Perkin 1 Abstracts: Biocatalysis in Organic Synthesis

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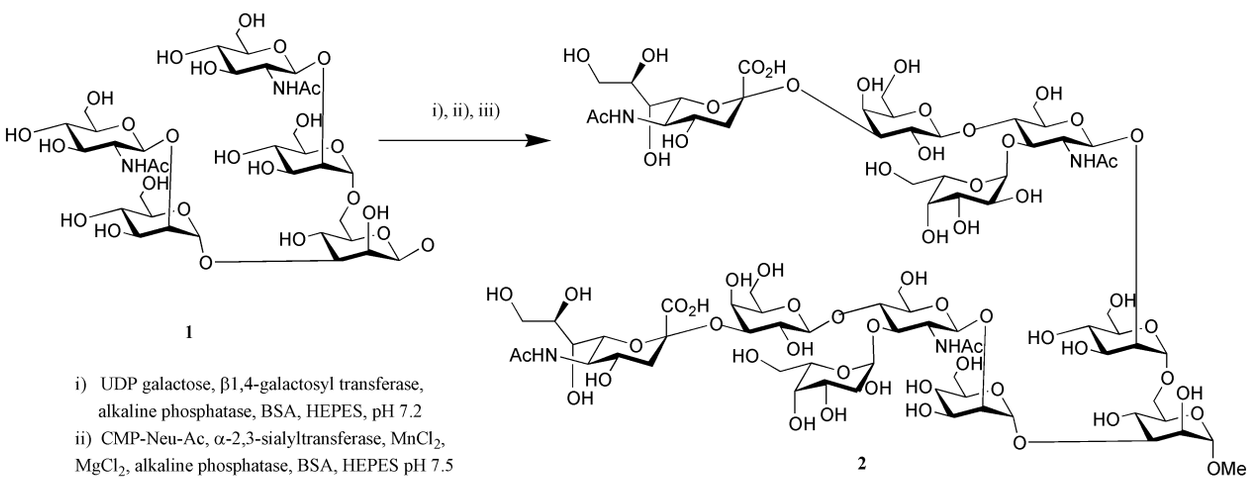
Perkin 1 Abstracts: Biocatalysis in Organic Synthesis aims to cover recent literature concerning the applications of enzymes and micro-organisms as catalysts in organic synthesis. The abstracts will emphasise the key synthetic step(s) that are mediated by the biocatalyst. Emerging technologies for biocatalyst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

Synthesis of dimeric sialyl Lewis X structures ***Galactosyl transferase/fucosyl transferase***

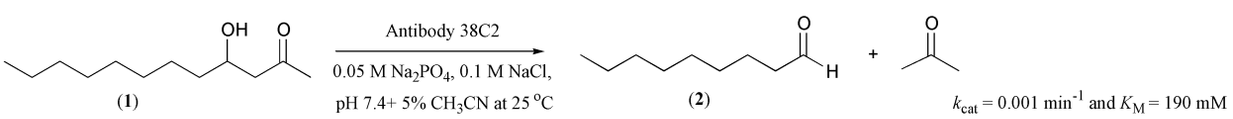


i) UDP galactose, β1,4-galactosyl transferase, alkaline phosphatase, BSA, HEPES, pH 7.2
ii) CMP-Neu-Ac, α-2,3-sialyltransferase, $MnCl_2$, $MgCl_2$, alkaline phosphatase, BSA, HEPES pH 7.5
iii) GDP-L-galactose, α-1,3-fucosyltransferase V, $MnCl_2$, alkaline phosphatase, BSA, MES pH 6.0.

A. Düffels, L. G. Green, R. Lenz, S. V. Ley, S. P. Vincent and C.-H. Wong, *Bioorg. Med. Chem.*, 2000, **8**, 2519.

Undecasaccharide **2** was elaborated in three enzymatic steps from the pentasaccharide **1**. The sialylation was achieved in a high yield of 99%. The undecasaccharide **2** is being tested for binding affinity toward E-selectin.

Rapid detection of antibody catalysis by luminescent bacteria ***Aldolase antibody 38C2***

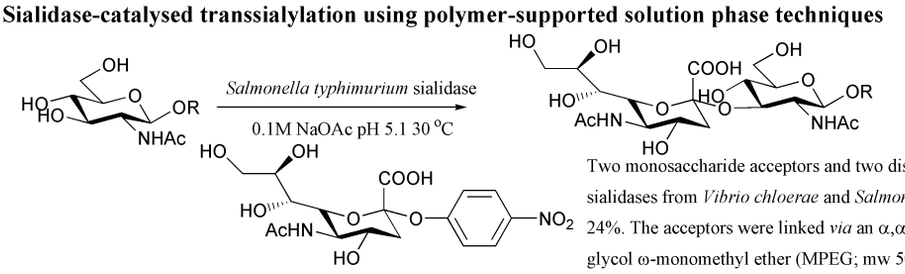


k_{cat} = 0.001 min^{-1} and K_M = 190 mM

H. Shulman, A. Eberhard, C. Eberhard, S. Ulitzur and E. Keinan, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 2353.

VhM42, an aldehyde negative mutant of *Vibrio harveyi*, was used to detect nonanal (**2**) produced in the retro aldol fragmentation of 4-hydroxydodecan-2-one (**1**). *Vibrio harveyi* produces light as a result of the luciferase-catalysed reaction of reduced flavin mononucleotide, with a long chain aldehyde and oxygen. VhM42 produces light in the presence of exogenous aldehyde. Detection of $3x10^{-8}$ M of antibody 38C2 is feasible. Detection of activity of 24H6 towards **1** was also reported.

Sialidase-catalysed transsialylation using polymer-supported solution phase techniques ***Sialidase***



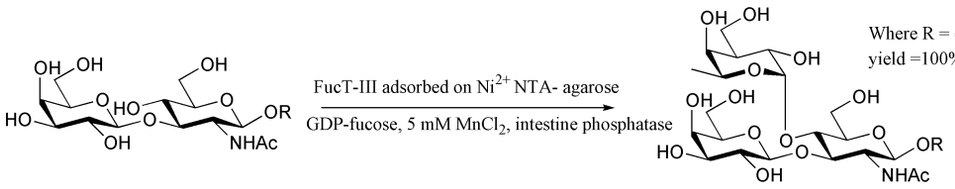
Where R = DOX- MPEG Yield = 24% after 2 transsialylation steps

D. Schmidt and J. Thiem, *Chem. Commun.*, 2000, 1919.

Two monosaccharide acceptors and two disaccharide acceptors were transsialylated using sialidases from *Vibrio chloerae* and *Salmonella typhimurium* in yields ranging from 12 to 24%. The acceptors were linked *via* an α,α′dioxyxylyl linker(DOX) to polyethylene glycol ω-monomethyl ether (MPEG; mw 5000), a polymer which is soluble under reaction conditions but insoluble during work-up in *tert*-butyl methyl ether. Unreacted polymer bound acceptor molecules were removed using β-galactosidase from *Bacillus circulans* and β–*N*-acetylhexosaminidase from *Aspergillus niger.*

Outstanding stability of immobilized recombinant α(1→3/4)-fucosyltransferases

Fucosyltransferase

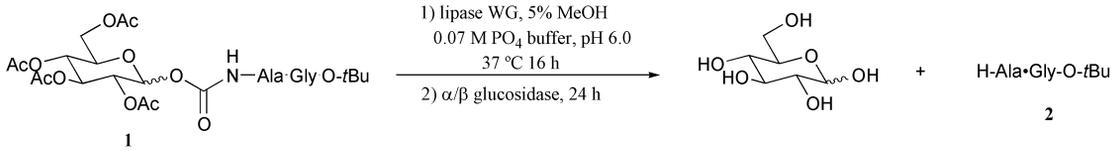


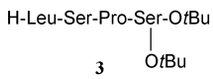
Immobilisation of α(1→3/4)-fucosyltransferase (FucT-III) on Ni^{2+}-agarose increased the enzyme activity by a factor of 15-20 and improved enzyme stability under incubation conditions ($t_{1/2}$ = 3 weeks at 37 °C).

C. Auge, A. Malleron, H. Tahrat, A. Marc, J.-L. Goergen, M. Cerutti, W. F. A. Steelant, P. Delannoy and A. Lubineau, *Chem. Commun*., 2000, 2017.

Enzyme labile protecting groups in peptide synthesis

Lipase/glucosidase/galactosidase



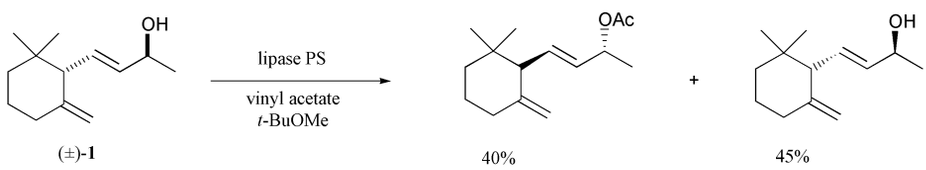


A one-pot, two step enzymatic method for the removal of protecting groups was reported. Peptide conjugate **1** was transformed to the peptide **2** in 64% yield. For larger peptides, a β-galactose-derived urethane was employed, and deprotection accomplished using galactosidases. Using this methodology, the tetrapeptide **3** was synthesised.

A. G. Gum, T. Kappes-Roth and H. Waldmann, *Chem. Eur. J*., 2000, **6**, 3714.

Resolution of γ-ionone derivatives

Lipase

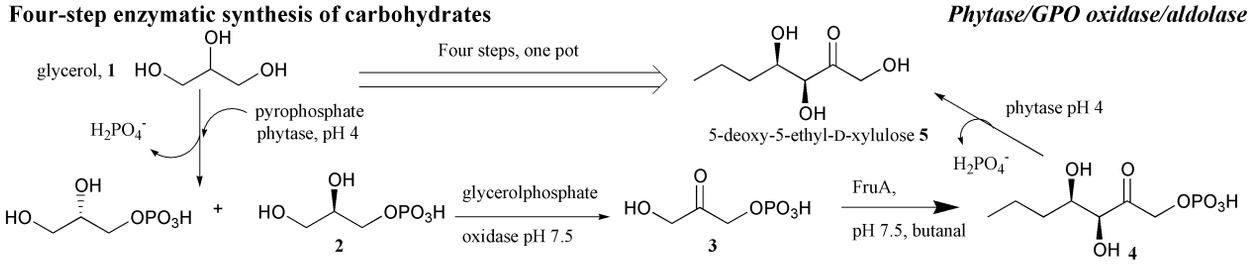


A chemoenzymatic approach to (+)- and (–)-γ-ionone was reported. The racemic form of alcohol **1** gave a 1:1 mixture of enantiomerically pure diastereomeric acetates on treatment with *Pseudomonas cepacia* lipase enzyme. Hence, both diastereomers were resolved by crystallisation, then diastereomer **1** was resolved using the same enzyme.

C. Fuganti, S. Serra and A. Zenoni, *Helv. Chim. Acta,* 2000, **83**, 2761.

Four-step enzymatic synthesis of carbohydrates

Phytase/GPO oxidase/aldolase

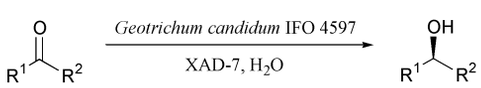


A four step enzymatic cascade was devised for the synthesis of 5-deoxy-5-ethyl-D-xylulose **5** from glycerol. 1) Phosphorylation of glycerol (pH 4) 2) aerobic oxidation (pH 7.5) 3) aldolase C–C bond formation (pH 7.5) 4) dephosphorylation (pH 4). The phytase on/off switch by pH was therefore used to control phosphorylation/dephosphorylation.

R. Schoevaart, F. van Rantwijk and R. A. Sheldon, *J. Org. Chem.*, 2000, **65**, 6940.

Asymmetric reduction of ketones by *Geotrichum candidum* in the presence of Amberlite™

Geotrichum candidum

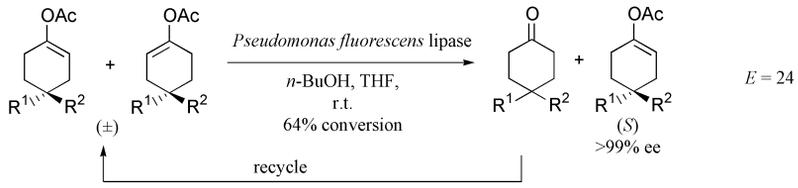


A range of aliphatic ketones were reduced with yields in the range 14-98% and ee in the range 18 to >99%. A range of acetophenone derivatives and aromatic ketones were reduced, under argon, with yields in the range 32 to 99% and ee in the range 84 to >99%. Reduction of a range of ketones on a 300 mg scale was reported.

K. Nakamura, M. Fujii and Y. Ida, *J. Chem. Soc., Perkin Trans. 1*, 2000, 3205.

Resolution of enol acetates

Lipase



$E = 24$

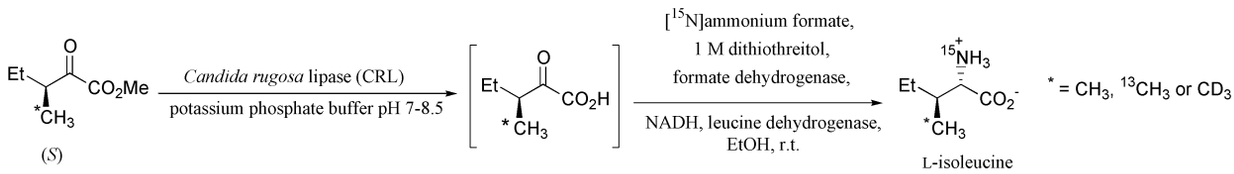
R^1 = Ph, R^2 = CN

Substrates with varying R^1 and R^2 groups were screened. It was found that the highest selectivity was obtained for substrates containing a 4-cyano and 4-aryl or a 4-benzyloxy substituent.

G. Allan, A. J. Carnell, M. L. E. Hernandez and A. Pettman, *J. Chem. Soc., Perkin Trans. 1*, 2000, 3382.

Synthesis of isotopically labelled L-α-amino acids

Lipase



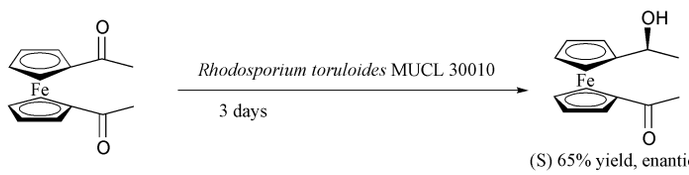
L-isoleucine
74% yield, 90% de

Similar enzymatic hydrolysis-reductive amination sequences were used to prepare other L-α-amino acids e.g. *allo*-isoleucine, threonine, *allo*-threonine and valine.

J. R. Harding, R. A. Hughes, N. M. Kelly, A. Sutherland and C. L. Willis, *J. Chem. Soc., Perkin Trans. 1*, 2000, 3406.

Enantioselective yeast reduction of diacetylferrocene

Reductase



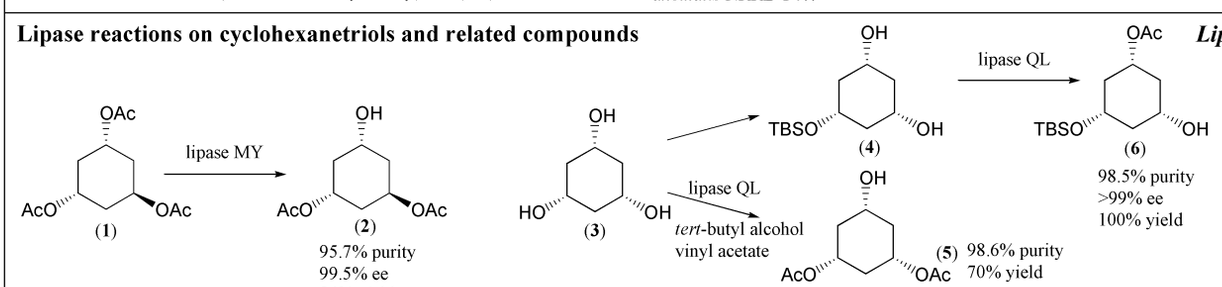
(S) 65% yield, enantiomerically pure

The enantioselective enzymatic reduction of diacetylferrocene was achieved using 4 yeast strains. Of these, *R. toruloides* MUCL 30010 gave enantiomerically pure (*S*)-ketoalcohol, and *Saccharomyces montanas* CBS 6772 gave the (*R*) enantiomer with lower ee. Other effective strains were *R. glutinis* NRRL Y1091 and *Pichia anomalis* NRRL Y40.

L. Veum, H. Brouard, P. Meffre, M. Larchevêque, D. Buisson, E. Demousseau and R. Azerad, *Tetrahedron*: *Asymmetry*, 2000, **11**, 4055.

Lipase reactions on cyclohexanetriols and related compounds

Lipase

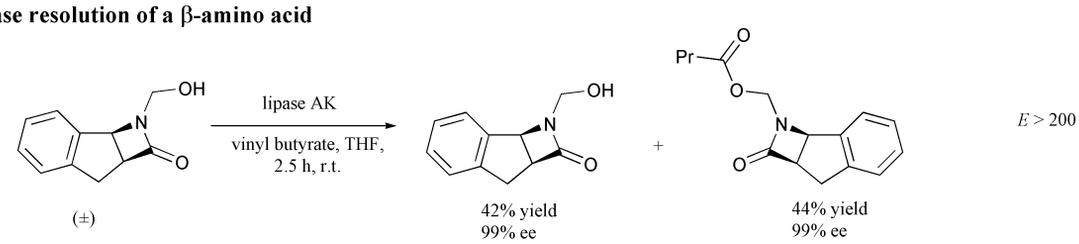


A number of lipases were screened for suitability for the selective hydrolysis of the triacetate (**1**). Of these, lipase MY was most effective. The resulting diacetoxy alcohol (**2**) was used in the synthesis of Retiferol. The other enantiomers were obtained *via* the enzymatic steps shown. In each case lipase QL was found to be most effective.

B. Wirz, H. Iding, and H. Hilpert. *Tetrahedron*: *Asymmetry*, 2000, **11**, 4171.

Lipase resolution of a β-amino acid

Lipase



$E > 200$

42% yield
99% ee

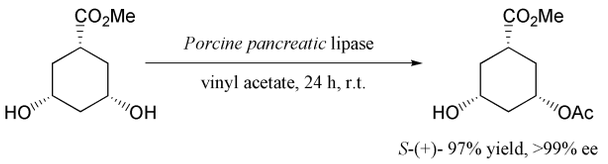
44% yield
99% ee

Other solvents, lipase PS, and vinyl acetate were also tested, but found to be less effective. Subsequent steps in the synthesis gave all 4 enantiomers of 1-aminoindane-2-carboxylic acid hydrochloride separately.

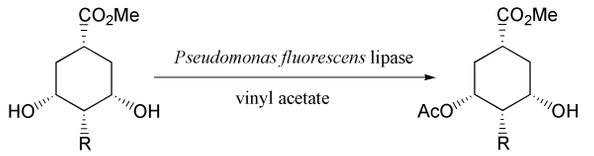
F. Fülöp, M. Palkó, J. Kámán, L. Lázár and R. Sillanpää, *Tetrahedron*: *Asymmetry*, 2000, **11**, 4179.

Asymmetrization of all-*cis*-3,5-dihydroxy-1-(methoxycarbonyl)cyclohexane

Lipase



S-(+)- 97% yield, >99% ee



R = Me, Et

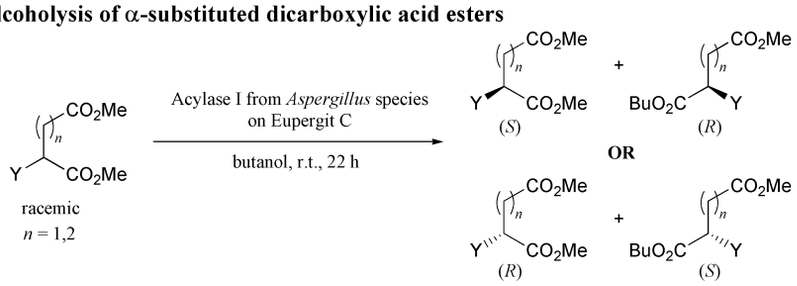
R = Me: *R*-(–)-92% yield, >99% ee
R = Et: *R*-(–)-67% yield, >99% ee

Y. Zhao, Y. Wu, P. De Clercq, M. Vandewalle, P. Maillos and J.-C. Pascal, *Tetrahedron: Asymmetry*, 2000, **11**, 3887.

Hydrolysis of the chemically synthesised 4-alkyl-diesters led to the opposite enantiomeric series. Interestingly, hydrolysis of the 4-unsubstituted diester resulted in the same enantiomeric series as the transesterification reaction.

Alcoholysis of α-substituted dicarboxylic acid esters

Acylase I



Y = Me, MeO, PrO, Cl, Br
E = 2-17, conversion = 5-45%

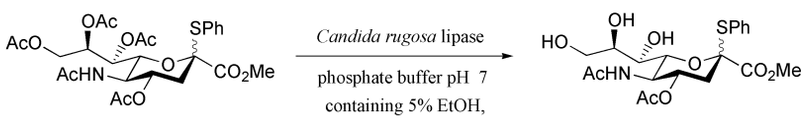
Y = NH_2, NHAc, PrCOHN, CbzNH, HO, AcO, $PrCO_2$
E = 2-100, conversion = 7-85%

A. Liljeblad, J. Lindborg and L.T. Kanerva, *Tetrahedron: Asymmetry*, 2000, **11**, 3957.

Excellent regioselectivity is observed with butanolysis occurring exclusively at the sterically more hindered α-ester group. Enantioselectivity is dependent on the nature of Y.

Chemoenzymatic preparation of 4-*O*-acetylsialic acid derivative

Lipase



β/α = 5/1

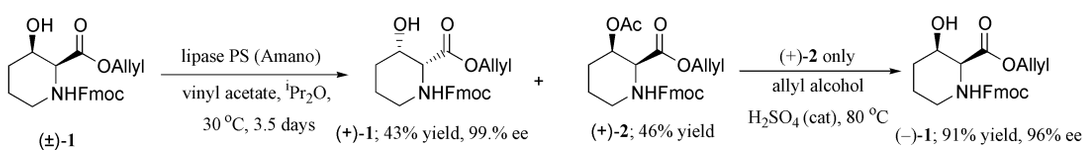
α; 14% yield
β; 65% yield

T.-L. Shih, M.-C. Cheng and S.-H. Wu, *Tetrahedron Lett.*, 2000, **41**, 7921.

The lipase selectively removes the primary acetyl group at C-9, then the secondary acetyl groups at C-8 and C-7 migrate to the C-9 position where they are hydrolysed by the lipase.

Resolution of *cis*-β-hydroxypipecolic acid

Lipase

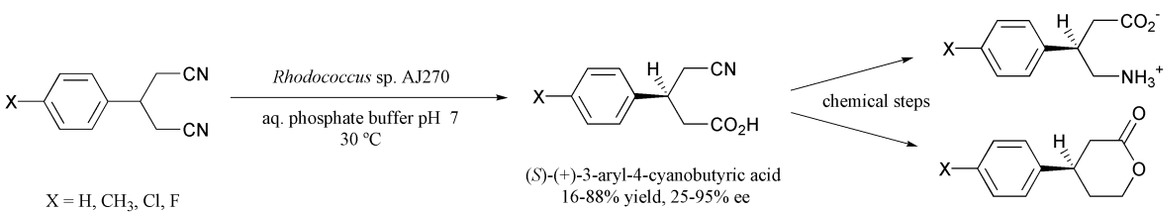


J. D. Scott and R. M. Williams, *Tetrahedron Lett.*, 2000, **41**, 8413.

Deprotected (–)-**1** is a key intermediate in the synthesis of the antitumour antibiotic, tetrazomine.

Desymmetrization of 3-arylglutaronitriles

***Rhodococcus* species**



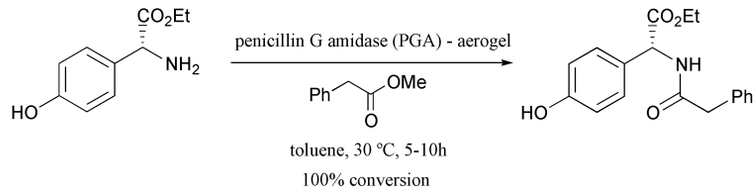
X = H, CH_3, Cl, F

(*S*)-(+)-3-aryl-4-cyanobutyric acid
16-88% yield, 25-95% ee

M.-X. Wang, C.-S. Liu, J.-S. Li and O. Meth-Cohn, *Tetrahedron Lett.*, 2000, **41**, 8549.

The chemical conversion and enantioselectivity is dependent on X and the reaction conditions. Using acetone as additive results in a greater enantioselectivity but a decrease in yield.

Aerogels as novel supports for enzyme adsorption ***Amidase/Protease***



A. Basso, L. De Martin, C. Ebert, L. Gardossi, A. Tomat, M. Casarci and O. Li Rosi, *Tetrahedron Lett.*, 2000, **41**, 8627.

An increase in the initial reaction rate was observed when compared to adsorption of PGA on Celite. Adsorption of the proteases thermolysin and α-chymotrypsin resulted in incomplete conversion after 144 hours. Entrapment of PGA during aerogel synthesis results in a complete loss of amidase activity.