Perkin 1 Abstracts: Biocatalysis in Organic Synthesis

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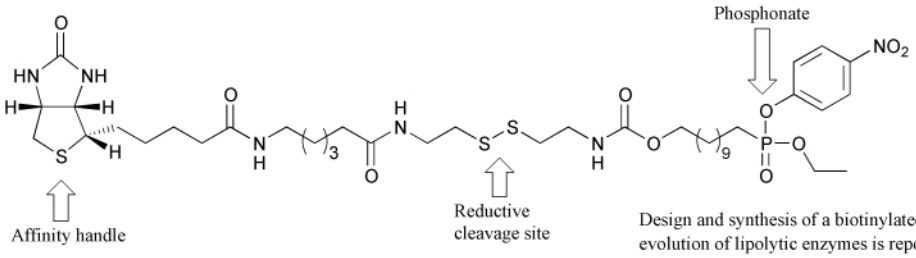
Perkin 1 Abstracts: Biocatalysis in Organic Synthesis aims to cover recent literature concerning the applications of enzymes and micro-organisms as catalysts in organic synthesis. The abstracts will emphasise the key synthetic step(s) that are mediated by the biocatalyst. Emerging technologies for biocatalyst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

Suicide inhibitor for directed evolution of lipases

Lipase

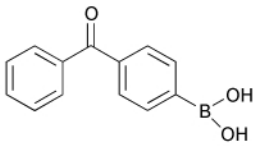


Design and synthesis of a biotinylated suicide inhibitor to be used for directed evolution of lipolytic enzymes is reported. The phosphonate inhibitor mimics esters and triglyceride substrates. Interaction of the inhibitor with *Humicola lanuginosa* lipase has been demonstrated by ELISA using horseradish peroxidase conjugated anti-lipase antibodies from streptavidin.

H.-J. Deussen, S. Danielsen, J. Breinholt and T. V. Borchert, *Bioorg. Med. Chem.*, 2000, **8**, 507.

Active-site photoprobe for serine proteases

Serine Protease



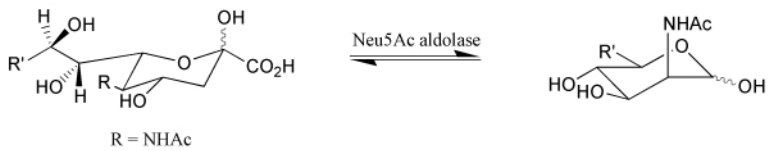
Boronic acid benzophenone photoprobe (BBP)

BBP was used as an active site directed photoprobe to study chemically modified mutant subtilisin (S116C) from *Bacillus lentus*. BBP binding in hydrophobic mutants S166C-$SCH_2C_6H_5$ and S166C-S-$CH_2C_6H_{11}$ was reduced by 86 fold and 9 fold, respectively, compared to wild type. Binding in charged mutants S166C-S-$CH_2CH_2SO_3^{2-}$ and S-166C-S-$CH_2CH_2NH^{3+}$was reduced by 170 fold and 4 fold, respectively, compared to wild type. Photolysis of WT-SBL-BBP complex inactivated the enzyme through crosslinking to glycine 127 in the S1 pocket. Photolysis of BBP complexes of charged mutants and S166C-$SCH_2C_6H_5$ showed no inactivation or crosslinking of the enzyme. Photolysis of S166C-S-$CH_2C_6H_{11}$ BBP complex inactivated 50% of enzyme and formed covalent crosslinks to glycine 127.

G. DeSantis, C. Paech and J. B. Jones, *Bioorg. Med. Chem.*, 2000, **8**, 563

C-9 modified *N*-acetylneuraminic acid derivatives

Aldolase



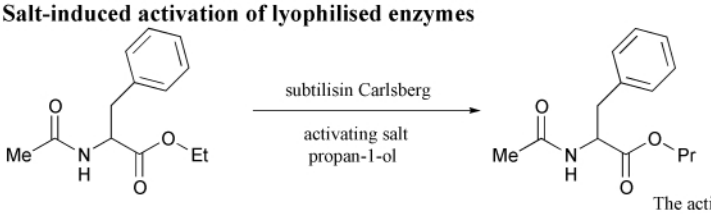
R = NHAc

Range of C-9 analogues of *N*-acetylneuraminic acid (R' = CH_2OH, CH_2OMe, CH_2OMOM, CH_2OAc, CH_2OBz, $CH{=}CHCO_2H$) evaluated as substrates for Neu5Ac aldolase. The rates of cleavage were significantly reduced by sterically demanding substituents. When R' = $CH{=}CHCO_2H$ then the compound is an inhibitor of the aldolase.

M. J. Kiefel, J. C. Wilson, S. Bennett, M. Gredley and M. von Itzstein, *Bioorg. Med. Chem.*, 2000, **8**, 657.

Salt-induced activation of lyophilised enzymes

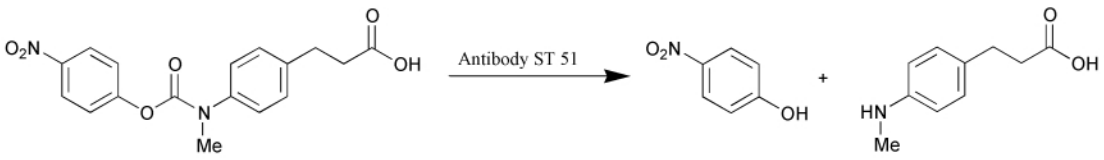
Protease



The activation of subtilisin Carlsberg by several salts, was evaluated as a function of the Jones-Dole *B* coefficient to investigate the effect of salt kosmotropicity on enzyme activity. In general, the water content, active site content and transesterification activity of subtilisin Carlsberg containing >96% w/w salt increased with increasing kosmotropicity of the activating salt. Degrees of activation relative to the salt-free enzyme ranged from 33-fold for chaotropic sodium iodide to 2480-fold for kosmotropic sodium acetate.

M. T. Ru, S. Y. Hirokane, A. S. Lo, J. S. Dordick, J. A. Reimer and D. S. Clark, *J. Am. Chem. Soc.*, 2000, **122**, 8, 1565.

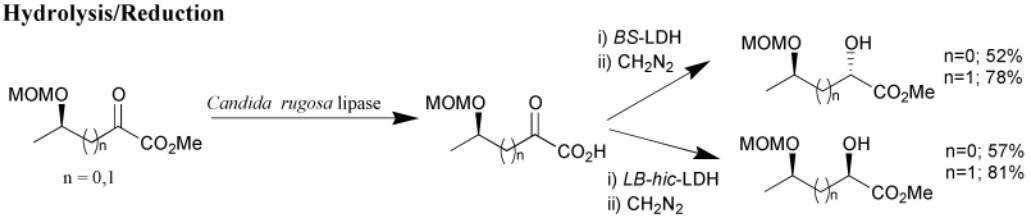
Hydrolysis of an *N*-methylcarbamate by a catalytic antibody ***Catalytic antibody***



At pH 9.0 k_{cat} = 9.1 x $10^{-2}h^{-1}$, K_m = 2.6 x 10^2 μM, and k_{cat}/K_m= 3.5 x10^{-4}μ$M^{-1}h^{-1}$. The rate enhancement k_{cat}/K_{uncat} = 6.5 x 10^3. The antibody displayed no activity towards ester or amide analogues. ST 51 was generated by raising a monoclonal antibody to a phosphoramidate transition state analogue.

A. N. Dinault, M. J. Chen, A. Marks, R. A. Batey and S. D. Taylor, *Chem. Commun.* 2000, 385.

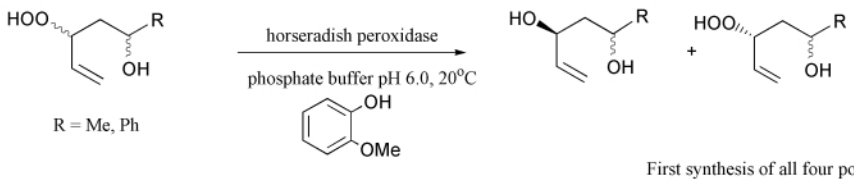
Hydrolysis/Reduction ***Lipase/ Dehydrogenase***



Bacillus stearothermophilus (*BS*-LDH) and *Lactobacillus delbrueckii* (*LB-hic*-LDH). One-pot dual enzyme system for transformation of 2-oxo acids with substitution at the C-3 or C-4 position into enantiopure 2,3 and 2,4 dihydroxyacids. With more complex trisubstituted substrates significant decompositon was observed.This procedure has been applied to the synthesis of protected 2,6,7- trihydroxyhept-3-enoic acids.

G. Bhalay, S. Clough, L .McLaren, A. Sutherland and C. L.Willis, *J. Chem. Soc., Perkin Trans. 1*, 2000, 901.

Enantioselective reduction of hydroperoxides ***Peroxidase***



First synthesis of all four possible optically active stereoisomers of the hydroperoxy alcohols and their corresponding 1,3-diol. Peroxidase route most efficient and more applicable to different R substituents, however only the *R*-configured hydroperoxides are obtained by this method. Alternative approach based upon using lipases is also reported.

W. Adam, C. R. Saha-Möller and K. S. Schmid, *J. Org. Chem.*, 2000, **65**, 1431.

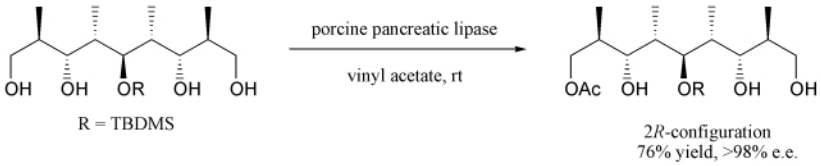
Amide synthesis ***Protease***

OGp = 4-guanidinophenyl ester

Clostripain (EC 3.4.22.8), is a cysteine protease commercially available from Fluka. The enzyme has been shown to be a useful catalyst for the acylation of a wide range of amines, amino alcohols and non α-amino acids. Bz-Arg-OEt and Bz-Phe-OGp were used as model acyl donors to determine kinetics and conversions. The enzyme displays high catalytic activity for amide bond formation, but relatively low activity towards the reverse cleavage reaction. In view of the wide substrate specificity, the enzyme may have use in the synthesis of peptide isosteres.

R. Günther, A. Stein and F. Bordusa, *J. Org. Chem.*, 2000, **65**, 1672.

Desymmetrisation of *meso*-polyol ***Lipase***



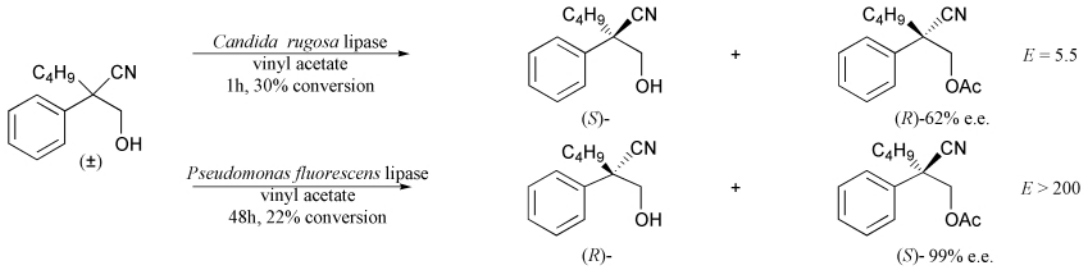
2*R*-configuration
76% yield, >98% e.e.

Lipase from *Pseudomonas fluorescens* (95% e.e.) and *Pseudomonas cepacia* (93% e.e.) also gave 2*R* isomer. Product is an intermediate in the total synthesis of rifamycin S.

R. Chênevert and Y. S. Rose, *J. Org. Chem.*, 2000, **65**, 1701.

Resolution of primary alcohols

Lipase

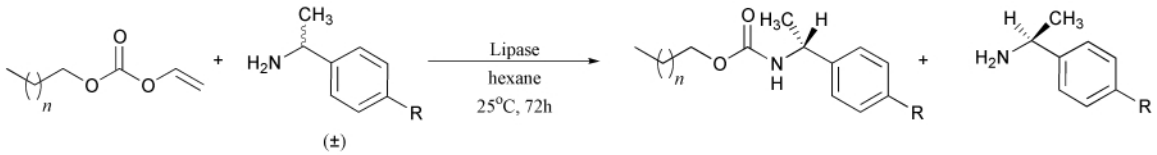


D. S. Im, C. S. Cheong, S. H. Lee, B. H. Youn and S. C. Kim, *Tetrahedron*, 2000, **56**, 1309.

Products transformed to analogues of Systhane®, a systemic fungicide.

Alkoxycarbonylation of amines

Lipase

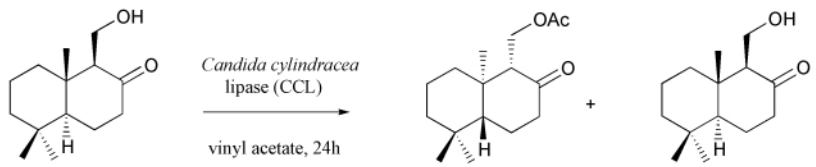


M. Soledad de Castro, P. Domínguez and J. V. Sinisterra, *Tetrahedron*, 2000, **56**, 1387.

A number of commercial lipases were screeened for their ability to perform the alkoxycarbonylation of amines. The effect of the different lipases on yield and enantioselectivity was investigated, with AK lipase being the most effective (37% yield, 95% e.e.). All lipases examined showed (*R*)-stereospecificity with the exception of lipases from *Rhizopus niveus* and *Pseudomonas cepacia.*

Resolution of Drimane and Labdane Intermediates.

Lipase

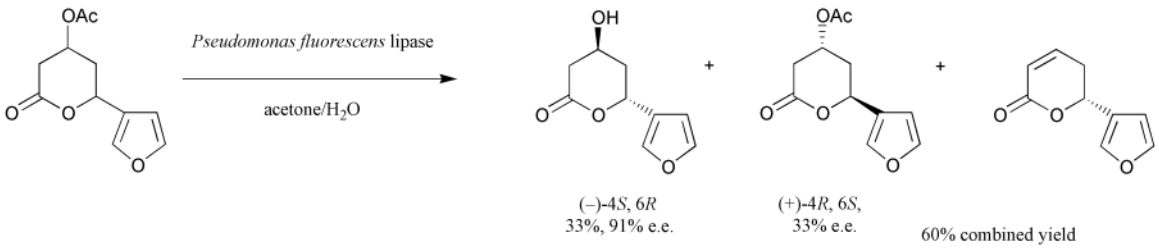


A. T. Anilkumar, U. Sudhir, S. Joly and M. S. Nair, *Tetrahedron*, 2000, **56**, 1899.

E.e of (−)-ketoalcohol product was improved to 82% by a further CCL catalysed transesterification in vinyl acetate.

Resolution of pyranofuranones.

Lipase

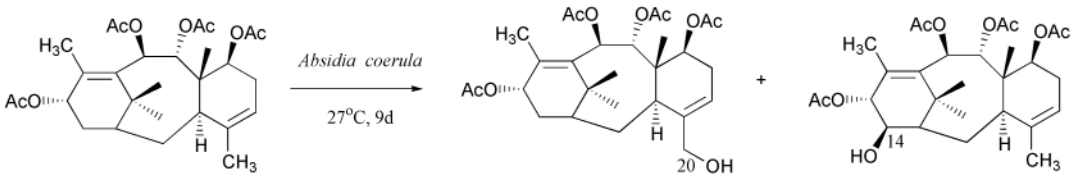


M. De Rosa, A. Soriente, G. Sodano and A. Scettri, *Tetrahedron*, 2000, **56**, 2095.

Resolved pyranofuranones are synthons for Manoalide and Cacospongionolide B.

20-Hydroxylation of taxoids

Oxygenase

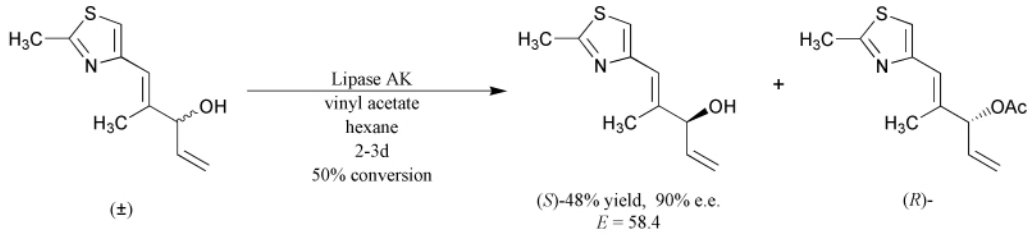


S. Hu, D. Sun and A. I. Scott, *Tetrahedron Lett.*, 2000, **41**, 1703.

First example of a microbial 20-hydroxylation of a taxoid. The 20-hydroxylated taxoid may be an intermediate in the taxol biosynthesis. The 14β-hydroxylation product was also observed (3% yield).

C12-C21 fragment of epothilones

Lipase

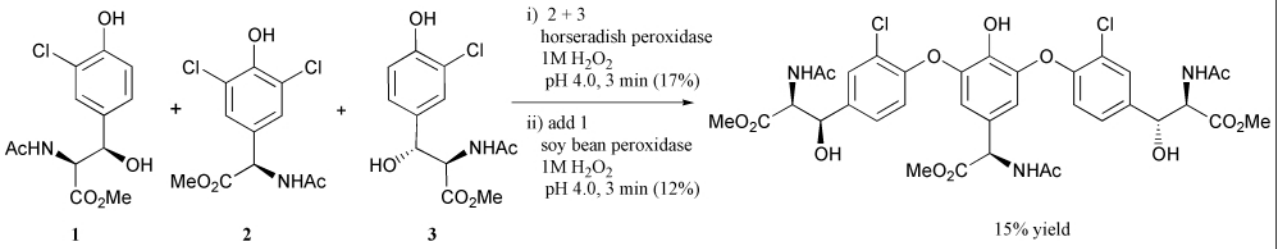


B. Zhu and J. S. Panek, *Tetrahedron Lett.*, 2000, **41**, 1863.

Three allyl alcohols were successfully resolved using Lipase AK. *E*-values ranged from 45.5 - 115.1.

Synthesis of bis-diaryl ether

Peroxidase

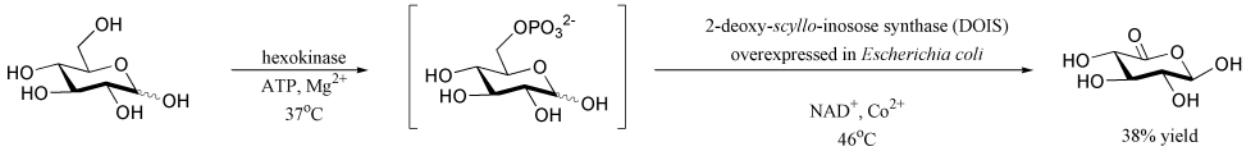


I. Malnar and C.J. Sih, *Tetrahedron Lett.*, 2000, **41**, 1907.

Some homo C-O and C-C coupling was also observed.

Conversion of glucose to catechol

Synthase / Kinase

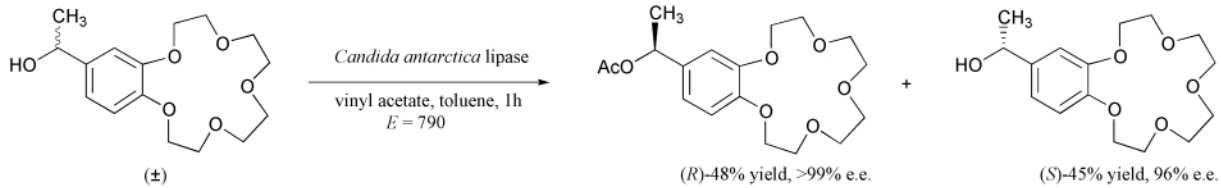


K. Kakinuma, E. Nango, F. Kudo, Y. Matsushima and T. Eguchi, *Tetrahedron Lett.*, 2000, **41**, 1935.

BtrC gene produces the enzyme DOIS, which in conjunction with hexokinase transforms D-glucose to 2-deoxy-*scyllo*-inosose (DOI) in up to 38% yield. DOI was reductively dehydrated to catechol in 59% yield.

Resolution of benzo-15-crown-5-ether derivatives

Lipase

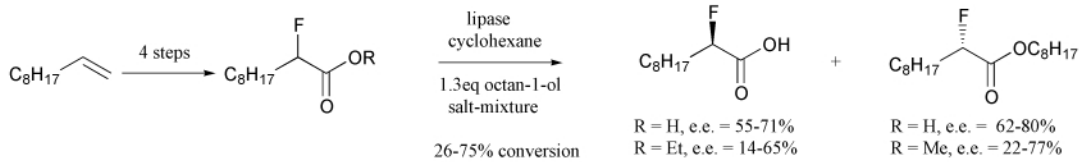


T. Kijima, T. Moriya, E. Kondoh and T. Izumi, *Tetrahedron Lett.*, 2000, **41**, 2125.

Benzo-15-crown-5-ethers have been shown to be substrates of various lipases, with *C. antarctica* being the most effective. A number of acyl donors can be used with *E*-values ranging from 289-544.
Note: Paper states (*R*)-configuration for the ester, however configuration is shown as (*S*) in diagram.

Resolution of 2-fluorodecanoic acid

Lipase

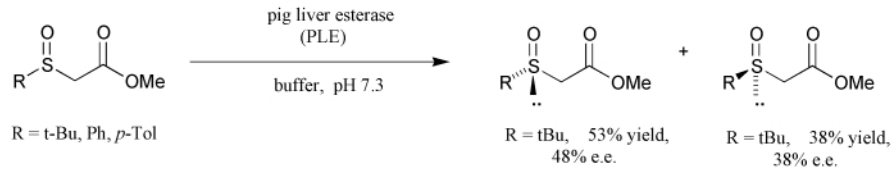


F. Tranel and G. Haufe, *Tetrahedron: Asymmetry*, 2000, **11**, 889.

Range of e.e.'s & conversion depending on R and lipase. R = $CH_3$and C_2H_5: Lipases: *Candida antarctica* (CAL), *Candida rugosa* (CRL) and *Pseudomonas cepacia* (PCL).

Resolution of *S*-chiral and prochiral sulfinyl substrates

Esterase

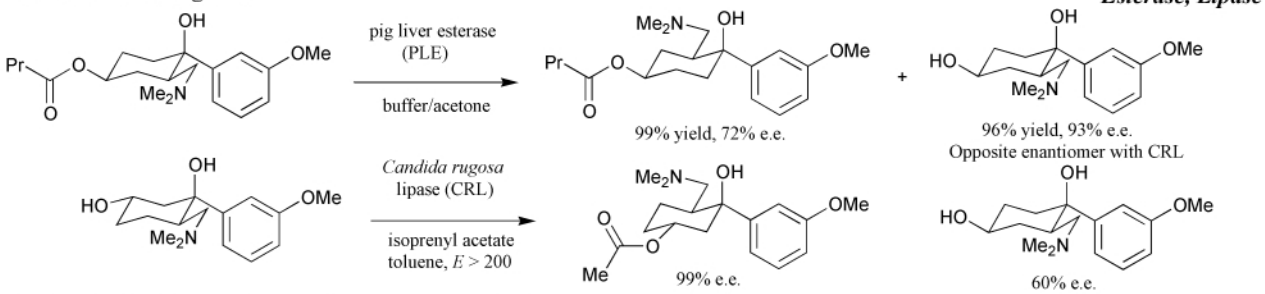


Application of the Jones model of pig liver esterase to prochiral sulfinyl substrates is reported. Moderate e.e. values of substrate and product are due to competition of oxygen atom and lone pair for front polar and back polar pockets of PLE.

P. Kielbasinski, *Tetrahedron: Asymmetry*, 2000, **11**, 911.

Resolution of analgesics

Esterase, Lipase

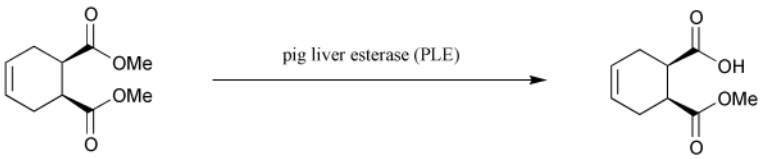


H-J. Gais, C. Griebel and H. Buschmann, *Tetrahedron: Asymmetry*, 2000, **11**, 917.

Successful resolution of aromatic ester of *O*-desmethyltramadol also performed.

Asymmetric ester hydrolysis using immobilised esterase in a hollow fibre ultrafiltration membrane

Esterase

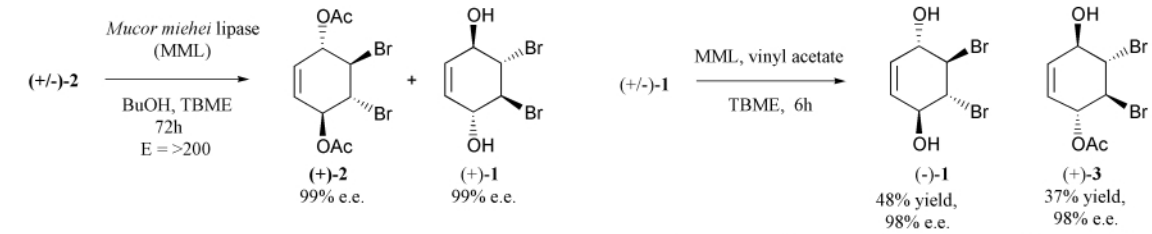


PLE immobilised using polysulfone ultrafiltration hollow fibres. Simultaneous reaction and separation in a two-phase system of hexane/0.1M phosphate buffer. After 25 days, enzyme had lost no immobilised activity (equivalent to 62% free form activity). Product was isolated with >97% e.e.

H. A. Sousa, J. G. Crespo and C. A. M. Afonso, *Tetrahedron: Asymmetry*, 2000, **11**, 929.

Resolution of 2,3-dibromocyclohex-5-ene-1,4-diol

Lipase

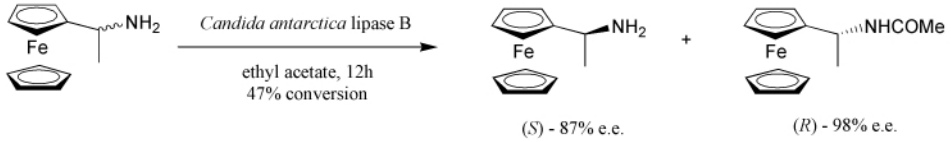


C. Sanfilippo, A. Patti and G. Nicolosi, *Tetrahedron: Asymmetry*, 2000, **11**, 1043.

Some diester formed in transesterification process. (+)-**1** and (−)-**1** serve as synthons for the conduritol series.

Resolution of (±)-1-ferrocenylethylamine.

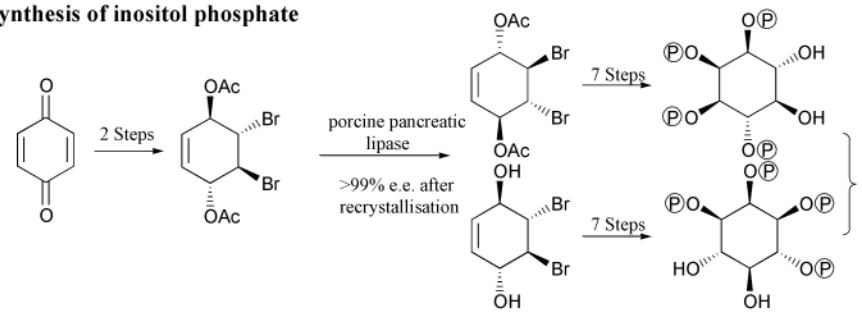
Lipase



L.E. Iglesias, F. Rebolledo and V. Gotor, *Tetrahedron: Asymmetry*, 2000, **11**, 1047.

For this transformation, E>200. Reactions using ethyl formate as acyl donor in 1,4-dioxane were faster, but E value was lower (30).

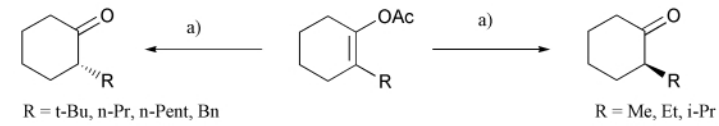
Synthesis of inositol phosphate — ***Phytases***



Enzymatic dephosphorylation. Various phytases give range of dephosphorylated products including Ins(1,2,3)P_3 (isomeric purity 99%), Ins(1,2,4)P_3 (75%), Ins(2,3,6)P_3 (100%).

O. Plettenburg, S. Adelt, G. Vogel and H.-J. Altenbach, *Tetrahedron: Asymmetry*, 2000, **11**, 1057.

Asymmetric hydrolysis of enol esters — ***Esterase***



R = t-Bu, n-Pr, n-Pent, Bn

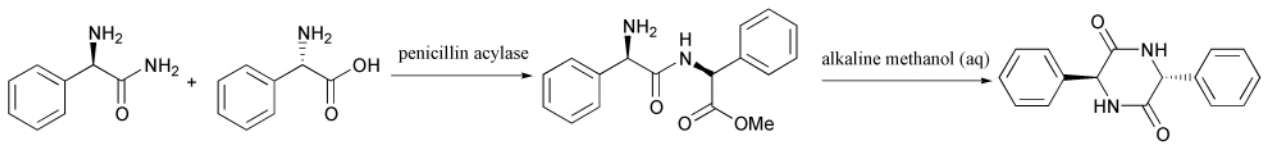
R = Me, Et, i-Pr

(a) Esterase I or Esterase II from *Marchantia polymorphia*

Two esterases had opposite enantioselectivity; both reversed stereoselectivity with increased bulk of R.

T. Hirata, K. Shimoda and T. Kawano, *Tetrahedron: Asymmetry*, 2000, **11**, 1063.

Peptide synthesis — ***Penicillin Acylase***



D- or L- enantiomer

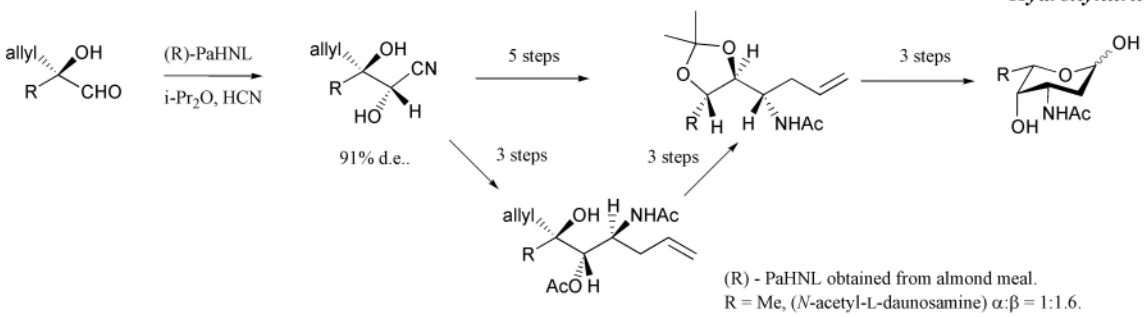
69% Yield, D-, L- or L-, L-.

trans isomer from D-, L-.
cis isomer from L-, L-.

L. M. van Langen, F. van Rantwijk, V. K. Svedas and R. A. Sheldon, *Tetrahedron: Asymmetry*, 2000, **11**, 1077.

trans-Isomer formed in pure form. *cis*-Form contained small amount of *trans*.

C-C bond formation — ***Hydroxynitrile lyase***

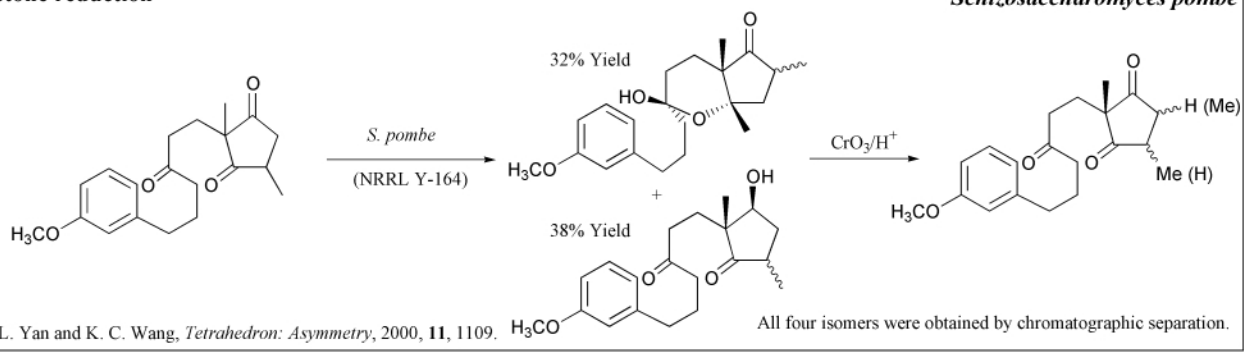


91% d.e..

(R) - PaHNL obtained from almond meal.
R = Me, (*N*-acetyl-L-daunosamine) α:β = 1:1.6.
R = $(CH_3)_2CHCH_2$ α:β = 1:1.2.

F. Effenberger and J. Roos, *Tetrahedron: Asymmetry*, 2000, **11**, 1085.

Ketone reduction — ***Schizosaccharomyces pombe***

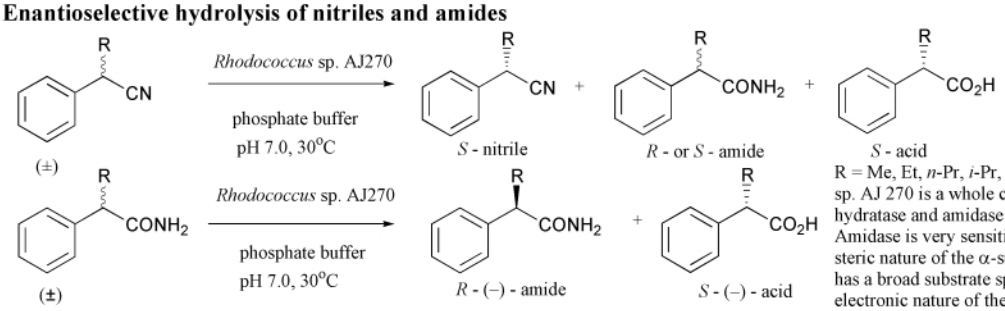


All four isomers were obtained by chromatographic separation.

J.-L. Yan and K. C. Wang, *Tetrahedron: Asymmetry*, 2000, **11**, 1109.

Enantioselective hydrolysis of nitriles and amides

Nitrile hydratase/ Amidase

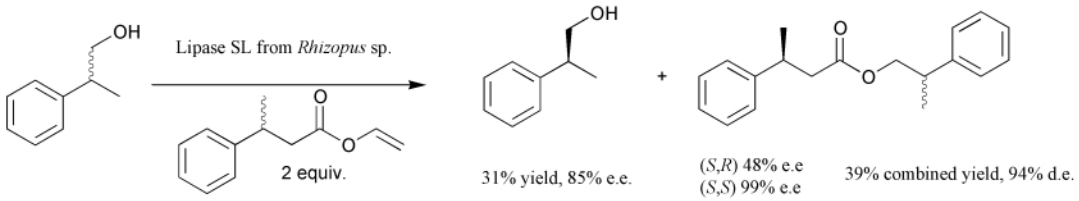


R = Me, Et, *n*-Pr, *i*-Pr, *n*-Bu, MeO, MeS. *Rhodococcus* sp. AJ 270 is a whole cell system containing both nitrile hydratase and amidase enzymes.
Amidase is very sensitive to both the electronic and steric nature of the α-substituent while nitrile hydratase has a broad substrate spectrum irrespective of the electronic nature of the α-substituent. The amidase exhibits higher *R*-selectivity for amides than the nitrile hydratase.

M.-X. Wang, G. Lu, G.-J. Ji, Z.-T. Huang, O. Meth-Cohn and J. Colby, *Tetrahedron: Asymmetry*, 2000, **11**, 1123.

Resolution of primary alcohol using chiral acyl donor

Lipase



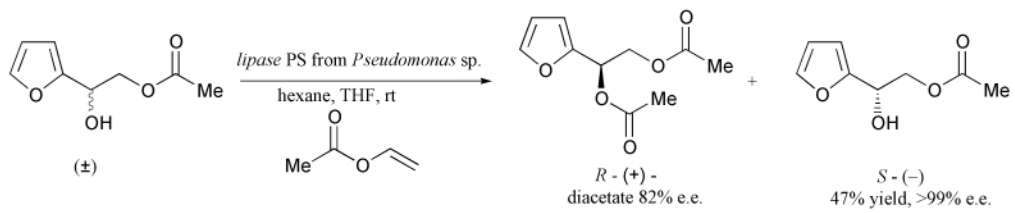
diisopropyl ether 1.5h
47% conversion
E=98

The use of a racemic acyl donor containing a stereogenic center β to the carbonyl group gave dramatic increases in enantioselectivity. Only one enantiomer of the acyl donor reacted allowing the use of racemic acyl donors. A variety of solvents, lipases and acyl donors were studied with *E* values ranging from 1 to 98.

K. Hirose, H. Naka, M. Yano, S. Ohashi, K. Naemura and Y.Tobe, *Tetrahedron: Asymmetry*, 2000, **11**, 1199

Resolution of 2-(2-furyl)-2-hydroxyethyl acetate

Lipase

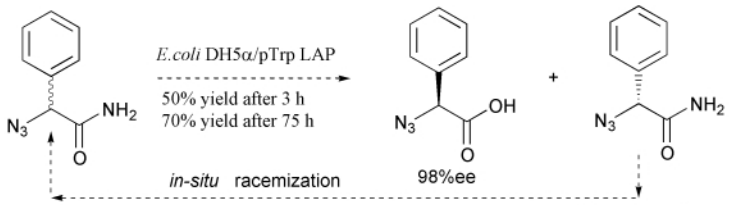


E.e. of diacetate was increased by deacetylation to the corresponding diol and *R*-acetylation with vinyl acetate in the presence of *Lipase* PS to give *R*-diacetate in 98% e.e.

J. E. Kaminska, K. Smigielski, D. Lobodzinska and J. Góra, *Tetrahedron: Asymmetry*, 2000, **11**, 1211.

Resolution of α-azido acids and amides

Aminopeptidase



Transformation performed using whole cells from *Escherichia. coli* containing the *Pseudomonas putida* L-aminopeptidase gene (*pepA*). Aliphatic substrates do not show *in-situ* racemisation *e.g.* hydrolysis of (+/-) 2-azidohexanoic acid gave 50% (*S*)-2-azidohexanoic acid after 20h (e.e.>99.8%).

C. W. Tornøe, T. Sonke, I. Maes, H. E. Schoemaker and M. Meldal *Tetrahedron: Asymmetry*, 2000, **11**, 1239.