Perkin 1 Abstracts: Biocatalysis in Organic Synthesis

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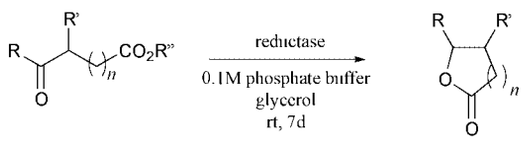
Perkin 1 Abstracts: Biocatalysis in Organic Synthesis aims to cover recent literature concerning the applications of enzymes and micro-organisms as catalysts in organic synthesis. The abstracts will emphasise the key synthetic step(s) that are mediated by the biocatalyst. Emerging technologies for biocatalyst design and optimisation will also be included.

Microbial reductions of γ- and δ-keto acids and their esters

Reductase



1 *n* = 1, R" = H (a-**e**)
2 *n* = 1, R" = Et (a-**e**)
3 *n* = 2, R" = H (**e**)
4 *n* = 2, R" = Et (**e**)

	R	R'
a	C_5H_{11}	H
b	C_5H_{11}	Me
c	Ph	H
d	Ph	Me
e	- $(CH_2)_4$ -	

A series of yeasts were investigated for their ability to reduce γ- and δ-keto acids. The hydroxy acids produced spontaneously cyclise to give the much sought after γ- and δ-lactones in enantiomerically pure form. The most versatile yeast found was *Saccharomyces cerevisiae*. The yeasts *Kluyveromyces marxianus*, *Pichia etchellsii*, *P. glucozyma* and *P. minuta* were good alternatives for enantioselective reduction of **1c**, **2a**, **2b** and **2e** respectively.

C. Forzato, R. Gandolfi, F. Molinari, P. Nitti, G. Pitacco and E. Valentin, *Tetrahedron: Asymmetry*, 2001, **12**, 1039.

Glycosylation of subtilisin protease causes broadening in stereospecificity in peptide synthesis

Subtilisin mutant

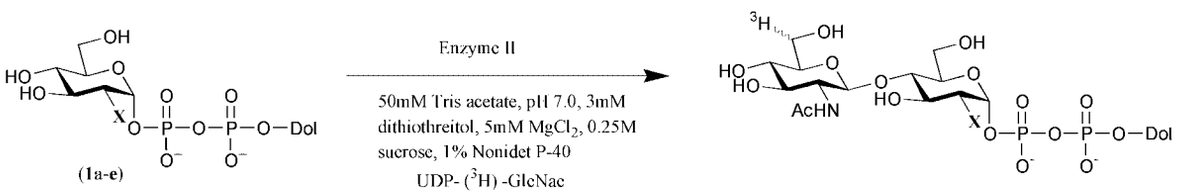
a: -$(CH_2)_2$-O-β-D-Glc$(Ac)_2$, 80% yield
b: -$(CH_2)_2$-O-β-D-Glc$(Ac)_3$, 77% yield
c: -$(CH_2)_2$-O-β-D-Gal$(Ac)_3$, 72% yield
d: -$(CH_2)_2$-O-β-D-Gal, 70% yield

Site-selective glycosylation at position 166 of serine protease subtilisin *Bacillus lentus* (SBL) produced S166C-S-a-**d**. SBL did not accept D-amino acids as acyl donors. In contrast to this, all of the glyco-SBLs S166C-s-a-**d** were able to catalyse the coupling of these amino acids.

K. Matsumoto, B. G. Davis and J. B. Jones, *Chem. Commun.*, 2001, 903.

Specificity of *N*-acetylglucosaminyl(diphosphodolichol)*N*-acetylglucosaminyl transferase (Enzyme II)

Transferase



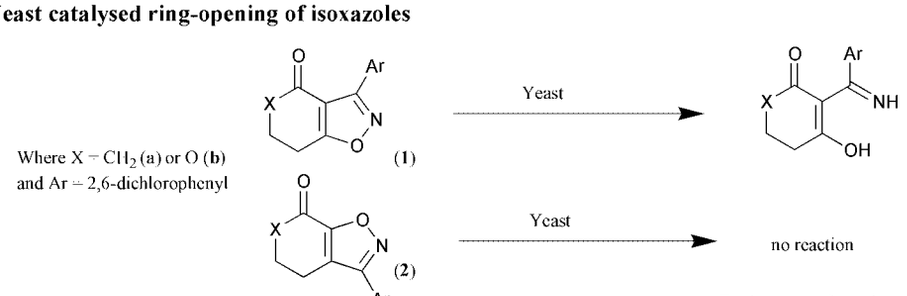
X = $NHCOCH_3$ (**a**), F (**b**), OEt (**c**), $NHCOCF_3$ (**d**) or NH_2 (**e**)

1(b-d) were shown to be inhibitors when studied in competition with the natural substrate **1**(**a**). Enzyme II was found to be highly specific for its glycosyl acceptor and the acetimido group shown to be a key functional determinate for this glycosylation reaction.

V. W.-F. Tai, M. K. O'Reilly and B. Imperiali, *Bioorg. Med. Chem.*, 2001, **9**, 1133.

Yeast catalysed ring-opening of isoxazoles

Yeast

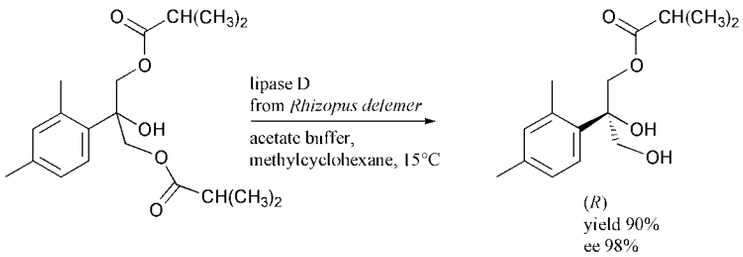


Furthermore, electrolysis of **1a** and **1b** gave **3a** and **3b** in yields of 61 and 66% respectively. By contrast electrolysis of **2a** and **2b** gave complex mixtures of compounds.

C. J. Easton, G. A. Heath, C. M. M. Hughes, C. K. Y. Lee, G. P. Savage, G. W. Simpson, E. R. T. Tiekink, G. J. Vuckovic and R. D. Webster, *J. Chem. Soc., Perkin Trans.1*, 2001, 1168.

Synthesis of a diol *via* desymmetrisation

Lipase

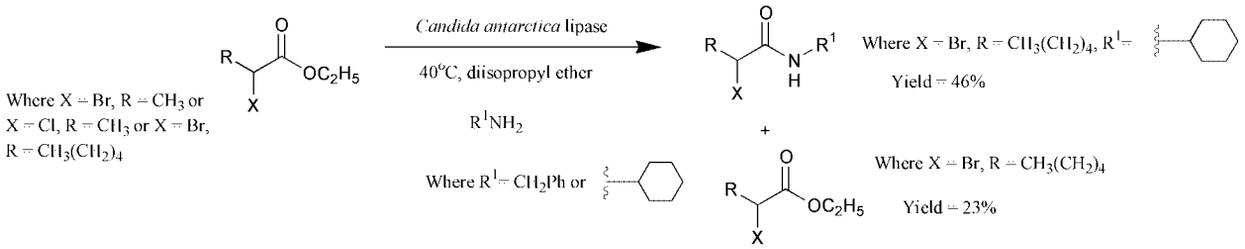


Y. Yasohara, K. Miyamoto, N. Kizaki, J. Hasegawa and T. Ohashi, *Tetrahedron Lett*, 2001, **42**, 3331.

The diol is an intermediate in the synthesis of some antifungal agents. A number of diesters (*e.g.* methyl, butyl) were also tested, with the best results obtained for the larger esters.

Synthesis of optically enriched α-haloamides

Lipase

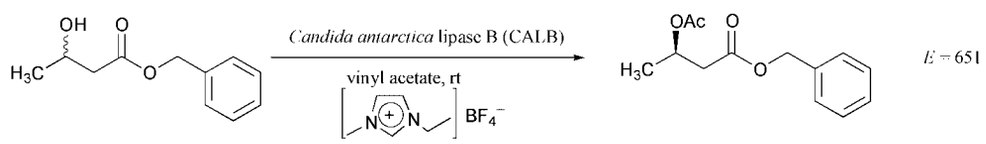


A. Azim, S. K. Sharma, C. E. Olsen and V. S. Parmar, *Bioorg. Med. Chem.*, 2001, **9**, 1345.

A range of α-haloamides were synthesized with yields in the range 40 - 46%.

Biocatalysis in ionic liquids

Lipase

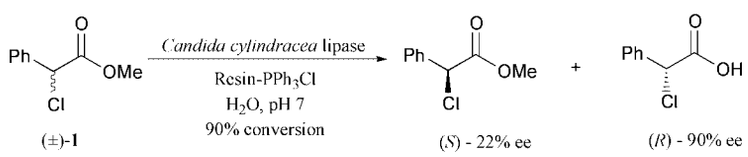


K.-W. Kim, B. Song, M.-Y. Choi and M.-J. Kim, *Org. Lett.*, 2001, **3**, 1507.

Lipase-catalysed transesterifications in ionic liquids proceeded with enhanced enantioselectivity when compared to the reactions carried out in organic solvents, *e.g.* THF and toluene.

Enzymatic hydrolysis and selective racemisation reactions of α-chloro esters

Lipase

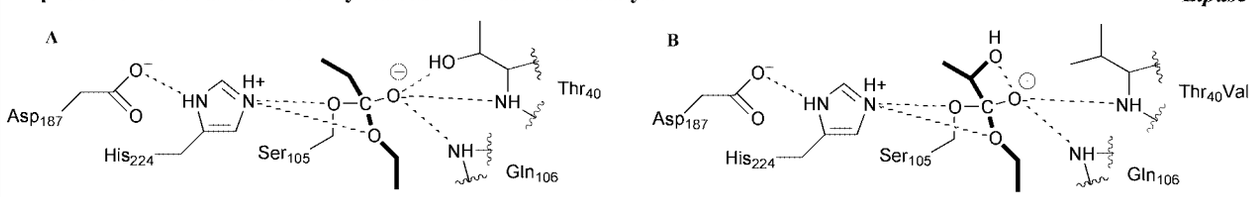


L. Haighton and J. M. J. Williams, *Synthesis*, 2001, **6**, 943.

Dynamic kinetic resolution of (±)-**1** was achieved by combining the enzyme resolution and α-chloro ester racemisation (mediated by resin-bound phosphonium chloride).

Improvement of enantioselectivity *via* substrate assisted catalysis

Lipase

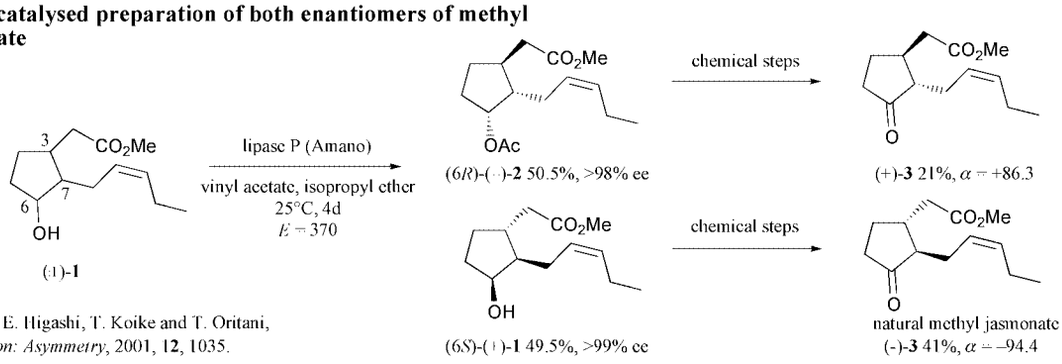


A. Magnusson, K. Hult and M. Holmquist, *J. Am. Chem. Soc.*, 2001, **123**, 4354.

A mutant of *Candida antarctica* lipase B (CALB), Thr40Val, shows improved enantioselectivity for the hydrolysis of ethyl 2-hydroxypropanoate over the wild-type enzyme. Transition state stabilisation of the oxyanion intermediate is accomplished by the hydroxy group of the substrate (**B**) rather than the threonine hydroxy, in the wild-type mechanism of the hydrolysis of ethyl propanoate (**A**).

Lipase catalysed preparation of both enantiomers of methyl jasmonate

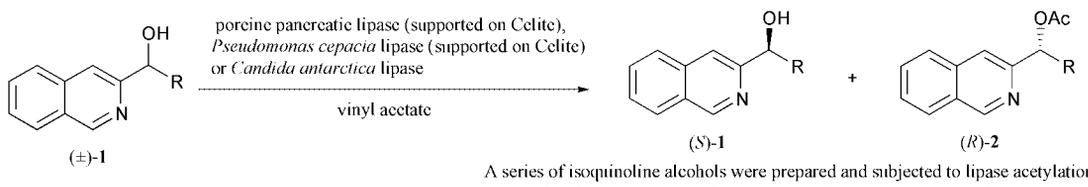
Lipase



H. Kiyota, E. Higashi, T. Koike and T. Oritani, *Tetrahedron: Asymmetry*, 2001, **12**, 1035.

Lipase catalysed resolution of isoquinoline alcohols

Lipase



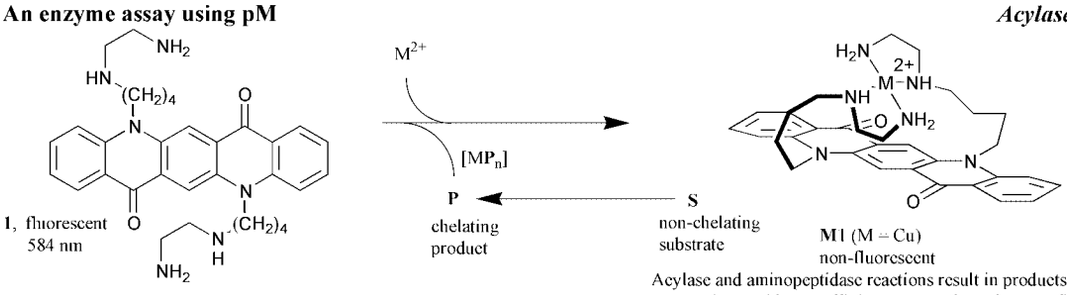
a R = Me
b R = Et
c R = C≡CH
d R = C=CH$_2$

A series of isoquinoline alcohols were prepared and subjected to lipase acetylation by three different lipases. In all cases the size of R group was influential and none of the lipases accepted R = Ph, *i*Pr, C≡C(CH$_2$)$_2$CH$_3$ or C≡C-SiMe$_3$. The ee for the acetate was >92% except for **1c** using PPL or PCL. The ee for the alcohol was dependent on conversion. At >50% conversion, ee >95%. Chemically synthesised acetates were subjected to lipase hydrolysis to obtain the (*R*)-alcohol and (*S*)-acetate. The same trends were observed. Stereoselective functionalisation of the *O*-protected alcohols was investigated.

G. Guanti and R. Riva, *Tetrahedron: Asymmetry*, 2001, **12**, 1185.

An enzyme assay using pM

Acylase/Aminopeptidase

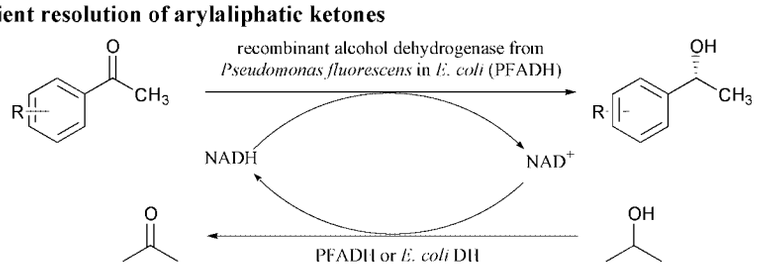


Acylase and aminopeptidase reactions result in products **P** which chelate divalent copper ions with an affinity greater than the non-fluorescent complex **M1**. Subsequent release of the fluorescent species **1** therefore gives a rapid assay for such enzymatic reactions for application in high-throughput screens.

G. Klein and J.-L. Reymond, *Angew. Chem., Int. Ed.*, 2001, **40**, 1771.

Efficient resolution of arylaliphatic ketones

Alcohol dehydrogenase



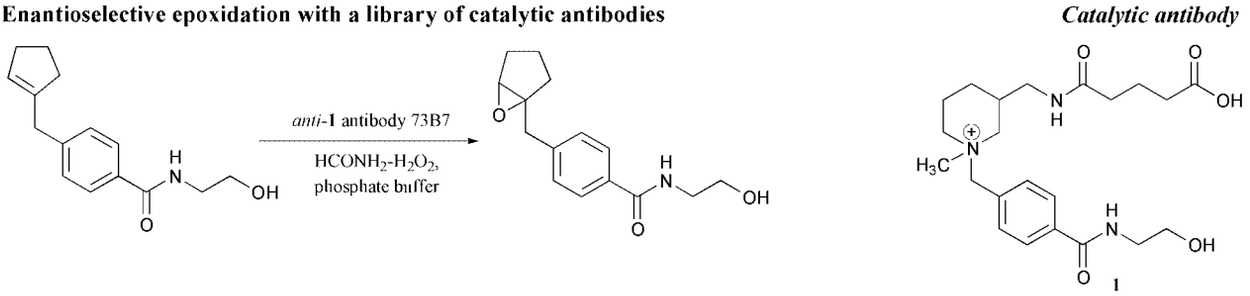
R	conv.	e.e.
H	95	92
4-Me	82	42
2-MeO	31	99
3-MeO	89	92
4-MeO	38	45
4-F	91	91
4-Cl	29	79

P. Hildebrandt, T. Riermeier, J. Altenbuchner and U. T. Bornscheuer, *Tetrahedron: Asymmetry*, 2001, **12**, 1207.

Nitro- or amino-substituents are not accepted as substrates. Temperature optimum was found to be 10-20°C, and up to 20% (v/v) isopropanol was accepted by the system.

Enantioselective epoxidation with a library of catalytic antibodies

Catalytic antibody

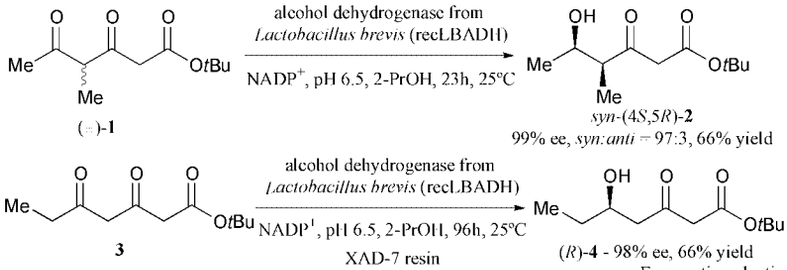


Y. Chen and J.-L. Reymond, *Synthesis*, 2001, **6**, 934.

Epoxidation by antibody 73B7 shows complementary enantioselectivity to that of antibody 20B11.

Enzymatic reduction of hydrophobic β,δ-diketo esters

Dehydrogenase

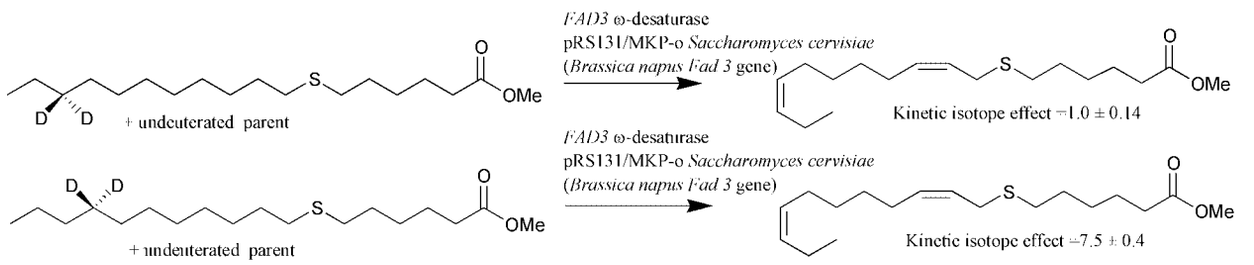


Enzymatic reduction of (±)-1 was carried out under dynamic kinetic resolution conditions. (*R*)-**4** is a precursor to (*R*)-6-ethyl-5,6-dihydropyran-2-one, a naturally occurring fragrance.

M. Wolberg, A. Ji, W. Hummel and M. Müller, *Synthesis*, 2001, **6**, 937.

Cryptoregiochemistry of a *Brassica napus* fatty acid desaturase (*FAD3*)

Desaturase

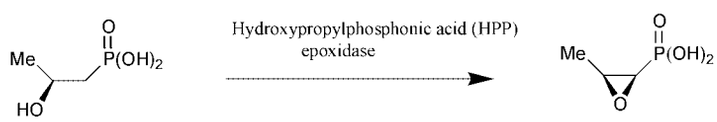


C.K. Savile, D.W. Reed, D. Meesapyodsuk, P.S. Covello and P.H. Buist, *J. Chem. Soc., Perkin Trans. 1*, 2001, 1116.

The kinetic isotope effect obtained indicates that C-15 is the site of initial oxidation in ω-3 desaturation.

The epoxidation of fosfomycin

Epoxidase

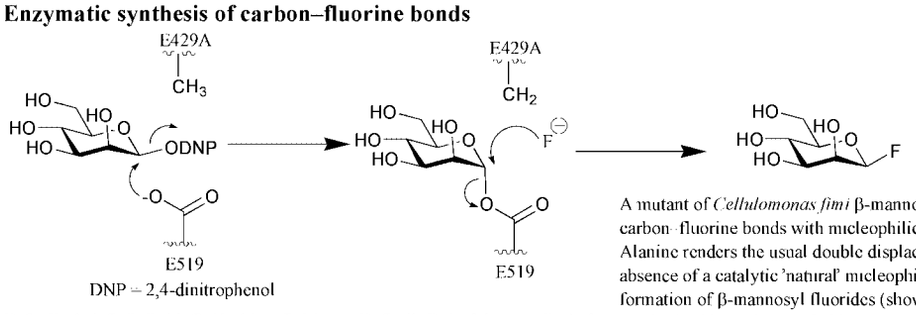


The gene encoding HPP epoxidase, Fom4, which transforms hydroxypropylphosphonic acid **1** to fosfomycin **2**, was cloned and expressed in *Escherichia coli* M15. The pure enzyme required NAD(P)H and exogenous ferrous ion for activity. Addition of a flavoprotein reductase to the *in vitro* epoxidation system greatly increased activity. Labelling experiments with ^{18}O confirmed that the hydroxy oxygen is retained in the epoxide, suggesting an unusual mechanism of biological epoxidation, involving a net dehydrogenation.

P. Liu, K. Murakami, T. Seki, X. He, S.-M. Yeung, T. Kuzuyama, H. Seto and H. Liu, *J. Am. Chem. Soc.*, 2001, **123**, 4619.

Enzymatic synthesis of carbon–fluorine bonds

Glycosidase

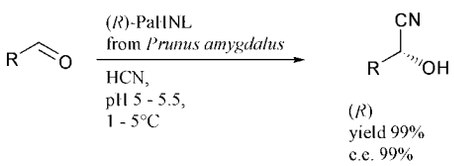


A mutant of *Cellulomonas fimi* β-mannosidase (Man2A) catalyses the formation of carbon–fluorine bonds with nucleophilic fluoride. Mutation of Glutamate 429 to Alanine renders the usual double displacement mechanism inoperable. In the absence of a catalytic 'natural' nucleophile, ROH, the addition of fluoride results in formation of β-mannosyl fluorides (shown). α-Glycosyl fluorides are also synthesised in analogous fashion using Man2A or E358G mutant of *Agrobacterium* sp. β-glucosidase (Abg).

D. L. Zechel, S. P. Reid, O. Nashiru, C. Mayer, D. Stoll, D. L. Jakeman, R. A. J. Warren and S. G. Withers, *J. Am. Chem. Soc.*, 2001, **123**, 4350.

Synthesis of chiral (*R*)-cyanohydrins

Hydroxy nitrile lyase

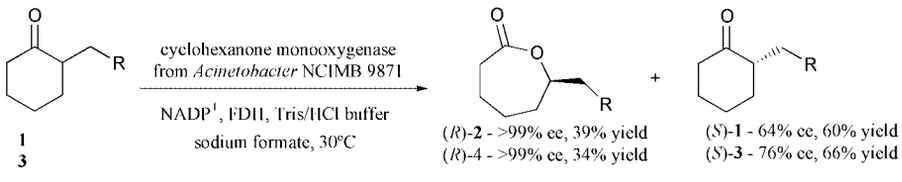


Numerous aldehydes which were considered to be difficult substrates since the competing non enzymatic reaction resulted in racemisation and lower yields, were tested. By using a tailored aqueous-organic two phase system, high ees and yields were achieved.

P. J. Gerrits, J. Marcus, L. Birikaki and A. van der Gen, *Tetrahedron: Asymmetry*, 2001, **12**, 971.

Baeyer–Villiger oxidation of 2-substituted ketones

Monooxygenase



1
3

(±)-**1** R = COOEt
(±)-**3** R = CH_2OAc

(*R*)-**2** - >99% ee, 39% yield
(*R*)-**4** - >99% ee, 34% yield

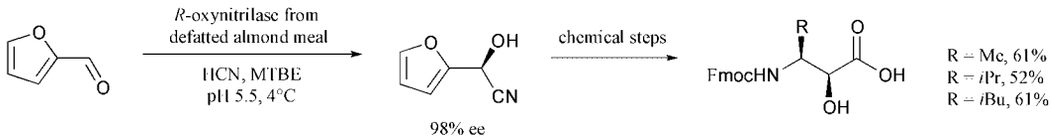
(*S*)-**1** - 64% ee, 60% yield
(*S*)-**3** - 76% ee, 66% yield

U. Schwarz-Linek, A. Krödel, F-A. Ludwig, A. Schulze, S. Rissom, U. Kragl, V.I. Tishkov and M. Vogel, *Synthesis*, 2001, **6**, 947.

Lactones (*R*)-**2** and (*R*)-**4** are precursors in the synthesis of lipoic acid.

Chemoenzymatic synthesis of *N*-protected α-hydroxy-β-amino acids

R-Oxynitrilase



98% ee

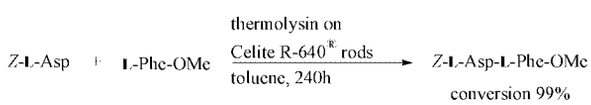
R = Me, 61%
R = *i*Pr, 52%
R = *i*Bu, 61%

R. A. Tromp, M. van der Hoeven, A. Amore, J. Brussee, M. Overhand, G. A. van der Marel and A. van der Gen, *Tetrahedron: Asymmetry*, 2001, **12**, 1109.

The α-hydroxy-β-amino acids were then used in the construction of a novel α-hydroxylated β-hexapeptide without prior protection of the α-hydroxy group. NMR studies show that, in pyridine, a stable secondary structure is formed that does not appear to be helical in character.

Synthesis of *Z*-L-aspartyl-L-phenylalanine methyl ester at controlled water activity

Protease

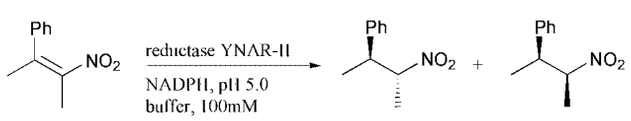


L. De Martin, C. Ebert, L. Gardossi and P. Linda, *Tetrahedron Lett.*, 2001, **42**, 3395.

By adsorbing the enzyme, thermolysin onto Celite R-640[R] rods it was possible to control hydration of the protein. Water activity ranged from 0.73 - 0.78 and yields of greater than 90% were possible. L-Phe-OEt was also tested as an acylating agent.

Asymmetric reduction of a nitroalkene

Reductase



(2*R*, 3*R*)
de 35%
ee > 98%

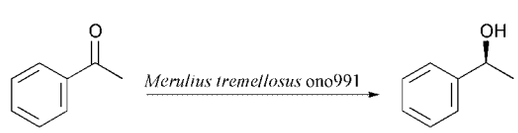
(2*S*, 3*R*)
ee 97%

Y. Kawai, Y. Inaba, M. Hayashi and N. Tokitoh, *Tetrahedron Lett.*, 2001, **42**, 3367.

Two reductases (YNAR-I and II) were isolated from Baker's Yeast. These were used to reduce (*Z*)-3-phenyl-2-nitrobut-2-ene. Both gave the nitroalkane in high enantioselectivity and moderate diastereoselectivity (improvement over the whole cell system). Use of NADH as coenzyme was also found to be viable but less effective than NADPH. A mechanism based on the reduction in D_2O was proposed.

Screening of whole cell asymmetric reductions of ketones

Reductase



1

2-(*S*)
yield 61%

A. Hage, D. G. I. Petra, J. A. Field, D. Schipper, J. B. P. A. Wijnberg, P. C. J. Kamer, J. N. H. Reek, P. W. N. M. van Leeuwen, R. Wever and H. E. Schoemaker, *Tetrahedron: Asymmetry*, 2001, **12**, 1025.

A variety of ketones were used in the screening of white-rot fungi. Using a model substrate (**1**), it was established that *Merulius tremellosus* was most effective. For each of the ketones, the reduction was also performed using a ruthenium(II) catalyst and an iridium(I) catalyst for comparison. The two systems were complementary in terms of reactivity or product configuration.