Perkin 1 Abstracts: Biocatalysis in Organic Synthesis

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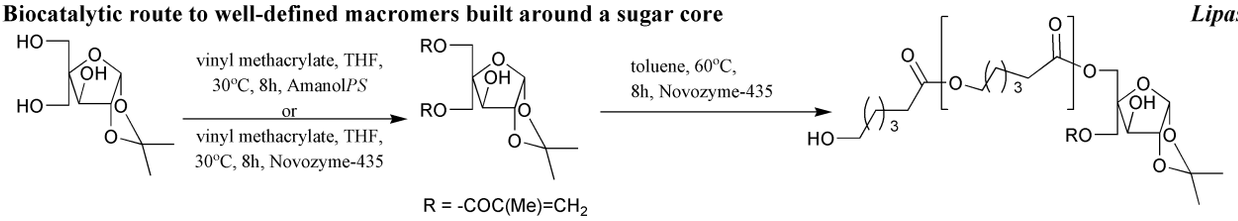
Perkin 1 Abstracts: Biocatalysis in Organic Synthesis aims to cover recent literature concerning the applications of enzymes and micro-organisms as catalysts in organic synthesis. The abstracts will emphasise the key synthetic step(s) that are mediated by the biocatalyst. Emerging technologies for biocatalyst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

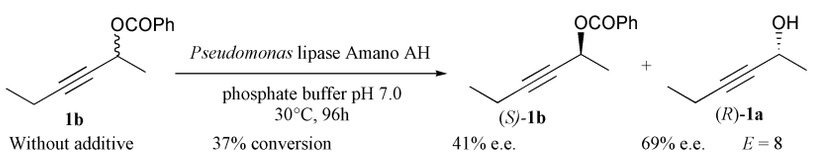
Biocatalytic route to well-defined macromers built around a sugar core ***Lipase***



Selective acrylation of 4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-pentofuranose by Amano *PS* occurred at the C-5 hydroxy and C-1' position with Novozyme-435. The acryl sugar was ring opened and polymerised in toluene with Novozyme-435 with the acryl sugar moiety linked to the carboxy terminus of a PCL chain. This was derivatised with oxalyl chloride which showed that <5% of the consumed ε-CL formed chains with a carboxy terminus.

R. Kumar and R. A. Gross, *J. Am. Chem. Soc.*, 2002, **124**, 1850.

The effect of polyphenols on the enantioselectivity of lipase-catalysed reactions ***Lipase***

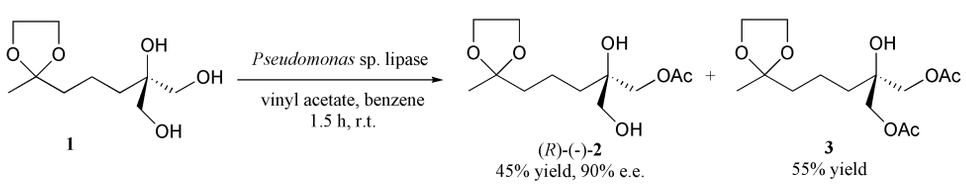


Without additive	37% conversion	41% e.e.	69% e.e.	$E = 8$
In the presence of (+)-catechin	23% conversion	29% e.e.	>99% e.e.	$E > 260$

Various catechin derivatives were screened and found to enhance the stereoselectivity of the hydrolysis of **1b** using lipase. In addition, some simple phenol derivatives were also found to increase the enantioselectivity.

K. Nakamura and K. Takenaka, *Tetrahedron: Asymmetry*, 2002, **13**, 415.

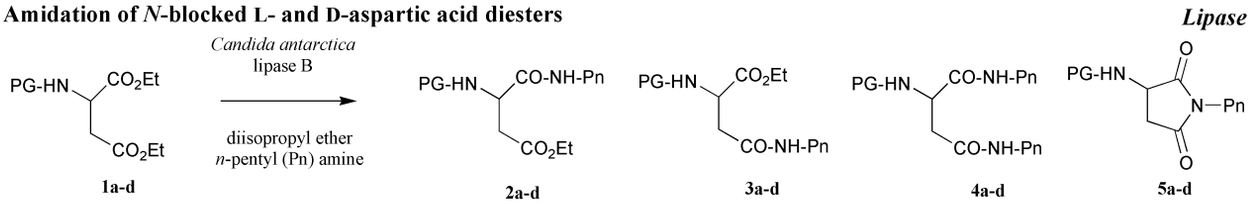
Chemo-enzymatic synthesis of (1*S*,5*R*)-(-)-frontalin ***Lipase***



R. Chênevert and D. Caron, *Tetrahedron: Asymmetry*, 2002, **13**, 339.

(*R*)-**2** was chemically transformed into frontalin in 95% yield.

Amidation of *N*-blocked L- and D-aspartic acid diesters ***Lipase***



PG = Protecting Group
a = CBz; b = Boc; c = Ac; d = PhAc

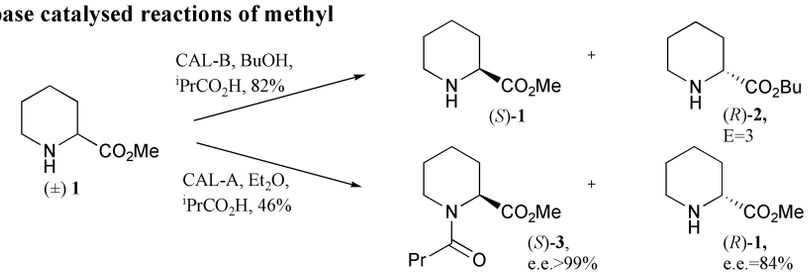
Possible products

Derivatives of aspartic acid **1a-d** were treated with *n*-pentylamine in the presence of lipase B from *Candida antarctica* to yield a mixture of the four possible products shown. L- derivatives underwent amidation at the α-position (**2a-d**) preferentially. D- *N*- amide derivatives were converted to β-monoamides (**3c** and **3d**).

S. Conde and P. López-Serrano, *Eur. J. Org. Chem.*, 2002, 922.

Enantioselective lipase catalysed reactions of methyl pipecolinate

Lipase

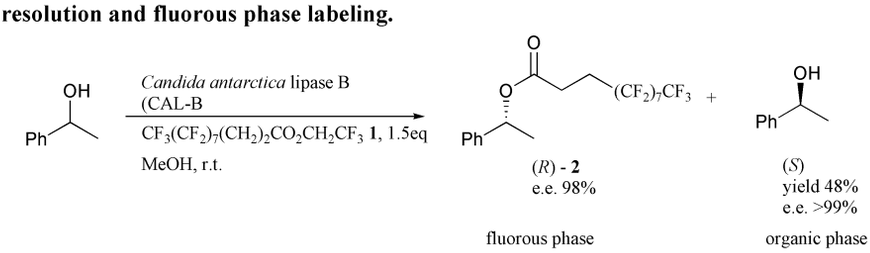


A. Liljeblad, J. Lindborg, A. Kanerva, J. Katajisto and L.T. Kanerva, *Tetrahedron Lett.*, 2002, **43**, 2471.

A study of the use of *Candida antarctica* lipase in the resolution of *N*-pipecolinate esters is reported. CAL-A was found to be a highly enantio- and chemoselective catalyst for the acylation of secondary amine, **1**, whereas CAL-B reacted chemoselectively at the ester function under the same conditions.

Kinetic resolution and fluorous phase labeling.

Lipase

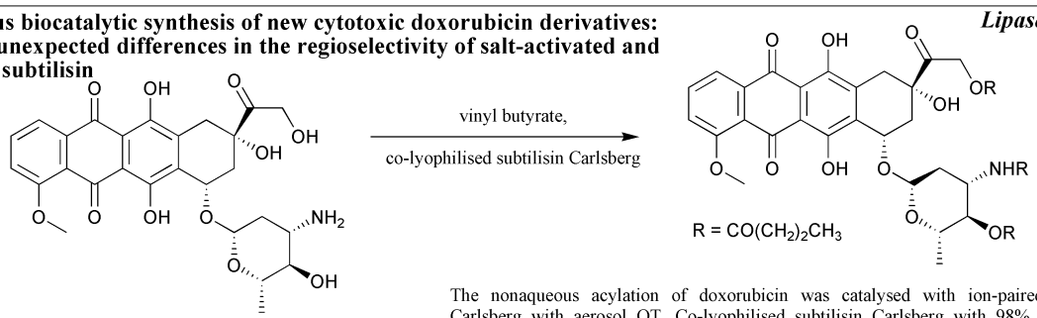


B. Hungerhoff, H. Sonnenschein and F. Theil, *J. Org. Chem.*, 2002, **67**, 1781.

The highly fluorinated ester **1** was synthesised in 5 steps and used as the acyl donor in a lipase catalysed resolution to yield ester **2**. The product mix was partitioned between *n*-C_6F_{14} and methanol. 3 other alcohols were effectively resolved in this way.

Nonaqueous biocatalytic synthesis of new cytotoxic doxorubicin derivatives: exploiting unexpected differences in the regioselectivity of salt-activated and solubilised subtilisin

Lipase/Protease

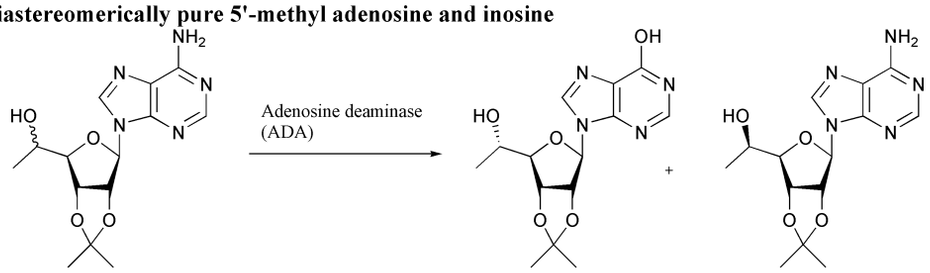


D. H. Altreuter, J. S. Dordick and D. S. Clark, *J. Am. Chem. Soc.*, 2002, **124**, 1871.

The nonaqueous acylation of doxorubicin was catalysed with ion-paired subtilisin Carlsberg with aerosol OT. Co-lyophilised subtilisin Carlsberg with 98% (w/w) KCl expanded the enzyme's regiospecificity to enable addition acylation at C4' hydroxy and C3' amine groups.

Preparation of diastereomerically pure 5'-methyl adenosine and inosine derivatives

Adenosine deaminase

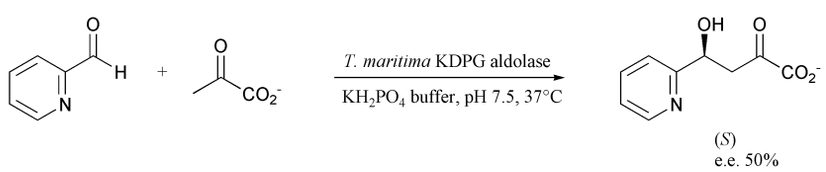


P. Ciuffreda, A. Loseto and E. Santaniello, *Tetrahedron* : *Asymmetry*, 2002, **13**, 239.

A study of the use of ADA to catalyse the deamination of adenosine analogs containing the ribose moiety modified at positions 2', 3' and 5', is reported. This process allows the preparation of diastereomerically pure (5'*S*)-inosine and unreacted (5'*R*)-adenosine derivatives.

Characterisation of *Thermotoga maritima* KDPG aldolase

Aldolase

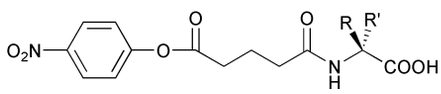


J. S. Griffiths, N. J. Wymer, E. Njolito, S. Niranjanakumari, C. A. Fierke and E.J. Toone, *Bioorg. Med. Chem.*, 2002, **10**, 545.

The KDPG aldolase from *T. maritima* has been cloned and over-expressed in *E. coli*. The scheme shown was used to establish stereospecificty of the enzyme. The activity of the retro-aldol cleavage was also studied. Substrate specificity in the synthetic direction was studied for a variety of aldehydes.

Remote chiral recognition in a reaction mediated by a catalytic antibody

Antibody



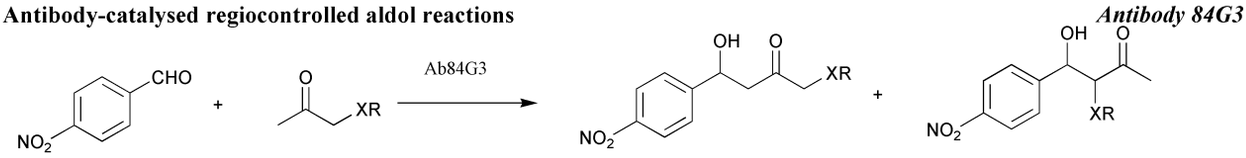
R = Me or H
R'= H or Me

L. J. D'Souza, B. Gigant, M. Knossow and B. S. Green, *J. Am. Chem. Soc.*, 2002, **124**, 2114.

D2.3 antibody preferentially binds the L-enantiomer of the chiral 4-nitrobenzyl ester hapten. The antibody catalyses its hydrolysis with a 20-fold improvement by a modification at the binding site from a glycine to an alanine.

Antibody-catalysed regiocontrolled aldol reactions

Antibody 84G3



1, X = CH_2, R = Me
2, X = CH_2, R = Et
3, X = O, R = Me
4**,** X = S, R = Me

5, X = CH_2, R = Me
6, X = CH_2, R = Et
7, X = O, R = Me
8, X = S, R = Me

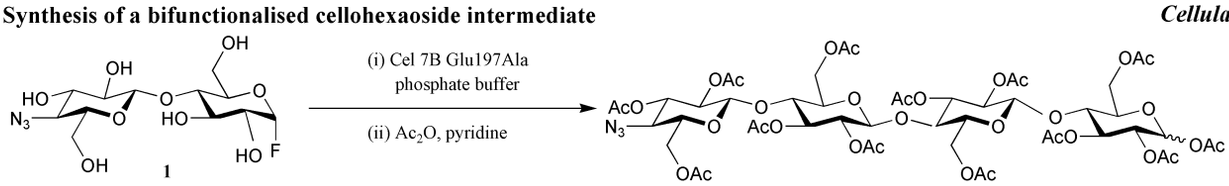
9, X = CH_2, R = Me
10, X = CH_2, R = Et
11, X = O, R = Me
12, X = S, R = Me

V. Maggiotti, M. Resmini and V. Gouverneur, *Angew. Chem., Int. Ed.*, 2002, **41**, 1012.

Antibody Ab84G3 catalysed the regioselective aldol coupling of *p*-nitro benzaldehyde to unsymmetrical ketones **1** to **4** to yield products of reaction at the less substituted carbon atom **5-8**. **9** to **12** were not formed. The enantioselectivity of addition was typically higher than 94%.

Synthesis of a bifunctionalised cellohexaoside intermediate

Cellulase



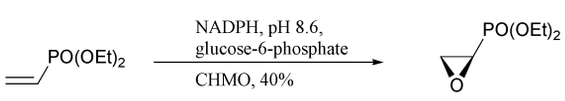
+ cellobiose

V. Boyer, S. Fort, T.P. Frandsen, M. Schülein, S. Cottaz and H. Driguez, *Chem. Eur. J.*, 2002, **8**, 1389.

The transglycosylation of α-glucosylfluoride **1** was achieved using the Glu197Ala 'glycosynthase' mutant of cellulase Cel7B from *Humicola insolens*. Condensation of **1** with an equimolar amount of cellobiose, followed by acetylation gave tetrasaccharide **2** with a yield of 66%**.**

First asymmetric epoxidation catalysed by cyclohexanone monooxygenase

Cyclohexanone monooxygenase



1

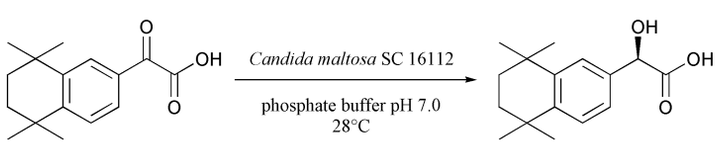
2
e.e.>98%
yield 40%

S. Colonna, N. Gaggero, G. Carrea, G. Ottolina, P. Pasta and F. Zambianchi, *Tetrahedron Lett.*, 2002, **43**, 1797.

The first highly enantioselective epoxidation reaction catalysed by cyclohexanone monooxygenase is reported. Diethyl vinylphosphonate, **1**, was converted to diethyl phosphonate epoxide, **2**, in moderate yield and excellent e.e.

Enantioselective microbial reduction of an aryl ketone

Dehydrogenases



1

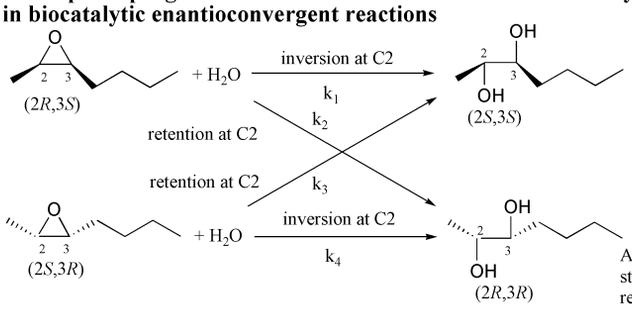
(*R*)-**2** - 56% yield, 99% e.e.

R. N. Patel, L. Chu, R. Chidambaram, J. Zhu and J. Kant, *Tetrahedron: Asymmetry*, 2002, **13**, 349.

(*R*)-**2** is an intermediate in the synthesis of (*R*)-3-fluoro-4-[[hydroxy(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)acetyl]amino]benzoic acid, a retinoic acid receptor gamma-specific agonist which is potentially useful as a dermatological and anti-cancer drug.

A computer program for the determination of stereoselectivity in biocatalytic enantioconvergent reactions

Epoxide Hydrolases/ Dehalogenases/Sulfatases/ Glycosidases

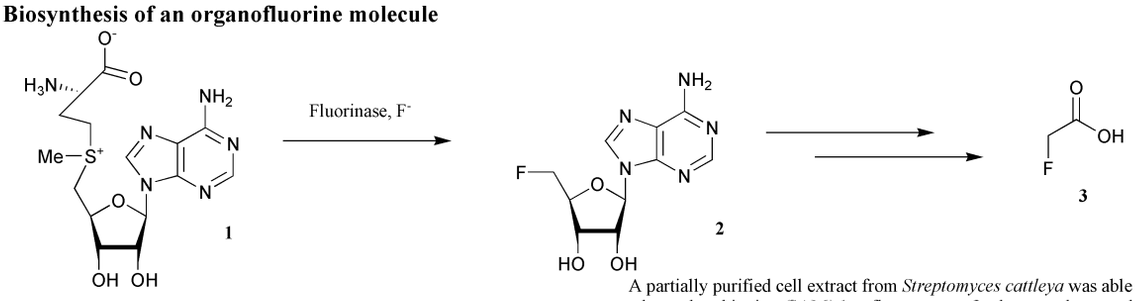


A parameter '*RI*' (Retention-Inversion ratio) was introduced to describe the stereoselectivity of biocatalytic asymmetric reactions which may proceed via different regio- or stereo-isomeric pathways. A computer program was developed for the treatment of the kinetics of such single-step processes.

K. Faber and W. Kroutil, *Tetrahedron: Asymmetry*, 2002, **13**, 377.

Biosynthesis of an organofluorine molecule

Fluorinase

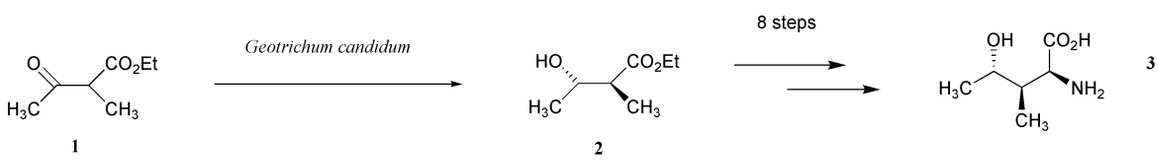


A partially purified cell extract from *Streptomyces cattleya* was able to transform *S*-adenosylmethionine *(*SAM*)* **1** to fluoroacetate **3** when supplemented with fluoride ions. The 'fluorinase' appeared to catalyse a nucleophilic attack at C-5' of SAM to give **2**. **2** may be metabolised to **3** *via* fluoroacetaldehyde.

D. O'Hagan, C. Schaffrath, S. L. Cobb, J. T. G. Hamilton and C. D. Murphy, *Nature*, 2002, **416**, 279.

Preparation of a precursor of (2*S*, 3*R*, 4*S*)-4-hydroxyleucine

Geotrichum candidum

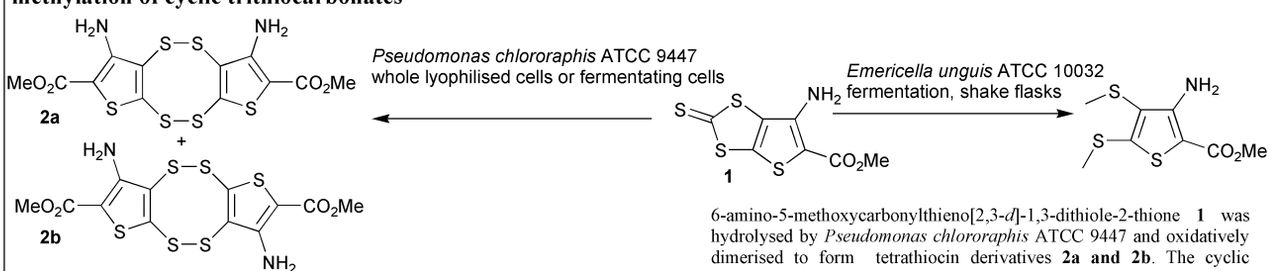


Ethyl 2-methylacetoacetate **1** was reduced by resting cells of *Geotrichum candidum* LCM I-2366 to give (2*S*, 3*S*)-3-hydroxy-2-methylbutanoate **2** in 91% yield with 97% diastereomeric excess and 92% enantiomeric excess. Eight further synthetic steps gave the insulinotropic α-amino acid (2*S*, 3*R*, 4*S*)-4-hydroxyleucine **3**.

Q. Wang, J. Ouazzani, N. André-Sasaki and P. Potier, *Eur. J. Org. Chem.*, 2002, 834.

First preparative biocatalytic hydrolysis and *S*-methylation of cyclic trithiocarbonates

Microbial

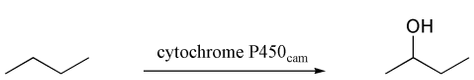


6-amino-5-methoxycarbonylthieno[2,3-*d*]-1,3-dithiole-2-thione **1** was hydrolysed by *Pseudomonas chlororaphis* ATCC 9447 and oxidatively dimerised to form tetrathiocin derivatives **2a and 2b**. The cyclic trithiocarbonate was also methylated by *Emericella unguis* ATCC 10032 involving cleavage followed by methylation of both thiol groups.

W. Kroutil, A. A. Stämpfli, R. Dahinden, M. Jörg, U. Müller and P. Pachlatko, *Tetrahedron*, 2002, **58**, 2589.

Butane and propane oxidation

Monooxygenase

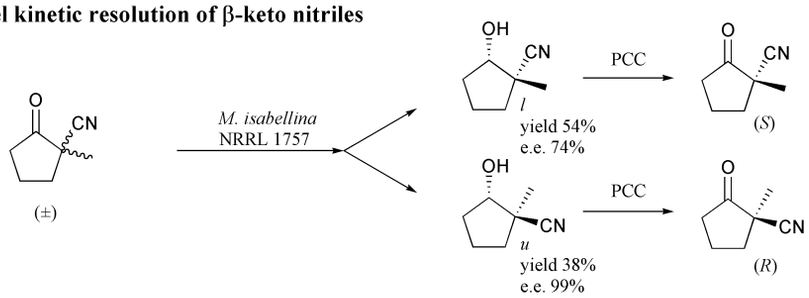


Using mutants of $P450_{cam}$, in which the active site volume was reduced, the oxidation of butane and propane was studied. Turnover rate and efficiency generally increased with decreasing active site volume. The highest turnover rate (750 min^{-1}) was observed for butane using a quadruple mutant. This value is comparable to the camphor oxidation rate by the wild-type enzyme (1200 min^{-1}).

S. G. Bell, J.-A. Stevenson, H. D. Boyd, S. Campbell, A. D. Riddle, E. L. Orton and L.-L. Wong, *Chem. Commun.*, 2002, 490.

Parallel kinetic resolution of β-keto nitriles

Mortierella isabellina

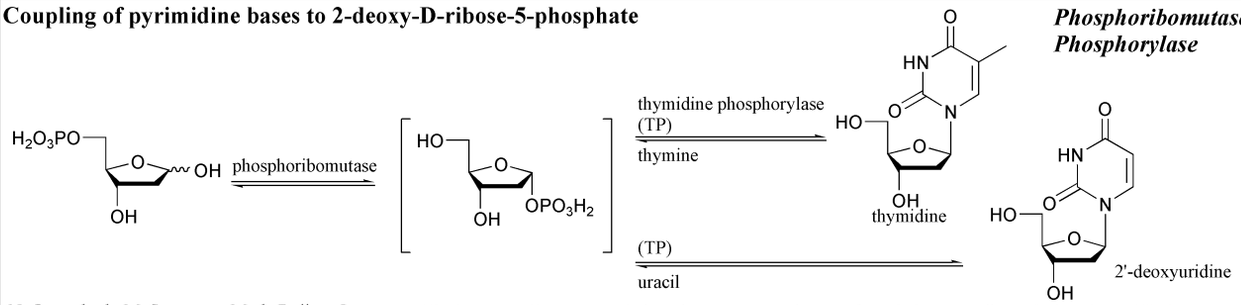


After separating the alcohol diastereomers by chromatography, oxidation gave the (*R*)- and (*S*)-ketones. Enhancement of the e.e. of the *l* alcohol was achieved by a repeat of the reduction using the (*S*)-ketone, giving the alcohol in 44% yield and 96% e.e. Similar results were obtained for the analogous 6 membered ketones.

J. R. Dehli and V. Gotor, *J. Org. Chem.*, 2002, **67**, 1716.

Coupling of pyrimidine bases to 2-deoxy-D-ribose-5-phosphate

Phosphoribomutase, Phosphorylase

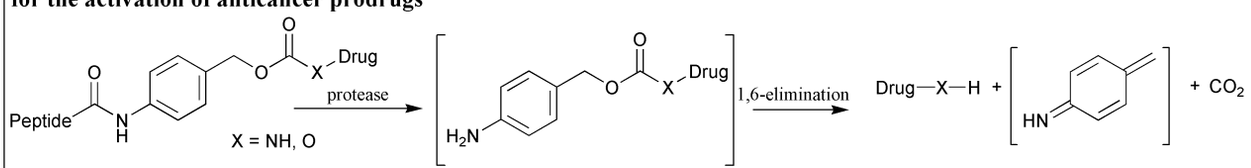


The base coupling was carried out in two enzymatic steps from 2-deoxy-D-ribose-5-phosphate. TP shows no activity with cytosine and 4 chemical steps were used to convert 2'-deoxyuridine to 2'-deoxycytidine. It was possible using this route to synthesise a variety of labeled pyrimidine 2'-deoxynucleosides.

N. Ouwerkerk, M. Steenweg, M. de Ruijter, J. Brouwer, J. H. van Boom, J. Lugtenburg and J. Raap, *J. Org. Chem.*, 2002, **67**, 1480.

Protease-mediated fragmentation of *p*-aminobenzyl ethers: a new strategy for the activation of anticancer prodrugs

Protease

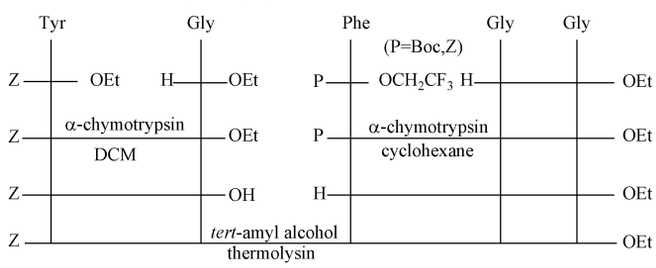


N-protected peptide benzyloxycarbonyl-valine-citrulline (*Z*-val-cit), was attached to amino groups of *p*-amidobenzyl ether derivatives of 1-naphthol and *N*-acetylnorephedrine. Upon treatment with cathepsin B peptide hydrolysis occured with 1-naphthol release. On the basis of these results etoposide and combretastatin were attached to *Z*-val-cit-*p*-amidobenzyl alcohol through ether linkages. Thus forming peptide drug derivatives that were stable in aqueous buffer and serum and underwent ether fragmentation upon treatment with cathepsin B.

B. E. Toki, C. G. Cerveny, A. F. Wahl and P. D. Senter, *J. Org. Chem.*, 2002, **67**, 1866.

Synthesis of a precursor of bioactive pentapeptide OGP(10-14)

Protease

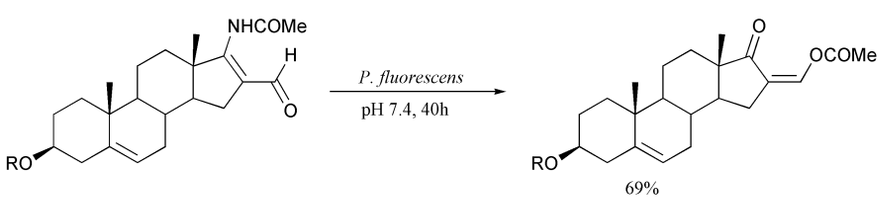


The enzymatic synthesis of a precursor of the bioactive pentapeptide OGP(10-14) using proteases in organic solvents is described. The effect of different solvents, water content and addition of triethylamine on the reaction was also investigated.

P. Liu, G. Tian, K.-S. Lee, M.-S. Wong and Y. Ye, *Tetrahedron Lett.*, 2002, **43**, 2423.

A facile 1,5-rearrangement of β-formylenamides

Pseudomonas fluorescens

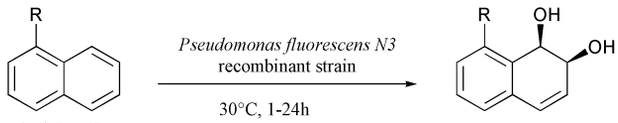


The use of *Pseudomonas fluorescens* strain RRLJ 134 to catalyse the 1,5-rearrangement of β-formylenamides to the corresponding β-acetoxyenones is reported. This microbe was also found to be an efficient catalyst for the cleavage of acetates.

U. Bora, M. Longchar, A. Chetia, B. S. D. Kumar, R. C. Boruah and J. S. Sandhu, *Tetrahedron Lett.*, 2002, **43**, 2269.

Chemo-enzymatic synthesis of (2*R*)-8-substituted-2-aminotetralins

Pseudomonas fluorescens



1: a) R = H
b) R = C_2H_5
c) R = OCH_3
d) R = $COOCH_3$
e) R = Cl
f) R = NO_2

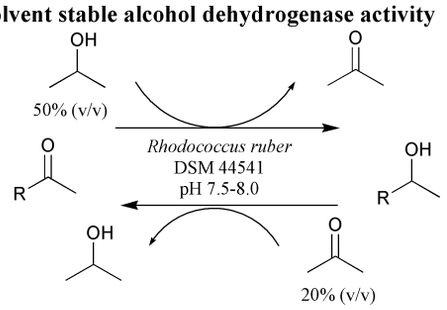
2

F. Orsini, G. Sello, E. Travaini and P. Di Gennaro, *Tetrahedron: Asymmetry*, 2001, **13**, 253.

Substrate **2d** is a precursor in the synthesis of (2*R*)-2-amino-8-methoxy-1,2,3,4-tetrahydronaphthalene.

A solvent stable alcohol dehydrogenase activity

Rhodococcus ruber



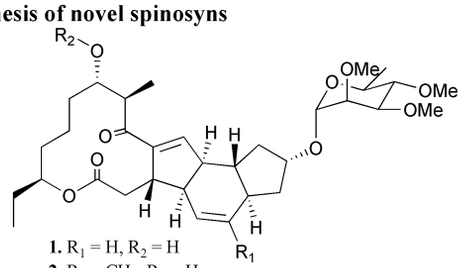
1, R = -$(CH_2)_2$-CH=CMe_2
2, R = *n*-C_6H_{13}
3, R = Ph

W. Stampfer, B. Kosjek, C. Moitzi, W. Kroutil and K. Faber, *Angew. Chem., Int. Ed.*, 2002, **41**, 1014.

Lyophilised cells of *Rhodococcus ruber* DSM 44541 were reported to be a highly efficient catalyst for the preparative reduction/oxidation of ketones/alcohols in the presence of high concentrations of substrate and co-substrate for cofactor regeneration. Ketone **1** (125 gL^{-1}) was reduced to the corresponding (*S*)-alcohol with 99% e.e. in phosphate buffer containing 50% propanol.

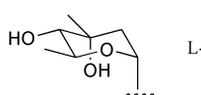
Biosynthesis of novel spinosyns

Saccharopolyspora erythraea



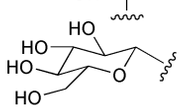
1. R_1 = H, R_2 = H
2. R_1 = CH_3, R_2 = H

3. R_1 = H, R_2 =
4. R_1 = CH_3, R_2 =



L-mycarose

5. R_1 = H, R_2 =
6. R_1 = CH_3, R_2 =



D-glucose

S. Gaisser, C. J. Martin, B. Wilkinson, R. M. Sheridan, R. E. Lill, A. J. Weston, S. J. Ready, C. Waldron, G. D. Crouse, P. F. Leadlay and J. Staunton, *Chem. Commun.*, 2002, 618.

The novel spinosyns (3-6) were obtained on feeding a mix of 1 and 2 to the mutant *Saccharopolyspora erythaea* strain SGT2pSGSpnP.