Perkin 1 Abstracts: Biocatalysis in Organic Synthesis

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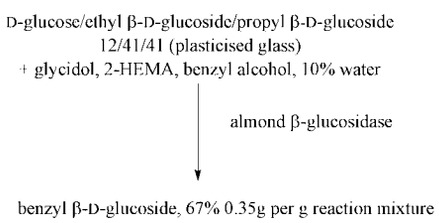
Perkin 1 Abstracts: Biocatalysis in Organic Synthesis aims to cover recent literature concerning the applications of enzymes and micro-organisms as catalysts in organic synthesis. The abstracts will emphasise the key synthetic step(s) that are mediated by the biocatalyst. Emerging technologies for biocatalyst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

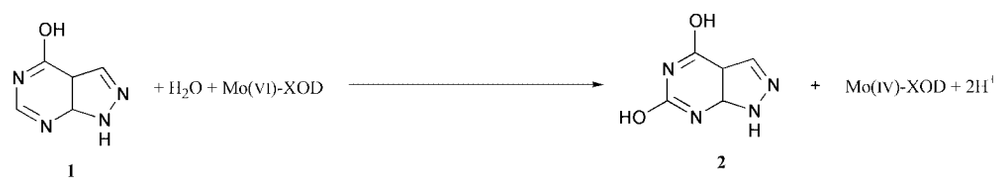
Enzymatic glycosylation in plasticised glass phases ***Glycosidases***



The antagonistic problems of low glycosidase activity in the absence of water and low glycosidase acceptor solubility in water have been addressed using plasticised glass phases as reaction media. These media are mixtures of monosaccharide, alkyl glycosides and various hydrophobic or hydrophilic compounds. They support high concentrations of acceptor and sugar donor, and enzyme activity is optimum at a water level of 10%. Excellent productivities were observed in 17 example reactions using almond β-glucosidase. Four β-galactosidases and two α–galactosidases were also employed successfully in the new media. Reaction products were shown to be a result of transglycosylation rather than reverse hydrolysis.

I. Gill and R. Valivety, *Angew. Chem., Int. Ed.*, 2000, **39**, 3804.

Control of xanthine oxidase activity by light ***Xanthine oxidase***

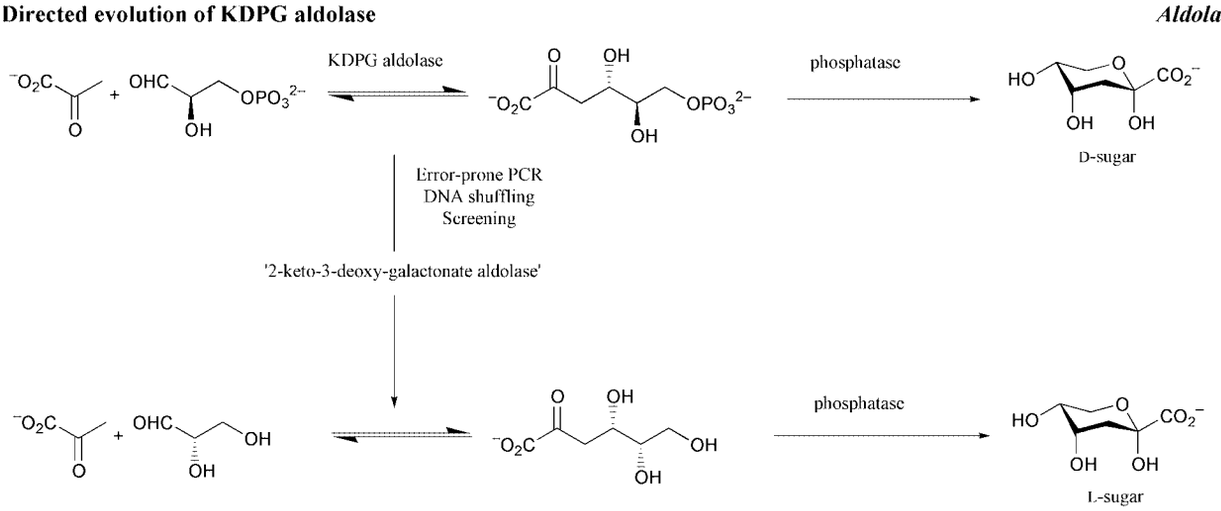


1 **2**

Xanthine oxidase (XOD) is inhibited by alloxanthine **2**, the oxidation product of allopurinol **1**. When exposed to light, enzymatic activity is regained at levels of up to 2000%. Light triggers the release of the inhibitor by photoexcitation of the Mo(IV) metal centre of the inhibited enzyme. Consequent electron transfer from the metal to other cofactors in the enzyme, Fe–S centres and FAD, results in conversion of Mo(IV) to Mo(V) or Mo(VI).

L. A. Tai and K. C. Hwang, *Angew. Chem., Int. Ed.*, 2000, **39**, 3886.

Directed evolution of KDPG aldolase ***Aldolase***

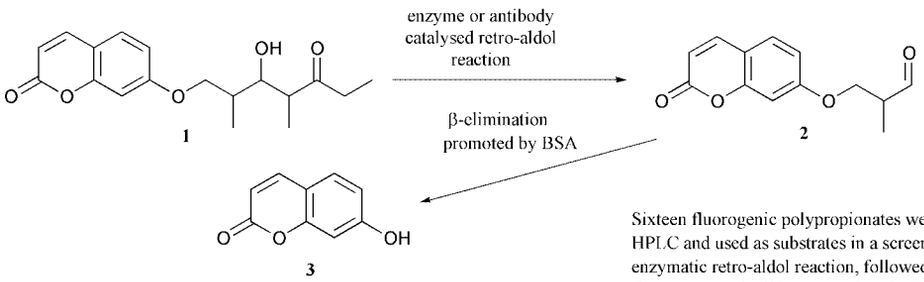


KDPG aldolase was evolved *in vitro* using standard PCR mutagenesis methods. Two separate experiments yielded mutants with improved activity toward 1) non-phosphorylated substrates (mutant KA3, 70% improved activity over wild-type) and 2) L-sugars (mutant KA3-L2, 6-fold enhancement in activity over wild-type). Amino acid analysis of mutants revealed that the site of mutations leading to the improvement of the new desired properties were not easy to rationalise.

S. Fong, T. D. Machajewski, C. C. Mak and C. H. Wong, *Chem. Biol.*, 2000, **7**, 873.

Fluorogenic substrates for screening for stereoselective aldolases

Aldolase

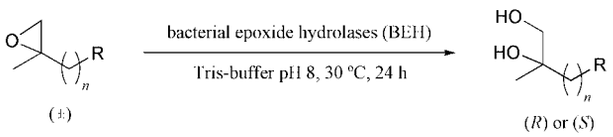


Sixteen fluorogenic polypropionates were synthesised, separated by preparative HPLC and used as substrates in a screen for stereoselective aldolase activity. The enzymatic retro-aldol reaction, followed by facile β-elimination, leads to the fluorescent umbelliferone product **3**. The screening protocol was successful in selecting for the aldolase antibody 38C2, with a defined (*S*)-*anti* aldol selectivity.

R. Pérez-Carlón, N. Jourdain and J.-L. Reymond, *Chem. Eur. J.*, 2000, **6**, 4154.

Hydrolysis of 2,2-disubstituted oxiranes

Bacterial epoxide hydrolases



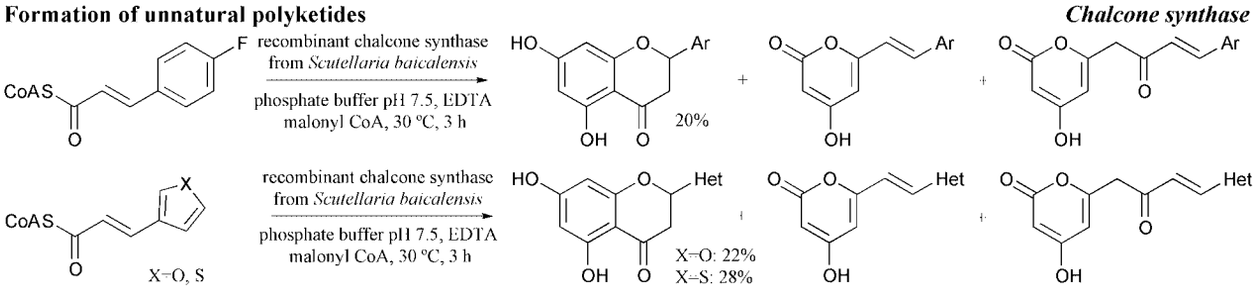
R = OH, $OCH_2CH{=}CH_2$, $OCH_2CH_2CH_3$, $OCH_2C_6H_5$, OTBDMS, $OSiEt_3$, CN, $OCH_2O(CH_2)_2OCH_3$, $CH(OEt)_2$, N_3.

The 2,2-disubstituted oxiranes were screened against eleven different bacteria, with activity and selectivity dependent on both substrate and biocatalyst. Increasing polarity of the R group resulted in decreased reaction rate and selectivity, to the point that a free hydroxy group shows no reaction. Increasing chain length also decreased the selectivity of the reaction.

A. Steinreiber, I. Osprian, S. F. Mayer, R. V. A. Orru and K. Faber, *Eur. J. Org. Chem.*, 2000, 3703.

Formation of unnatural polyketides

Chalcone synthase

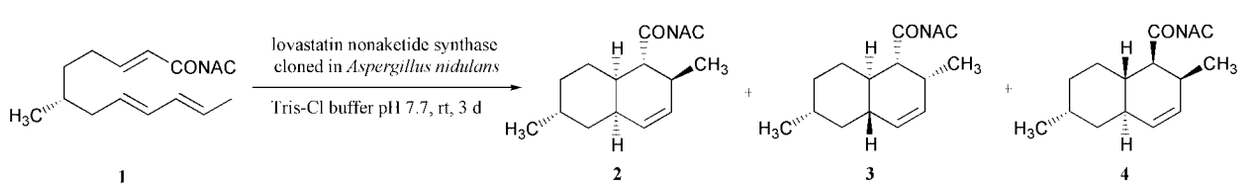


First demonstration of the formation of novel, unnatural polyketides containing a heteroatom moiety. A mechanism is proposed for the synthesis of products and by-products.

I. Abe, H. Morita, A. Nomura and H. Noguchi, *J. Am. Chem. Soc.*, 2000, **122**, 11242.

Intramolecular Diels–Alder catalysed by lovastatin nonaketide synthase

Lovastatin nonaketide synthase



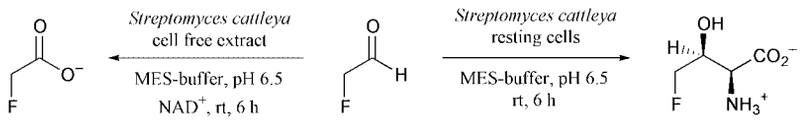
NAC=$SCH_2CH_2NHCOCH_3$

Lovastatin nonaketide synthase (LNKS) converted triene **1** into **2**, **3** and **4** in a 15:15:1 ratio. Non-enzymatic cyclisation, and inactivation of LNKS prior to incubation resulted in a 1:1 mixture of **2** and **3**. Non-enzymatic products are formed *via* a transition state with C-6 methyl in a favourable pseudoequatorial position, whereas **4** must proceed through a transition state with C-6 methyl in a crowded pseudoaxial position.

K. Auclair, A. Sutherland, J. Kennedy, D. J. Witter, J. P. Van den Heever, C. R. Hutchinson and J. C. Vederas, *J. Am. Chem. Soc.*, 2000, **122**, 11519.

Fluoroacetaldehyde: a biosynthetic precursor to fluoroacetate and 4-fluorothreonine

Streptomyces cattleya

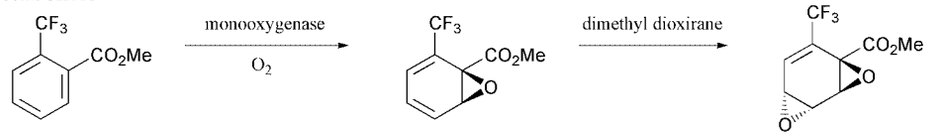


Labelling studies show that fluoroacetaldehyde is incorporated intact into 4-fluorothreonine, perhaps *via* an aldol-type condensation. However, it has been shown that glycine is not the condensing nucleophile.
The formation of fluoroacetate was shown to be dependent of NAD^+, and an *S. cattleya* cell free extract is also active on a number of other aldehydes, indicating a relatively non-specific aldehyde dehydrogenase.

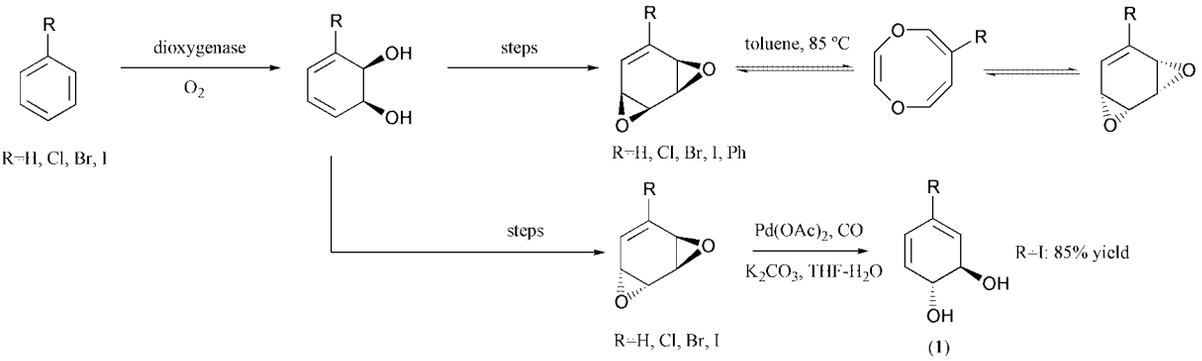
S. J. Moss, C. D. Murphy, J. T. G. Hamilton, W. C. McRoberts, D. O'Hagan, C. Schaffrath and D. B. Harper, *Chem. Commun.*, 2000, 2281.

Chemical modification of arene oxide and *cis*-dihydrodiol metabolites

Monooxygenase/dioxygenase



Arene oxide metabolites yield isolable *anti*-arene dioxides upon chemical oxidation. However, racemisation through an oxepine intermediate can and does occur in certain instances.

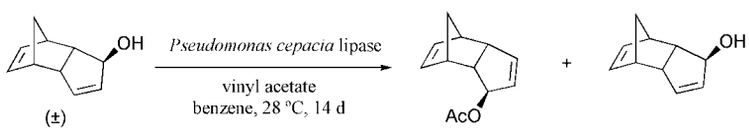


Enantiopure *syn*- and *anti*- mono- and poly-arene dioxides were synthesised from *cis*-dihydrodiol metabolites. The *anti*-benzene dioxides are precursors in a much improved synthesis of the biologically interesting *trans*-3,4-dihydrodiols (**1**). The *syn*-benzene dioxides do not undergo the palladium-catalysed transformation. Racemisation of the *syn*-benzene dioxides with four chiral centres has been shown to occur thermally.

D. R. Boyd, N. D. Sharma, C. R. O'Dowd and F. Hempenstall, *Chem. Commun.*, 2000, 2151.

Chemoenzymatic approaches to the decahydro-*as*-indacene ring-system

Lipase



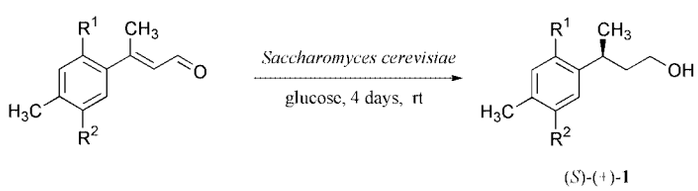
44% yield, 74% ee **1** - 39% yield, 72% ee

M. Banwell, D. Hockless, B. Jarrott, B. Kelly, A. Knill, R. Longmore and G. Simpson, *J. Chem. Soc., Perkin Trans. 1*, 2000, 3555.

The alcohol **1** is an intermediate in the synthesis of decahydro-*as*-indacenes relevant to the construction of commercially produced pesticides, spinosyns A and D.

Reduction of unsaturated aldehydes

Saccharomyces cerevisiae



(*S*)-(+)-**1**

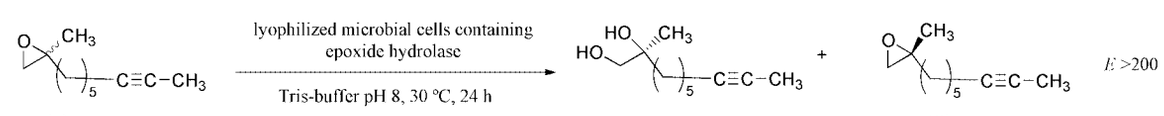
R^1, R^2 = OMe; 56% yield, >98% ee
R^1 = OMe, R^2 = H; 49% yield, >98% ee
R^1 = H, R^2 = OMe; 56% yield, >98% ee

C. Fuganti and S. Serra, *J. Chem. Soc., Perkin Trans. 1*, 2000, 3758.

Enantiopure (*S*)-**1** is a chiral building block for the synthesis of bisabolane sesquiterpenes.

Resolution of 2,2-disubstituted oxiranes

Epoxide hydrolase

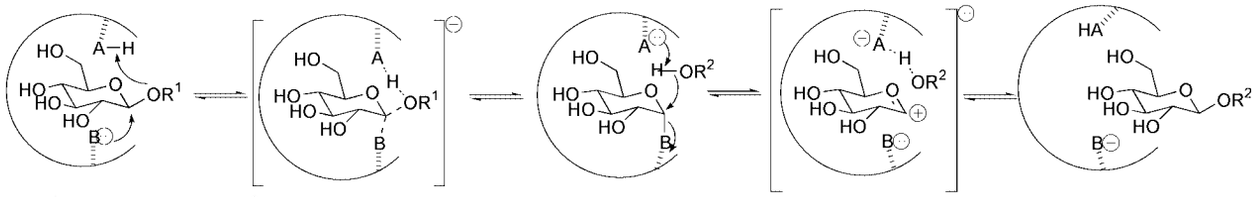


Various substrates were screened to examine the enantioselectivity (*E* value) of bacterial epoxide hydrolases. A significant selectivity enhancement of more than a ten-fold increase of *E*-values was achieved by the appropriate choice of the C–C multiple bond, *i.e.* by choosing an alkene or alkyne moiety or by variation of the *E*/*Z*-configuration of olefinic substrates.

I. Osprian, W. Stampfer and K. Faber, *J. Chem. Soc., Perkin Trans. 1*, 2000, 3779.

Why are some alcohols easy to glucosylate with β-glucosidases while others are not?

Glucosidase



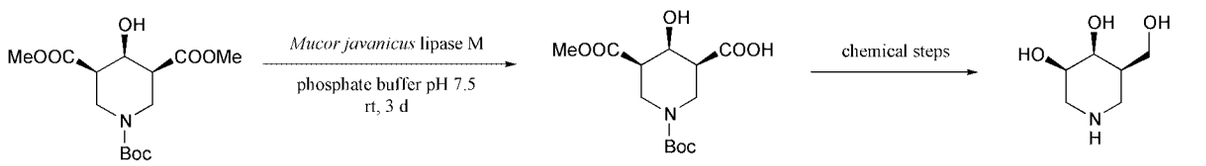
R^1OH = water or aglycon, R^2OH = aglycon or water,
AH = acidic amino acid side chain, B^- = basic amino acid side chain.

B. Mattheus de Roode, H. Zuilhof, M. C. R. Franssen, A. van der Padt and A. de Groot, *J. Chem. Soc., Perkin Trans. 2*, 2000, 2217.

The reactivity or lack thereof of almond β-glucosidase towards 16 aglycons is explained using quantum chemical methods. The successful enzymatic glucosylation of an aglycon appears to be mainly dependent on the nucleophilicity of the aglycon. This method expains why vinylic and phenolic aglycons are unreactive towards glucosylation with β-glycosidase.

Chemoenzymatic synthesis of isogalactofagomine

Lipase



(3*R*,5*S*)-95%, 99% e.e.

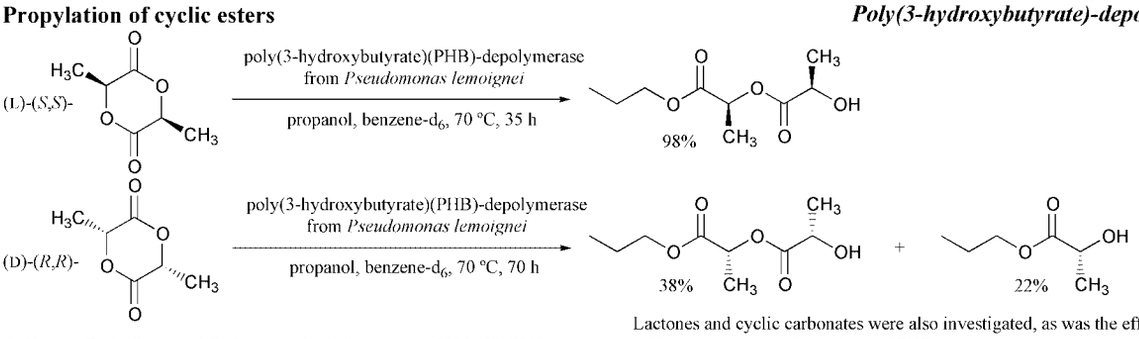
10% isogalactofagomine

X. Liang, A. Lohse and M. Bols, *J. Org. Chem.*, 2000, **65**, 7432.

Reported synthesis shows improvements over previous methods.

Propylation of cyclic esters

Poly(3-hydroxybutyrate)-depolymerase

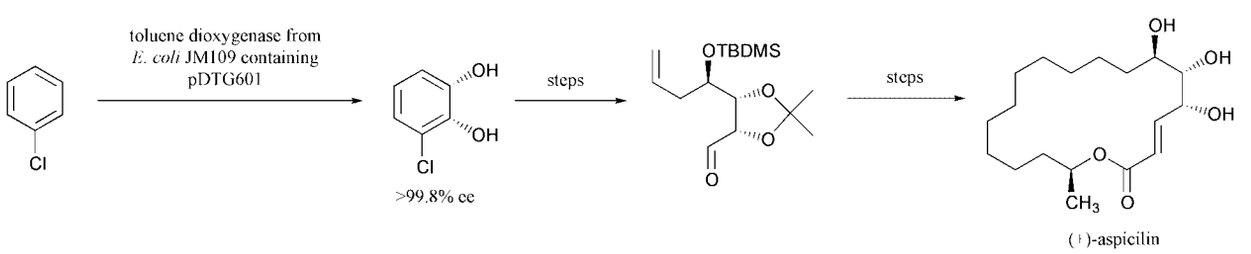


A. Kumar, R. A. Gross and D. Jendrossek, *J. Org. Chem.*, 2000, **65**, 7800.

Lactones and cyclic carbonates were also investigated, as was the effect of different solvents on the activity of PHB-depolymerase.

Chemoenzymatic synthesis of (+)-aspicilin

Toluene dioxygenase



>99.8% ee

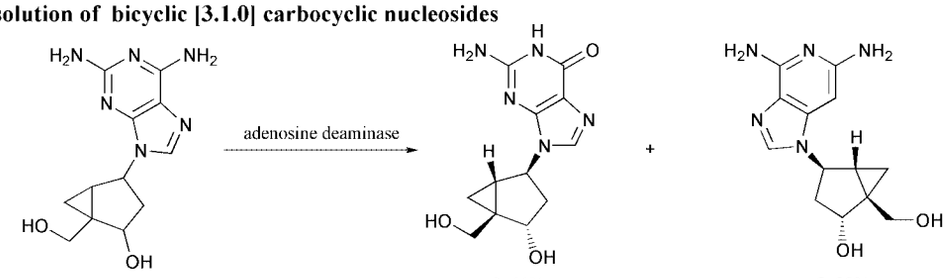
(+)-aspicilin

M. G. Banwell and K. J. McRae, *Org. Lett.*, 2000, **2**, 3583.

The utility of the valuable chiral diols generated by toluene dioxygenase is again demonstrated in this elegant synthesis of the macrocyclic lactone (+)-aspicilin.

Resolution of bicyclic [3.1.0] carbocyclic nucleosides

Adenosine deaminase



(±)-**1**

2, 46%

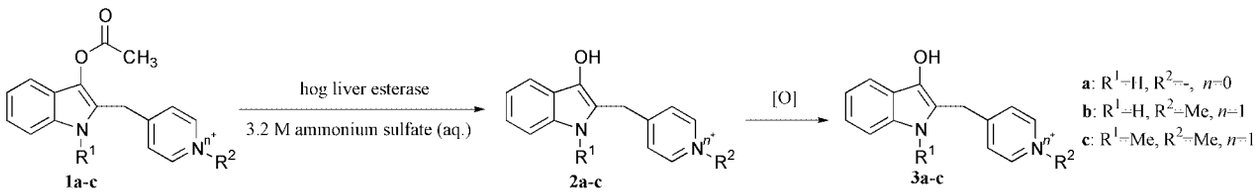
3, 39%

H. R. Moon, H. Ford Jr. and V. E. Marquez, *Org. Lett.*, 2000, **2**, 3793.

Enzymatic resolution of **1** was accomplished using adenosine deaminase to yield the desired product **2** in 46% yield. An adenine analogue was similarly resolved.

New indolyl substrates for chromogenic and fluorogenic detection of esterase activity in solution

Esterase

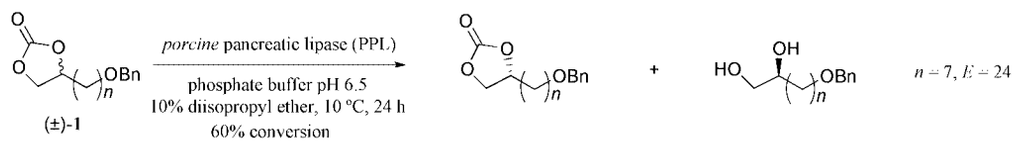


Hydrolysis by esterase followed by oxidation affords the coloured water soluble indolinones **3a-c** with absorption maxima at 478, 502 and 540 nm respectively. The absorption maxima of **3a-c** are significantly different from the absorption maxima of the substrate bound chromophore, therefore allowing for chromogenic detection. Hydrolysis of **1a** was also monitored using fluorescence spectroscopy since the intermediate **2a** is fluorescent.

H. J. Karlsson and G. Westman, *Tetrahedron*, 2000, **56**, 8939.

Enzymatic preparation of optically active 1,2-diols

Lipase

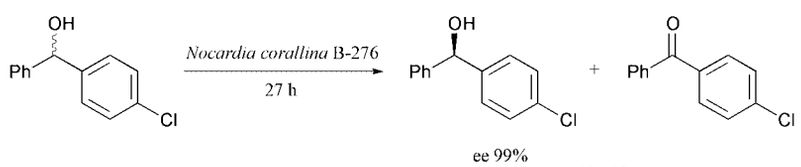


Other substrates bearing longer alkyl chains were examined. When $n = 10$, optically pure (*R*)-**1** was obtained (38% yield, $E = 27$).

M. Shimojo, K. Matsumoto and M. Hatanaka, *Tetrahedron*, 2000, **56**, 9281.

Kinetic resolution of some diaryl carbinols

Dehydrogenase

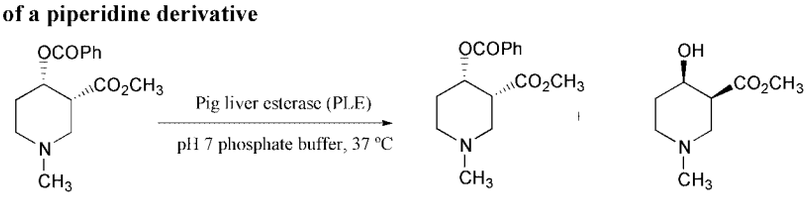


A range of substrates, in addition to the one shown, with various other substituents in different positions around the ring were tested. Reaction time, ee and the ratio of ketone/unreacted alcohol varied depending on the substrate used.

H. I. Pérez, , H. Luna, N. Manjarrez, A. Solis and Ma. A. Nuñez, *Tetrahedron: Asymmetry*, 2000, **11**, 4263.

Lipase resolution of a piperidine derivative

Esterase

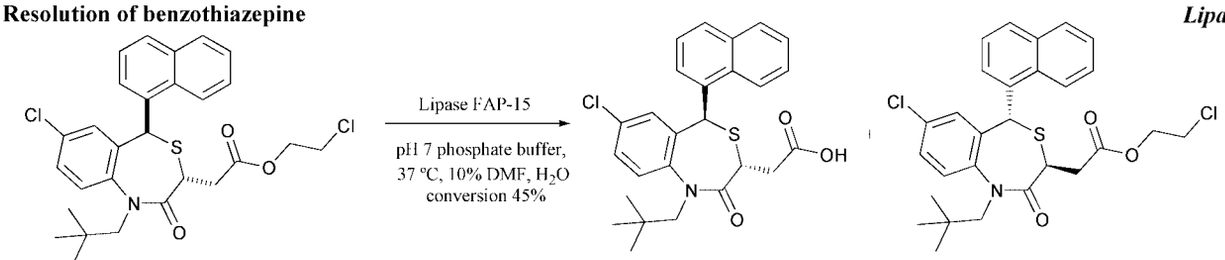


The PLE resolution was also performed on the *trans*- isomer, and with an *N*-isopropyl group in place of the *N*-methyl group. ee's varied between 95 and 100%. This transformation is part of a synthesis of some cocaine analogues.

M. Roberti, R. Rondanin, R. Ferroni, R. Baruchello, F. P. Invidiata, V. Andrisano, C. Bertucci V. Bertolasi, S. Grimaudo, M. Tolomeo and D. Simoni, *Tetrahedron: Asymmetry*, 2000, **11**, 4397.

Resolution of benzothiazepine

Lipase

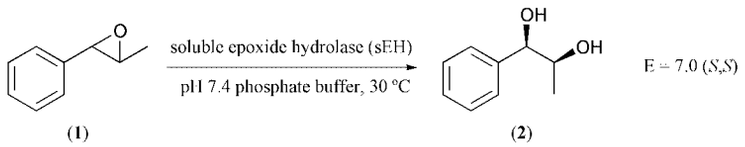


A number of different esters were subjected to hydrolysis by a variety of lipases. The best results were found for Lipase FAP-15, with chloro ester (**1**). The acid was used in the synthesis of some potential squalene synthetase inhibitors.

X. Yang, L. Buzon, E. Hamanaka and K. K.-C. Liu, *Tetrahedron: Asymmetry*, 2000, **11**, 4447.

Regio- and enantioselective hydrolysis of phenyloxiranes

Epoxide hydrolase

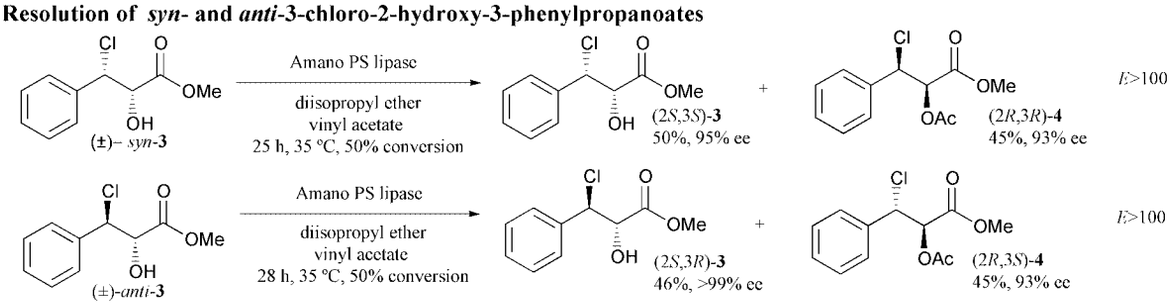


K. C. Williamson, C. Morisseau, J. E. Maxwell and B. D. Hammock, *Tetrahedron: Asymmetry*, 2000, **11**, 4451.

The regio- and enantioselectivity of various sEHs for some phenyloxiranes was investigated. The highest enantioselectivity was observed for human sEH on the compound shown. Regioselectivity was generally high. *e.g.* 98.3–100% water incorporation at the benzylic carbon of (**1**), with mouse sEH.

Resolution of *syn*- and *anti*-3-chloro-2-hydroxy-3-phenylpropanoates

Lipase

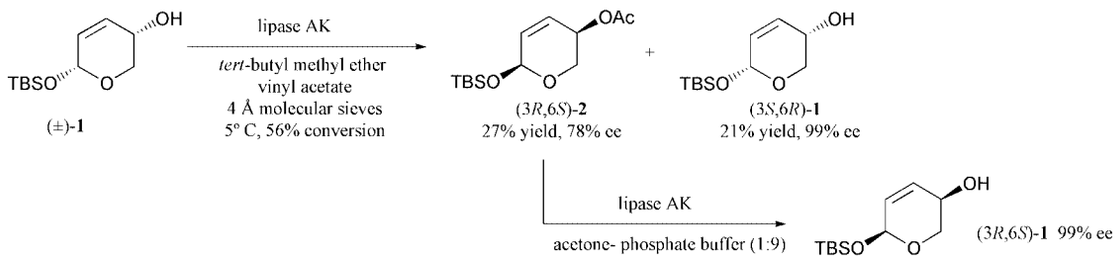


H. Hamamoto, V. A. Mamedov, M. Kitamoto, N. Hayashi and S. Tsuboi, *Tetrahedron: Asymmetry*, 2000, **11**, 4485.

All four diastereomers of **3** were converted to *N*-benzoyl (2*R*,3*S*)-3-phenylisoserine methyl ester, C-13 side chain analogues of paclitaxel.

Kinetic resolution of *cis*-6-(*tert*-butyldimethylsilyloxy)-3,6-dihydro-2*H*-pyran-3-ol

Lipase

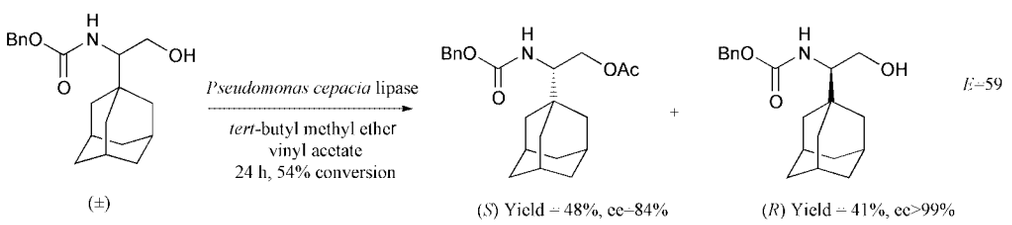


K. Sugawara, Y. Imanishi and T. Hashiyama, *Tetrahedron: Asymmetry*, 2000, **11**, 4529.

(3*S*,6*R*)-**1** and (3*R*,6*S*)-**1** were oxidised by manganese dioxide to provide the corresponding 6-silyloxypyran-3-one (6*R*) and (6*S*) respectively.

Resolution of *rac*-*N*-(benzyloxycarbonyl)-2-(1-adamantyl)-2-aminoethanol

Lipase

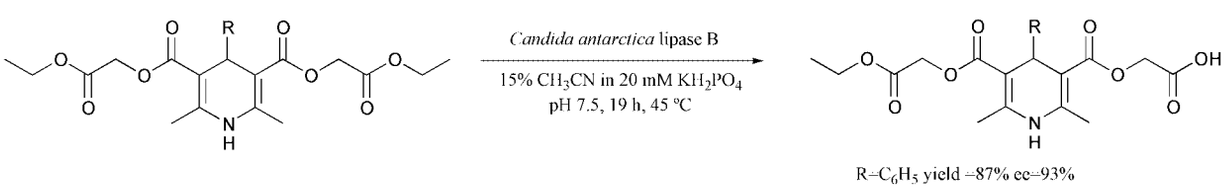


J. Clariana, S. García-Granda, V. Gotor, A. Gutiérrez-Fernández, A. Luna, M. Moreno-Mañas and A. Vallribera, *Tetrahedron: Asymmetry*, 2000, **11**, 4549.

The *R* enantiomer was converted in two steps to (*R*)-(1-adamantyl)glycine and one step to (*R*)-2-(1-adamantyl)-2-aminoethanol.

Hydrolysis of 4-substituted bis(ethoxycarbonylmethyl) 1,4-dihydropyridine-3,5-dicarboxylates

Lipase

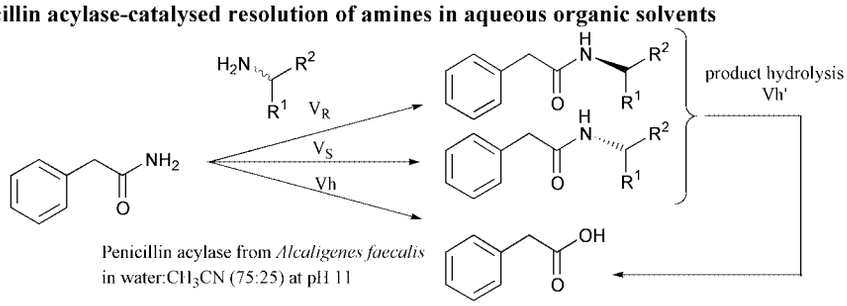


A. Sobolev, M. C. R. Franssen, N. Makarova, G. Duburs and A. de Groot *Tetrahedron: Asymmetry*, 2000, **11**, 4559.

A range of 1,4-dihydropyridines were hydrolysed with yields in the range 15-87% and ee in the range 1 to 97%. A range of reaction solvents were also studied.

Penicillin acylase-catalysed resolution of amines in aqueous organic solvents

Acylase



Penicillin acylase from *Alcaligenes faecalis* in water:CH_3CN (75:25) at pH 11

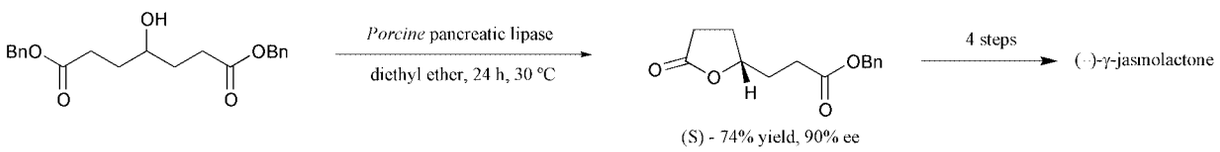
Where $R^1 = C_2H_4Ph$ and $R^2 = CH_3$ $E = 400$ (*R*), S/H (initial ratio between amide formation and formation of phenylacetic acid) = 4 and V_i (initial rate of amide formation in μmol per unit per hour) = 13

L. M. van Langen, N. H. P. Oosthoek, D. T. Guranda, F. van Rantwijk, V. K. Svedas and R. A. Sheldon, *Tetrahedron: Asymmetry*, 2000, **11**, 4593.

Penicillin acylase from *Alcaligenes faecalis* catalysed the phenylacetylation of amines by an order of magnitude more efficiently than penicillin acylase from *E. coli*.

Enantioselective synthesis of (–)-γ-jasmolactone

Lipase

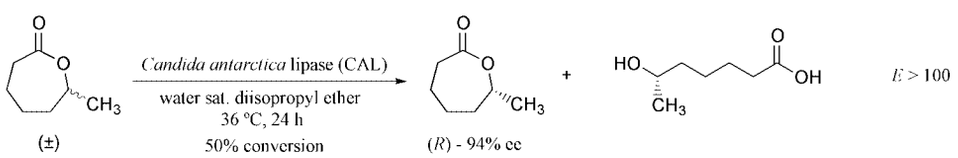


(S) - 74% yield, 90% ee

L. J. Missio and J. V. Comasseto, *Tetrahedron: Asymmetry*, 2000, **11**, 4609.

Resolution of racemic seven-membered substituted lactones

Lipase

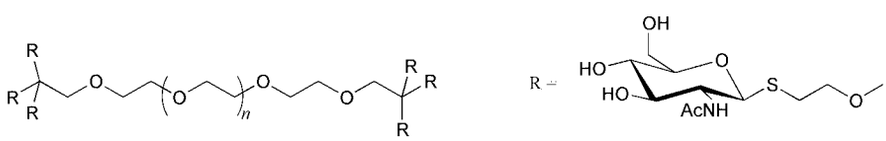


K. Shioji, A. Matsuo, K. Okuma, K. Nakamura and A. Ohno, *Tetrahedron Lett.*, 2000, **41**, 8799.

Other optically active 3-, 4-, 5-methyl substituted seven-membered lactones were prepared by CAL-catalysed resolution of the racemic lactones. Among these lactones, only 5-methylhexanolide shows (*R*)-selectivity to give the (*S*)-isomer.

Enzymatic manipulations of oligosaccharides on solid support

Transferase, epimerase

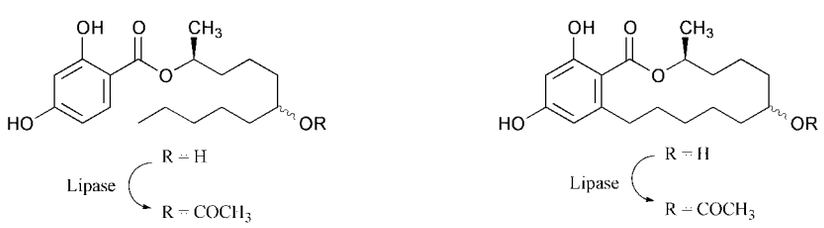


A. Lubineau, A. Malleron and C. Le Narvor, *Tetrahedron Lett.*, 2000, **41**, 8887.

A new solid support has been evaluated with respect to enzymatic glycosylation. Using bovine milk β(1-4)galactosyltransferase (2 U) and UDP-glucose/UDP-glucose 4-epimerase gave after cleavage, *N*-acetyllactosamine. Using Fuc TIII (0.2 U) gave a mixture of Lewis^x trisaccharide and *N*-acetyllactosamine.

Acylation of secondary alcohols in 14-membered lactones and acyclic analogues

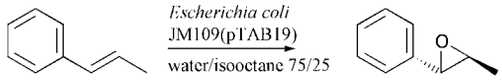
Lipase



E. Ljubović and V. Sunjić, *Tetrahedron Lett.*, 2000, **41**, 9135.

A comparative study of the acylation of a macrocyclic lactone and its acyclic form was performed using seven lipases. The macrocyclic form was acylated, predominately giving the 3*S*, 7*S* product, with de up to 99%. The acyclic form gave either diastereomer, depending on the lipase.

Epoxidation of styrene derivatives ***Monooxygenase***



ee >99%

Substrates with variations in ring substituents, ring heteroatoms and in the allylic portion were examined. Electron withdrawing substituents decreased the conversion rate, as did α- and β-substituted styrenes and most markedly, styrenes with a substituent at the 4 position on the ring. Substituent size also appeared to affect the conversion rate.

S. Bernasconi, F. Orsini, G. Sello, A. Colmegna, E. Galli and G. Bestetti, *Tetrahedron Lett.*, 2000, **41**, 9157.