirality from the cyanohydrin moiety to adjacent alkene units.[137]

The use of the (*R*)-oxynitrilase from almond meal and the (*S*)-oxynitrilase from *Sorghum bicolor* to convert aromatic aldehydes (such as *p*-acetoxybenzaldehyde) into optically active cyanohydrins is well established.[138] Two important developments in this area have been, first, the over-expression and employment of (*S*)-hydroxynitrilase from *Hevea brasiliensis* for the conversion of a wide range of aromatic aldehydes, some cyclohexane carbaldehydes and cinnamaldehyde into the corresponding cyanohydrins often in excellent yield and high enantiomeric excess.[139] Secondly, the hydroxynitrile lyase from *Manihot esculenta* (EC 4.1.2.37) has been over-expressed in *E. coli.* The enzyme catalyses the addition of HCN to alkyl-, aryl-, heteroaryl-aldehydes and methyl ketones to afford the (*S*)-cyanohydrins (yields 80–100%, ees 90–98%).[140]

More extensively substituted chiral materials may be derived from aldolase-catalysed reactions. For example, L-threonine aldolase from *Candida humicola* AKU 4586 catalyses the reaction between α-benzyloxybutanal and glycine to give the *erythro*-product **57** after 15 min, albeit in only 18% yield.[141]



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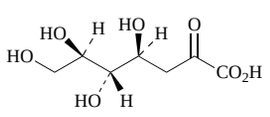
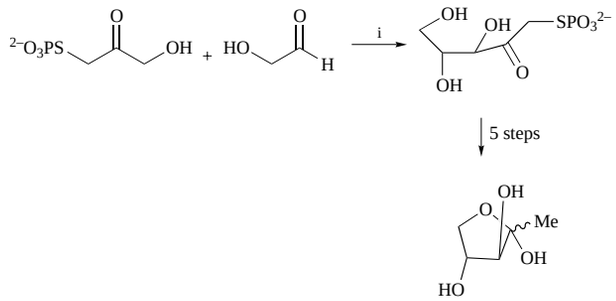


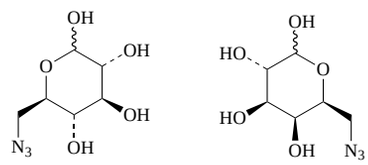
58

Fructose diphosphate aldolase has been used for the preparation of 1-deoxy-1-thioketose sugars as a route to some deoxy-sugars that were not previously accessible by enzymatic methods (**Scheme 31**).[142] 2-Keto-3-deoxy-6-phosphogluconate aldolases from *Pseudomonas putida*, *E. coli* and *Zymononas mobilis* accept low molecular weight, non-carbohydrate aldehydes (*e.g.* chloracetaldehyde) as substrates. The enzyme is useful for the production of a variety of polyhydroxy compounds such as the tetrol **58** from pyruvate and erythrose.[143] A full paper has been published on the use of different aldolases to produce compounds of the type **59** and **60** *en route* to tetrahydroxyperhydroazepines.[144]



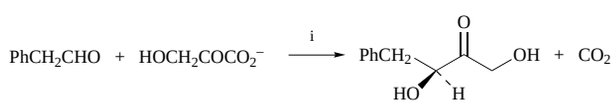
1-deoxy-D-xylulose

Scheme 31 *Reagents:* i, fructose diphosphate aldolase, H_2O



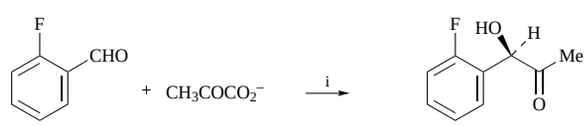
59 **60**

A transketolase expressed in *E. coli* has been used to couple hydroxypyruvate to a variety of aldehydes, *e.g.* phenylacetaldehyde, on a multi-gram scale (**Scheme 32**).[145] Pyruvate decarb-



Scheme 32 *Reagents:* i, *E. coli* transketolase, Mg^{2+}, TPP, H_2O

oxylase from *Zymomonas mobilis*, over-expressed in *E. coli*, has been used to link pyruvate to aromatic and heterocyclic aldehydes with ees 80–⩾97% (**Scheme 33**): it is noteworthy that the

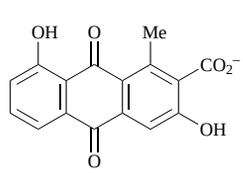


60% yield, >97% ee

Scheme 33 *Reagents:* i, pyruvate decarboxylase from *Zymomonas mobilis* over-expressed in *E. coli*

enzyme is able to use acetaldehyde as the C_2-donor as well as pyruvate.[146]

The synthesis of aromatic polyketides *in vitro* by Actinorhodin polyketide synthase has been demonstrated by conversion of malonyl-CoA into DMAC **61** by a cocktail of at least six different enzymes.[147]



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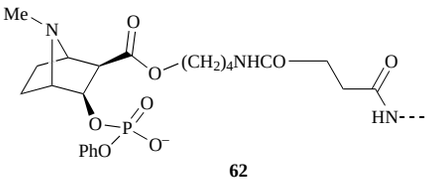
9 Enzyme mimetics

The use of catalytic antibodies in synthetic organic chemistry has some potential. Several advances have been made recently, as described below, but, generally, the research work is still confined to a few specialist laboratories.

An antibody raised to the hapten **62** catalyses the hydrolysis of cocaine (rate enhancement $>10^4$),[148] while an antibody raised to the hapten **63** catalyses the hydrolysis of the amino acid derivative **64** but not the (*R*)-enantiomer.[149]

The racemic hapten **65** induced two separate classes of



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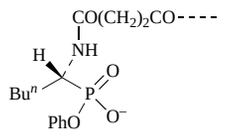

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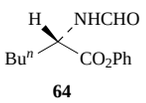
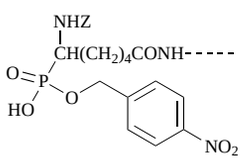


64

catalytic antibody which could hydrolyse L- or D-*N*-*Z*-amino acid esters preferentially. For example, a catalytic antibody labelled 7G12 gave the L-acid (96% ee) while the antibody 3G2 furnished the D-acid (94% ee) on hydrolysis of the (±)-ester **66**.



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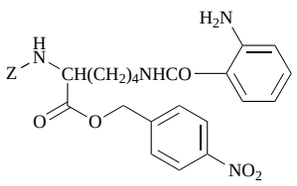
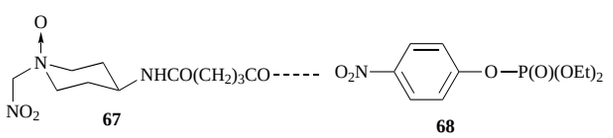


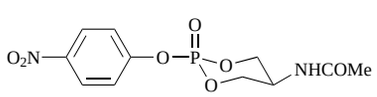
66

The former antibody showed a broad substrate specificity and, generally, a high enantioselectivity.[150]

The hapten **67** gave an antibody which catalysed the hydrolysis of paraoxon **68** (K_{cat} 4.0×10^{-4} at pH 7.8)[151] and another protein that catalysed the hydrolysis of the phosphotriester **69** (K_{cat} 2.65×10^{-3}).[152]



67 **68**



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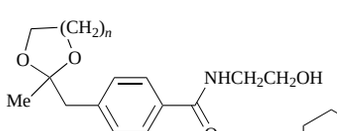
Antibodies catalysing the hydrolysis of inactivated acetals can be acquired by a very simple design. For example the hydrolysis of the acetals **70** was achieved by raising antibodies to the quaternary ammonium species **71**.[153] Two antibody aldolases, described previously, catalyse the decarboxylation of keto acids **72** and **73**. The rate enhancement (*ca.* 1.5×10^4) is impres-



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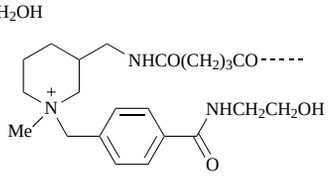

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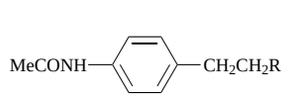
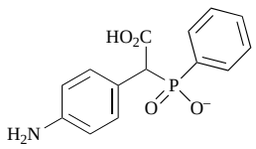


72 R = $COCH_2CO_2H$
73 R = $CH_2CH(CO_2H)COMe$

sive, while still 10^5-fold lower than that reported for acetoacetate decarboxylase.[154]

The hapten **74** was used to raise an antibody which catalysed



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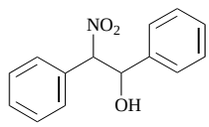
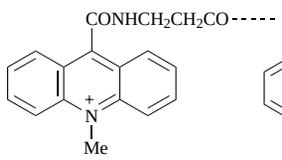


75

the retro-aldol (Henry) reaction of the alcohol **75** (rate of retroreaction *syn* > *anti*, by the ratio 2 : 1).[155]

An antibody labelled 9D9, raised against the hapten **76**, modestly catalyses the retro-Diels–Alder reaction of the polycyclic compound **77**.[156] This catalyst is the first of its kind: a



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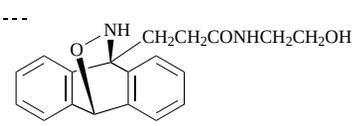
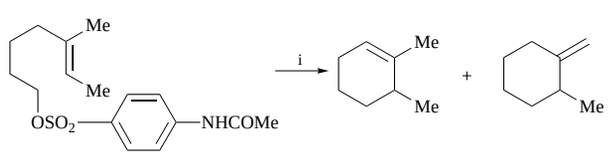


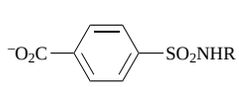
77

review on the possible existence of natural Diels–Alderases has been published.[157] An antibody has been isolated and characterised that catalyses a terpene-like electrophilic cyclisation (**Scheme 34**).[158]



Scheme 34 *Reagents:* i, IgG HAI-17G8

Antibodies raised to conjugates of the hapten **78** catalyse thiol additions to 4-nitrostyrene.[159]



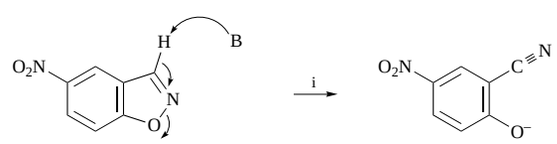
78

The formation of cyanohydrins in optically active form using the cyclic dipeptide cyclo-Phe-His has been investigated in more detail, particularly with regard to the best physical state of the catalyst and the relative importance of H-bonding motifs.[160]

The polypeptide poly(Asp-Leu-His-Leu-Ser-Leu) was constructed so as to simulate the catalytic triad present in serine proteases. The polymer hydrolyses the *p*-nitrophenyl ester of phenylalanine with modest enantioselectivity $K_L/K_D \geqslant 1.72$.[161]

The use of polyleucine for the asymmetric epoxidation of α,β-unsaturated ketones has been re-investigated.[162] Some of the optically active epoxides derived from this methodology have been used to prepare oxygen-containing heterocycles, for example *trans*- and *cis*-dihydroflavanols.[163]

Bovine serum albumin (BSA) recognises different enantiomers of racemic materials and has proved useful as a solid support for resolution of enantiomers by chromatography. Now the protein has been shown to preferentially bind to the P-form of the racemic helicene 2-hydroxymethylthieno[3,2-*e*:4,5-*e*′]di-[1]benzothiophene.[164] BSA also promotes the deprotonation of benzoisoxazoles (**Scheme 35**).[165] The enantioselective epoxid-



Scheme 35 *Reagents:* i, bovine serum albumin, H_2O, pH 10

ation of simple and functionalised alkenes using *m*-chloroperoxybenzoic acid in liposomes has been claimed.[166]

10 Conclusion and outlook

Papers detailing enzyme-catalysed conversions as key features contributed *ca.* 5% to the material published in 1996 in the Journals listed in reference 1. This percentage is likely to rise slightly as more laboratories try out enzyme-catalysed transformations and as the fine chemical industry utilises one or more biocatalysis steps as crucial elements in large scale preparations of commercially important compounds.

The range of enzyme-catalysed reactions that may be carried out on a substantial scale continues to expand and this should, increasingly, give synthetic organic chemists the opportunity to consider completely different strategies to manufacture target molecules.

11 References

1 Journals reviewed: *Tetrahedron: Asymmetry*, *Tetrahedron*, *Tetrahedron Lett*., *Chem. Commun.*, *Synthesis*, *Synlett*, *J. Chem. Soc*., *Perkin Trans. 1* and *2*, *J. Org. Chem*., *J. Am. Chem. Soc*., *Chem. Lett*., *Synth. Commun.*, *Angew. Chem*., *Int. Ed. Engl*., *Helv. Chim. Acta*, *Bull. Chem. Soc. Jpn*., *Bioorg. Med. Chem*., *Bioorg. Med. Chem. Lett*., *J. Chem. Res.*, *J. Med. Chem.*, *Can. J. Chem.*

2 *Biocatal. Biotransform*., *J. Mol. Catal. B: Enzym*., *Biotechnol. Lett.*, *Appl. Microbiol. Biotechnol.*

3 A. M. Koskinen and A. M. Klibanov, *Enzymatic Reactions in Organic Media*, Blackie, London, 1996.

4 A. J. Kirby, *Angew. Chem*., *Int. Ed. Engl*., 1996, **35**, 706.

5 *Biotransformation Abstracts*, 1996/7, **Volumes 10, 11**, Chapman and Hall, London.

6 Available from Synopsys Scientific Systems, Leeds, U.K.

7 Available from Chapman and Hall, Electronic Publishing Division, London and New York.

8 *Preparative Biotransformations*, eds. S. M. Roberts, K. Wiggins, G. Casy, S. Phythian, C. Todd and M. B. Nielsen, Wiley, Chichester, 1992 and annual updates.

9 B. Ganem, *Angew. Chem*., *Int. Ed. Engl.*, 1996, **35**, 937.

10 M. Schelhaas, S. Glomsda, M. Hänsler, H.-D. Jakubhe and H. Waldmann, *Angew. Chem.*, *Int. Ed. Engl*., 1996, **35,** 106.

11 T. Pohl and H. Waldmann, *Angew. Chem.*, *Int. Ed. Engl*., 1996, **35,** 1720.

12 O. Houille, T. Schmittberger and D. Uguen, *Tetrahedron Lett*., 1996, **37,** 625.

13 P. Clapés, I. Valverde, C. Jaime and J. L. Torres, *Tetrahedron Lett*., 1996, **37**, 417.

14 D. Basavaiah, S. Pandiaraju and K. Muthukumaran, *Tetrahedron: Asymmetry*, 1996, **7**, 13.

15 M. Kinoshita and A. Ohno, *Tetrahedron*, 1996, **52**, 5397.

16 V. S. Parmar, A. Singh, K. S. Bisht, N. Kumar, Y. N. Belokon, K. A. Kochetkov, N. S. Ikonnikov, S. A. Orlova, V. I. Tararov and T. F. Saveleva, *J. Org. Chem.*, 1996, **61**, 1223.

17 J. A. S. Howell, M. G. Palin, G. Jaouen, B. Malezieux, S. Top, J. M. Cense, J. Salin, P. McArdle, D. Cunningham and M. O'Gara, *Tetrahedron: Asymmetry*, 1996, **7**, 95.

18 C. Bonini, A. Giugliano, R. Racioppi and G. Righi, *Tetrahedron Lett*., 1996, **37**, 2487.

19 J. V. Allen and J. M. J. Williams, *Tetrahedron Lett*., 1996, **37**, 1859.

20 A. Patti, C. Sarfilippo, M. Piattelli and G. Nicolosi, *Tetrahedron: Asymmetry*, 1996, **7**, 2665.

21 E. Schoffers, A. Golebiowski and C. R. Johnson, *Tetrahedron*, 1996, **52,** 3769.

22 D. T. Chapman, D. H. G. Crout, M. Mahmoudian, D. I. C. Scopes and P. W. Smith, *Chem. Commun.*, 1996, 2415.

23 T. Miyazawa, S. Nakajo, M. Nishikawa, K. Imagawa, R. Yanagihara and T. Yamada, *J. Chem. Soc.*, *Perkin Trans. 1*, 1996, 2867.

24 C. Ebert, L. Gardossi and P. Linda, *Tetrahedron Lett.*, 1996, **37**, 9377.

25 K. Kobata, K. Yoshikawa, M. Kohashi and T. Watanabe, *Tetrahedron Lett*., 1996, **37**, 2789.

26 M. Adamczyk and J. Grote, *Tetrahedron Lett*., 1996, **37**, 7913.

27 L. T. Kanerva, P. Csomós, O. Sundholm, G. Bernáth and F. Fülöp, *Tetrahedron: Asymmetry*, 1996, **7**, 1705.

28 S. Takayama, W. J. Moree and C.-H. Wong, *Tetrahedron Lett.*, 1996, **37**, 6287.

29 B. Orsat, P. B. Alper, W. Moree, C.-P. Mak and C.-H. Wong, *J. Am. Chem. Soc*., 1996, **118**, 712.

30 I. Alfonso, C. Astorga, F. Rebolledo and V. Gotor, *Chem. Commun.*, 1996, 2471.

31 S. Puertas, F. Rebolledo and V. Gotor, *J. Org. Chem*., 1996, **61**, 6024.

32 Y.-F. Wang, K. Yakovlevsky and A. L. Margolin, *Tetrahedron Lett*., 1996, **37**, 5317.

33 C. Vorde, H.-E. Hogberg and E. Hedenstrom, *Tetrahedron: Asymmetry*, 1996, **7**, 1507.

34 C. A. Costello, A. J. Kreuzman and M. J. Zmijewski, *Tetrahedron Lett*., 1996, **37**, 7469.

35 S. J. Maddrell, N. J. Turner, A. Kerridge, A. J. Willetts and J. Crosby, *Tetrahedron Lett*., 1996, **37**, 6001.

36 K. Faber, M. Mischitz and W. Kroutil, *Acta Chem. Scand*., 1996, **50**, 249.

37 G. Bellucci, C. Chiappe, A. Cordini and G. Ingrosso, *Tetrahedron Lett*., 1996, **37**, 9089; G. Bellucci, C. Chiappe and A. Cordoni, *Tetrahedron: Asymmetry*, 1996, **7**, 197.

38 S. Pedragosa-Moreau, A. Archelas and R. Furstoss, *Tetrahedron Lett*., 1996, **37**, 3319.

39 S. Pedragosa-Moreau, A. Archelas and R. Furstoss, *Tetrahedron*, 1996, **52,** 4593; S. Pedragosa-Moreau, C. Morisseau, J. Zylber, A. Archelas, J. Baratti and R. Furstoss, *J. Org. Chem*., 1996, **61**, 7402.

40 W. Kroutil, M. Mischitz, P. Plachota and K. Faber, *Tetrahedron: Asymmetry*, 1996, **7**, 2041.

41 I. V. J. Archer, D. J. Leak and D. A. Widdowson, *Tetrahedron Lett*., 1996, **37**, 8819.

42 G. Vic, J. J. Hastings, O. W. Howarth and D. H. G. Crout, *Tetrahedron: Asymmetry*, 1996, **7**, 709; G. Vic, J. J. Hastings and D. H. G. Crout, *Tetrahedron: Asymmetry*, 1996, **7**, 1973.

43 S. Takayama, M. Shimazaki, L. Qiao and C.-H. Wong, *Bioorg. Med. Chem. Lett*., 1996, **6**, 1123.

44 T. Kimura, S. Takayama, H. Huang and C.-H. Wong, *Angew. Chem.*, *Int. Ed. Engl*., 1996, **35**, 2348.

45 S. Singh, M. Scigelova, G. Vic and D. H. G. Crout, *J. Chem. Soc*., *Perkin Trans. 1*, 1996, 1921.

46 S. Singh, M. Scigelova and D. H. G. Crout, *Chem. Commun*., 1996, 993.

47 G. Vic, M. Scigelova, J. J. Hastings, O. W. Howarth and D. H. G. Crout, *Chem. Commun*., 1996, 1473.

48 K. Okahata and T. Muri, *J. Chem. Soc.*, *Perkin Trans. 1*, 1996, 2861.

49 A. Zervosen and L. Elling, *J. Am. Chem. Soc*., 1996, **118**, 1836.

50 C. Unverzagt, *Angew. Chem.*, *Int. Ed. Engl*., 1996, **35,** 2350.

51 K. K.-C. Lin and S. J. Danishefsky, *Chem. Eur. J*., 1996, **2**, 1359.

52 G. Baisch and R. Öhrlein, *Angew. Chem.*, *Int. Ed. Engl*., 1996, **35,** 1813; M. M. Palcic and O. Hindsgaul, *Trends Glycosci. Glycotechnol.*, 1996, **8**, 37.

53 U. Sprengard, M. Schudok, W. Schmidt, G. Kretzschmar and H. Kunz, *Angew. Chem*., *Int. Ed. Engl*., 1996, **35,** 321.

54 G. Baisch, R. Öhrlein and A. Katopidis, *Bioorg. Med. Chem. Lett*., 1996, **6**, 2953.

55 A. Zervosen, A. Stein, A. Adrian and L. Elling, *Tetrahedron,* 1996, **52**, 2395.

56 M. Ors, A. Morcuende, M. I. Jiménez-Vacas, S. Valverde and B. Herradón, *Synlett*., 1996, 449.

57 Y. Kita, Y. Takebe, K. Murata, T. Naka and S. Akai, *Tetrahedron Lett*., 1996, **37,** 7369.

58 E. Guibé-Jampel, Z. Chalecki, M. Bassir and M. Gelo-Pujic, *Tetrahedron*, 1996, **52**, 4397.

59 S. Takayama, P. O. Livinston and C.-H. Wong, *Tetrahedron Lett*., 1996, **37**, 9271.

60 H. E. Master, R. V. Newadkar, R. A. Rane and A. Kumar, *Tetrahedron Lett.*, 1996, **37**, 9253.

61 T. Fukazawa, Y. Shimoji and T. Hashimoto, *Tetrahedron: Asymmetry*, 1996, **7**, 1649.

62 B. E. Carpenter, I. R. Hunt and B. A. Keay, *Tetrahedron: Asymmetry*, 1996, **7**, 3107; *cf.* a chemical method using PhCOBr, $SnBr_2$ and a chiral ligand, T. Oriyama, Y. Hori, K. Imai and R. Sasaki, *Tetrahedron Lett*., 1996, **37**, 8543.

63 P. Crotti, V. Di Bussolo, L. Favero, F. Minutolo and M. Pineschi, *Tetrahedron: Asymmetry*, 1996, **7**, 1347.

64 M. Barz, E. Herdtweck and W. R. Theil, *Tetrahedron: Asymmetry*, 1996, **7**, 1717.

65 P. Stead, H. Marley, M. Mahmoudian, G. Webb, D. Noble, Y. T. Ip, E. Piga, T. Rossi, S. M. Roberts and M. J. Dawson, *Tetrahedron: Asymmetry*, 1996, **7**, 2247.

66 G. Kennedy, T. Rossi and B. Tamburini, *Tetrahedron Lett*., 1996, **37**, 7441; D. Andreotti, T. Rossi and C. Marchioro, *Bioorg. Med. Chem. Lett*., 1996, **118**, 9884.

67 T. Sugai, H. Ikeda and H. Ohta, *Tetrahedron*, 1996, **52**, 8123.

68 H. Sakagami, K. Samiza, T. Kamibubo and K. Ogasawasa, *Synlett*, 1996, 163.

69 Y. Takagi, J. Teramoto, H. Kihara, T. Ifoh and H. Tsukube, *Tetrahedron Lett.*, 1996, **37**, 4991; see also T. Ifoh, Y. Takagi, T. Murakami, Y. Hiyama and K. Tsukube, *J. Org. Chem.*, 1996, **61**, 2158.
70 A. F. Drake, A. Garofalo, J. M. L. Hillman, V. Merlo, R. McCague, S. M. Roberts and A. J. Thorpe, *J. Chem. Soc.*, *Perkin Trans. 1*, 1996, 2739.
71 F. Theil, H. Sonnenschein and T. Kreher, *Tetrahedron: Asymmetry*, 1996, **7**, 3365.
72 J. W. J. F. Thuring, A. J. H. Klunder, G. H. L. Nefkens, M. A. Wegman and B. Zwanenburg, *Tetrahedron Lett.*, 1996, **37**, 4759; H. Van der Deen, A. D. Cuiper, R. P. Hof, A. van Oeveren, B. L. Feringa and R. M. Kellogg, *J. Am. Chem. Soc.*, 1996, **118**, 3801.
73 T. Yokomatsu, M. Sato and S. Shibuya, *Tetrahedron: Asymmetry*, 1996, **7**, 2743.
74 W. Kreiser, A. Wiggermann, A. Krief and D. Swinnen, *Tetrahedron Lett.*, 1996, **37**, 7119.
75 L. Domon, F. Vogeleisen and D. Uguen, *Tetrahedron Lett.*, 1996, **37**, 2773.
76 M. T. Reetz, A. Zonta, J. Simpelkaup and W. Konen, *Chem. Commun.*, 1996, 1397; *Biotechnol. Bioeng.*, 1996, **42**, 527.
77 N. Khalaf, C. P. Govardhan, I. J. Lalonde, R. A. Persichetti, Y.-F. Wang and A. L. Margolin, *J. Am. Chem. Soc.*, 1996, **118**, 5494; see also R. A. Persichetti, J. J. Lalonde, C. P. Govardhan, N. K. Khalaf and A. L. Margolin, *Tetrahedron Lett.*, 1996, **37**, 6507.
78 P. M. Dinh, J. A. Howarth, A. R. Hudnott, J. M. J. Williams and W. Harris, *Tetrahedron Lett.*, 1996, **37**, 7623.
79 J. Partridge, G. A. Hutcheon, B. D. Moore and P. J. Halling, *J. Am. Chem. Soc.*, 1996, **118**, 12 873.
80 K. Griebenow and A. M. Klibanov, *J. Am. Chem. Soc.*, 1996, **118**, 11 695.
81 K. Xu and A. M. Klibanov, *J. Am. Chem. Soc.*, 1996, **118**, 9815.
82 C. R. Wescott, H. Noritomi and A. M. Klibanov, *J. Am. Chem. Soc.*, 1996, **118**, 10 365.
83 H. Uyama, H. Kikuchi, K. Takeya, N. Hoshi and S. D. Kobayashi, *Chem. Lett.*, 1996, 10.
84 S. Matsumura, H. Beppu, K. Nakamura, S. Osanai and K. Tushima, *Chem. Lett.*, 1996, 795.
85 C. Abalain, D. Buisson and R. Azerad, *Tetrahedron: Asymmetry*, 1996, **7**, 2983.
86 Y. Y. Zhu and D. J. Burnell, *Tetrahedron: Asymmetry*, 1996, **7,** 3295.
87 R. Hayakawa, M. Shimizu and T. Fujisawa, *Tetrahedron Lett.*, 1996, **37**, 7533.
88 M. North, *Tetrahedron Lett.*, 1996, **37**, 1699.
89 H. Watanabe, T. Watanabe and K. Mori, *Tetrahedron*, 1996, **52,** 13 939.
90 K. Nakamura, K. Kitano, T. Matsuda and A. Ohno, *Tetrahedron Lett.*, 1996, **37**, 1629.
91 G. Fantin, M. Fogagnolo, P. P. Giovannini, A. Medici, P. Pedrini, F. Gardini and R. Lanciotti, *Tetrahedron*, 1996, **52**, 3547.
92 T. Sugai, Y. Ohtsuka and H. Ohta, *Chem. Lett.*, 1996, 233.
93 H. Ikeda, E. Sato, T. Sugai and H. Ohta, *Tetrahedron*, 1996, **52**, 8113.
94 K. Seelbach, B. Riebel, W. Hummel, M.-R. Kula, V. I. Tishkov, A. M. Egorov, C. Wandrey and U. Kragl, *Tetrahedron Lett.*, 1996, **37,** 1377.
95 N. M. Kelly, R. G. Reid, C. L. Willis and P. L. Winton, *Tetrahedron Lett.*, 1996, **37**, 1517.
96 J. Kikuchi, Y. Kamijyo, H. Etoh and Y. Murakami, *Chem. Lett.*, 1996, 427.
97 P. Ferraboschi, S. R. T. Elahi, E. Verza, F. Meroni-Rivolla and E. Santaniello, *Synlett*, 1996, 1176.
98 Y. Kawai, K. Saitou, K. Hida, D. H. Dao and A. Ohno, *Bull. Chem. Soc. Jpn.*, 1996, **69**, 2633.
99 M. Baruah, A. Buruah, D. Prajapati and J. S. Sandhu, *Synlett*, 1996, 1193.
100 V. Alphand, R. Furstoss, S. Pedragosa-Moreau, S. M. Roberts and A. J. Willetts, *J. Chem. Soc.*, *Perkin Trans. 1*, 1996, 1867.
101 M. T. Bes, R. Villa, S. M. Roberts, P. W. H. Wan and A. J. Willetts, *J. Mol. Catal. B: Enzym.*, 1996, **1**, 127.
102 J. D. Steward, K. W. Reed and M. M. Kayser, *J. Chem. Soc.*, *Perkin Trans. 1*, 1996, 755; J. D. Steward, K. W. Reed, J. Zhu, G. Chen and M. M. Kayser, *J. Org. Chem.*, 1996, **61**, 7652.
103 G. Ottolina, G. Carrea, S. Colonna and A. Ruckemann, *Tetrahedron: Asymmetry*, 1996, **7**, 1123.
104 J. Lebreton, V. Alphand and R. Furstoss, *Tetrahedron Lett.*, 1996, **37,** 1011.
105 D. R. Kelly, C. J. Knowles, J. G. Mahdi, M. A. Wright, I. N. Taylor, S. M. Roberts, P. W. H. Wan, G. Grogan, S. Pedragosa-Moreau and A. J. Willetts, *Chem. Commun.*, 1996, 2333.
106 D. R. Kelly, *Tetrahedron: Asymmetry*, 1996, **37**, 1149.
107 W. Adam, M. Lazarus, C. R. Saha-Möller and P. Schreier, *Tetrahedron: Asymmetry*, 1996, **7**, 2287.
108 M. E. Jones, P. A. England, D. A. Rouch and L.-L. Wong, *Chem.Commun.*, 1996, 2413; P. A. England, D. A. Rouch, A. C. G. Westlake, E. G. Bell, D. P. Nickerson, M. Webberley, S. L. Flitsch and L.-L. Wong, *Chem. Commun.*, 1996, 357.
109 D. R. Boyd, N. D. Sharma, N. I. Bowers, P. Goodrich, M. R. Groocock, A. J. Blacker, D. A. Clarke, T. Howard and H. Dalton, *Tetrahedron: Asymmetry*, 1996, **7**, 1559.
110 P. D. Bailey, S. D. Higgins, C. H. Ridyard, S. M. Roberts, G. M. Rosair, R. A. Whittaker and A. J. Willetts, *Chem. Commun.*, 1996, 1833; C. H. Ridyard, R. A. Whittaker, S. D. Higgins, S. M. Roberts, A. J. Willetts, P. D. Bailey and G. M. Rosair, *J. Chem. Soc.*, *Perkin Trans. 2*, 1996, 1811.
111 D. Herlem, J. Ouazzani and F. Khuong-Huu, *Tetrahedron Lett.*, 1996, **37**, 1241.
112 S. Hu, X. Tian, W. Zhu and Q. Fang, *Tetrahedron*, 1992, **52**, 8739.
113 A. de Raadt, H. Griengl, M. Petsch, P. Plachota, N. Schoo, H. Weber, G. Braunegg, I. Kopper, M. Kreiner, A. Zeiser and (in part) K. Kieslich, *Tetrahedron: Asymmetry*, 1996, **7**, 467, 473, 491.
114 T. Hudlicky and A. J. Thorpe, *Chem. Commun.*, 1996, 1993; T. Hudlicky, K. A. Abboud, D. A. Entwistle, R. Fan, R. Maurya, A. J. Thorpe, J. Bolonick and B. Myers, *Synthesis*, 1996, 897.
115 T. Hudlicky, K. K. Pitzer, M. R. Stabile, A. J. Thorpe and G. M. Whited, *J. Org. Chem.*, 1996, **61**, 4151.
116 S. M. Roberts, P. W. Sutton and L. Wright, *J. Chem. Soc.*, *Perkin Trans. 1*, 1996, 1157.
117 T. Hudlicky, K. A. Abboud, J. Bolonick, R. Maurya, M. L. Stanton and A. J. Thorpe, *Chem. Commun.*, 1996, 1717.
118 T. Hudlicky, X. Tian, K. Königsberger, R. Maurya, J. Rouden and B. Fan, *J. Am. Chem. Soc.*, 1996, **118,** 10 752.
119 M. G. Banwell and G. S. Forman, *J. Chem. Soc.*, *Perkin Trans. 1*, 1996, 2565.
120 C. H. Tran and D. H. G. Crout, *Tetrahedron: Asymmetry*, 1996, **7**, 2403.
121 H. A. J. Carless and Y. Dove, *Tetrahedron: Asymmetry*, 1996, **7**, 649; C. H. Tran, D. H. G. Crout, W. Errington and G. Whited, *Tetrahedron: Asymmetry*, 1996, **7**, 691.
122 T. Hudlicky, M. A. A. Endoma and G. Butora, *J. Chem. Soc.*, *Perkin Trans. 1*, 1996, 2187.
123 T. Hudlicky, M. A. A. Endoma and G. Butora, *Tetrahedron: Asymmetry*, 1996, **7**, 61.
124 D. R. Boyd, N. D. Sharma, I. N. Brannigan, S. A. Haughey, J. F. Malone, D. A. Clarke and H. Dalton, *Chem. Commun.*, 1996, 2361.
125 S. Colonna, N. Gaggero, P. Pasta and G. Ottolina, *Chem. Commun.*, 1996, 2303.
126 G. Ottolina, P. Pasta, D. Varley and H. L. Holland, *Tetrahedron: Asymmetry*, 1996, **7**, 3427.
127 D. R. Kelly, C. J. Knowles, J. G. Mahdi, I. N. Taylor and M. A. Wright, *Tetrahedron: Asymmetry*, 1996, **7**, 365.
128 V. Alphand, N. Gaggero, S. Colonna and R. Furstoss, *Tetrahedron Lett.*, 1996, **37**, 6117.
129 S. Colonna, N. Gaggero, G. Carrea and P. Pasta, *Tetrahedron: Asymmetry*, 1996, **7**, 565; *cf.* P. C. B. Page, R. D. Wilkes, E. S. Namwindwa and M. J. Witty, *Tetrahedron*, 1996, **52**, 2125.
130 S. G. Allenmark and M. A. Andersson, *Tetrahedron: Asymmetry*, 1996, **7**, 1089.
131 A. Zaks, A. V. Yabannavar, D. R. Dodds, C. A. Evans, P. R. Das and R. Malchow, *J. Org. Chem.*, 1996, **61**, 8692.
132 F. J. Lakner and L. P. Hager, *J. Org. Chem.*, 1996, **61**, 3923.
133 G. H. Müller and H. Waldmann, *Tetrahedron Lett.*, 1996, **37**, 3833.
134 D. Martini, G. Buono, J.-L. Montillet and G. Iacazio, *Tetrahedron: Asymmetry*, 1996, **7**, 1489.
135 F. Effenberger, *Enantiomer*, 1996, **1**, 359.
136 J. J. Duffield and A. C. Regan, *Tetrahedron: Asymmetry*, 1996, **7**, 663.
137 E. G. J. C. Warmerdam, A. M. C. H. Van den Nieuwendijk, J. Brussee and C. G. Kruse, *Recl. Trav. Chim. Pays-Bas*, 1996, **115**, 20; E. G. J. C. Warmerdam, A. M. C. H. Van den Nieuwendijk, J. Brussee, C. G. Kruse and A. van der Gen, *Tetrahedron: Asymmetry*, 1996, **7**, 2539; see also E. G. J. C. Warmerdam, R. D. van Rijn, J. Brussee, C. G. Kruse and A. van der Gen, *Tetrahedron: Asymmetry*, 1996, **7**, 1723.
138 E. Kiljunen and L. T. Kanerva, *Tetrahedron: Asymmetry*, 1996, **7**, 1105; see also B. Danieli, C. Barra, G. Carrea and S. Riva, *Tetrahedron: Asymmetry*, 1996, **7**, 1675.
139 M. Schmidt, S. Hervé, N. Klempier and H. Griengl, *Tetrahedron*, 1996, **52**, 7833.

140 S. Förster, J. Roos, F. Effenberger, H. Wajant and A. Sprauer, *Angew. Chem.*, *Int. Ed. Engl*., 1996, **35**, 437.
141 K. Shibata, K. Shingu, V. P. Vassilev, K. Nishide, T. Fujita, M. Node, T. Kajimoto and C.-H. Wong, *Tetrahedron Lett*., 1996, **37**, 2791.
142 R. Duncan and D. G. Drueckhammer, *J. Org. Chem*., 1996, **61**, 438.
143 M. C. Shelton, I. C. Cotterill, S. T. A. Novak, R. M. Poonawala, S. Sudarshan and E. J. Toone, *J. Am. Chem. Soc*., 1996, **118**, 2117.
144 F. Morís-Varas, X.-H. Qian and C.-H. Wong, *J. Am. Chem. Soc*., 1996, **118**, 7647.
145 K. G. Morris, M. E. B. Smith, N. J. Turner, M. D. Lilly, R. K. Mitra and J. M. Woodley, *Tetrahedron: Asymmetry*, 1996, **7**, 2185.
146 S. Bornemann, D. H. G. Crout, H. Dalton, V. Kren, M. Lobell, G. Dean, N. Thomson and M. M. Turner, *J. Chem. Soc.*, *Perkin Trans. 1*, 1996, 425; see also M. Lobell and D. H. G. Crout, *J. Chem. Soc*., *Perkin Trans. 1*, 1996, 1577.
147 C. W. Carreras, R. Pieper and C. Khosla, *J. Am. Chem. Soc*., 1996, **118**, 5158.
148 G. Yang, J. Chun, H. Arakawa-Uramoto, X. Wang, M. A. Gawinowicz, K. Zhao and D. W. Landry, *J. Am. Chem. Soc*., 1996, **118**, 5881.
149 H. Wade and T. S. Scanlan, *J. Am. Chem. Soc*., 1996, **118**, 6510.
150 F. Tanaka, K. Kinoshita, R. Tanimura and I. Fujii, *J. Am. Chem. Soc*., 1996, **118,** 2332.
151 B. J. Lavey and K. D. Janda, *J. Org. Chem*., 1996, **61**, 7633.
152 B. J. Lavey and K. D. Janda, *Bioorg. Med. Chem. Lett*., 1996, **6**, 1523.
153 D. Shabat, S. C. Sinha, J.-L. Reymond and E. Keinan, *Angew. Chem.*, *Int. Ed. Engl*., 1996, **35**, 2628.
154 R. Björnestedt, G. Zhong, R. A. Lerner and C. F. Barbas III, *J. Am. Chem. Soc*., 1996, **118**, 11 720; L. A. Highbarger, J. A. Gerlt and G. L. Kenyon, *Biochemistry*, 1996, **35**, 61.
155 M. E. Flanagan, J. R. Jacobsen, E. Sweet and P. G. Schultz, *J. Am. Chem. Soc*., 1996, **118**, 6078.
156 N. Bahr, R. Güller, J.-L. Reymond and R. A. Lerner, *J. Am. Chem. Soc*., 1996, **118**, 3550.
157 S. Laschat, *Angew. Chem.*, *Int. Ed. Engl*., 1996, **35**, 289.
158 J. Hasserodt, K. D. Janda and R. A. Lerner, *J. Am. Chem. Soc*., 1996, **118**, 11 654.
159 E. Fan, Y. Oei, E. Sweet, T. Uno and P. G. Schultz, *J. Am. Chem. Soc*., 1996, **118**, 5474.
160 Y. Shvo, M. Gal, Y. Becker and A. Elgavi, *Tetrahedron: Asymmetry*, 1996, **7**, 911.
161 Y. Fukushima, *Bull. Chem. Soc. Jpn*., 1996, **69**, 2269.
162 M. E. Lasterra-Sánchez, U. Felfer, P. Mayon, S. M. Roberts, S. R. Thornton and C. J. Todd, *J. Chem. Soc*., *Perkin Trans. 1*, 1996, 343; W. Kroutil, M. E. Lasterra-Sánchez, S. J. Maddrell, P. Mayon, P. Morgan, S. M. Roberts, S. R. Thornton, C. J. Todd and M. Tüter, *J. Chem. Soc*., *Perkin Trans. 1*, 1996, 2837.
163 H. van Rensburg, P. S. van Heerden, B. C. B. Bezuidenhoudt and D. Ferreira, *Chem. Commun*., 1996, 2747; T. Patonay, A. Lvai, C. Nemes, T. Timár, G. Tóth and W. Adam, *J. Org. Chem*., 1996, **61**, 5375.
164 K. Yamada, R. Ishii, H. Nakagawa and H. Kawazura, *Tetrahedron: Asymmetry*, 1996, **7**, 737.
165 K. Kikuchi, S. N. M. Thorn and D. Hilvert, *J. Am. Chem. Soc*., 1996, **118**, 8184.
166 A. Kumar and V. Bhakuni, *Tetrahedron Lett*., 1996, **37**, 4751.