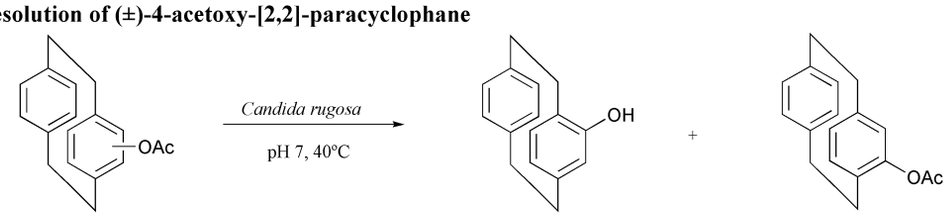
Perkin 1 Abstracts: Biocatalysis in Organic Synthesis

Gideon Grogan,[a] Selena Guilly,[b] Ian Jackson,[b] Denise McIntyre,[b] Reuben Carr,[b] Sabine Flitsch[b] and Nicholas Turner[b]

[a] *Department of Chemistry, University of York, Heslington, York, UK YO10 5DD*
[b] *Department of Chemistry, The University of Edinburgh, King's Buildings, West Mains Road, Edinburgh, UK EH9 3JJ*

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.

Kinetic resolution of (±)-4-acetoxy-[2,2]-paracyclophane ***Lipase***



(±) - **1**

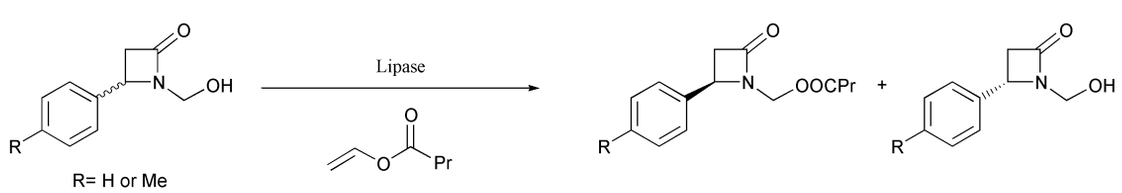
(+)-(*R*) - **2**
85% yield, ee 82%

(+)-(*S*) - **3**
92% yield, ee 83%

A study on the change in reactivity of crude *Candida rugosa* lipase with variation of reaction pH and temperature is reported. Kinetic resolution of racemic 4-acetoxy-[2,2]-paracyclophane **1**, with CRL yielded **2** and **3** as shown. Increase in temperature and pH resulted in increased yield, enantioselectivity and rate of reaction.

A. Cipiciani, F. Bellezza, F. Fringuelli and M. G. Silvestrini, *Tetrahedron: Asymmetry*, 2001, **12**, 2277.

Synthesis of 4-aryl-substituted β-lactam enantiomers ***Lipase***

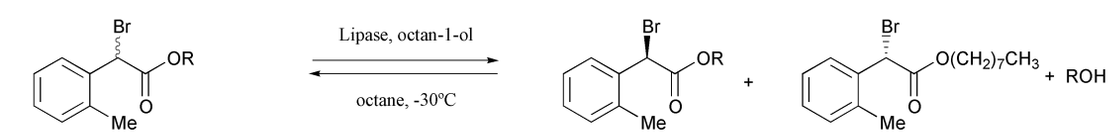


R= H or Me

Enantiopure azetidinones (ee's ≥ 96%) were prepared either *via* lipase-catalysed butyrylation of the primary hydroxy of *N*-hydroxymethylated β-lactams of (*R*)-configuration or by lipase-catalysed debutyrylation of *O*-butyryloxymethylazetidin-2-ones of (*R*)-configuration.

E. Forró and F. Fülöp, *Tetrahedron: Asymmetry*, 2001, **12**, 2351.

Resolution of 2-bromo-*o*-tolyl-carboxylic acid ***Lipase***

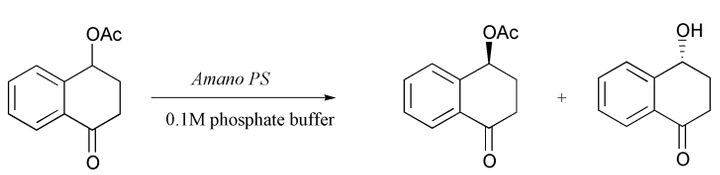


R= Me, Et, ^{n}Pr, ^{i}Pr and Bn

Lipases from *Rhizomucor miehei* and *Pseudomonas cepacia* were selected for the enantioselective transesterification of 2-bromo-*o*-tolylacetic acid.

D. Guieysse, C. Salagnad, P. Monsan and M. Remaud-Simeon, *Tetrahedron: Asymmetry*, 2001, **12**, 2473.

Kinetic resolution of 4-hydroxytetralone and 3-hydroxyindanone ***Lipase***



(±) - **1**

(–) - **1**
48% yield, >96% ee

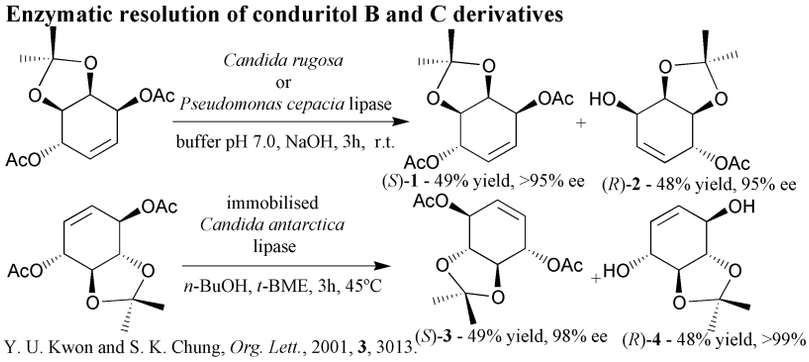
(–) - **2**
54% yield, 91% ee

An asymmetric synthesis of 4-hydroxy/acetoxytetralone, which uses enzymatic resolution methodology, is reported. The key enzymatic step involves hydrolysis of racemic 4-acetoxytetralone (±)-**1** to enantiomerically pure compounds (–)-**1** and (–)-**2**.

S. Joly and M. S. Nair, *Tetrahedron: Asymmetry*, 2001, **12**, 2283.

Enzymatic resolution of conduritol B and C derivatives

Lipase

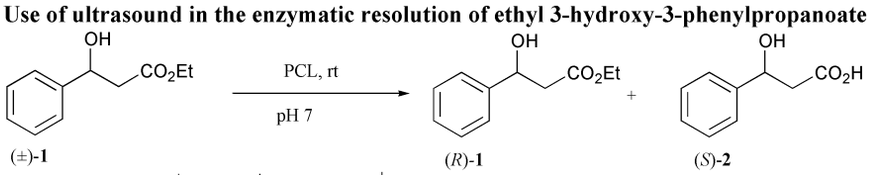


Y. U. Kwon and S. K. Chung, *Org. Lett.*, 2001, **3**, 3013.

First synthesis of all four possible enantiomeric pairs of conduritol stereoisomers.

Use of ultrasound in the enzymatic resolution of ethyl 3-hydroxy-3-phenylpropanoate

Lipase



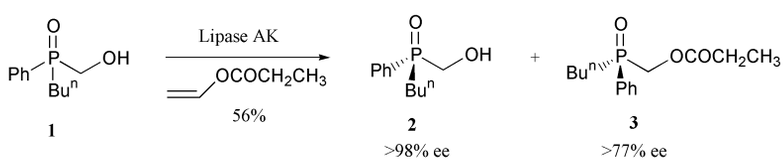
magnetic stirring	6h	60% yield 25% ee	22% yield 79% ee
ultrasound	3h	71% yield 26% ee	22% yield 81% ee

C. Magno, R. Ribeiro, E. N. Passaroto and E. C. S. Brenelli, *Tetrahedron Lett.*, 2001, **42**, 6477.

A study of the influence of ultrasound in the enzymatic hydrolysis of ethyl 3-hydroxy-3-phenylpropanoate **1** is presented. Although the rate of reaction increased, the application of ultrasound to the reaction mixture did not significantly alter the yield or enantiomeric excess of the reaction.

Kinetic resolution of P-chiral phosphorus compounds

Lipase

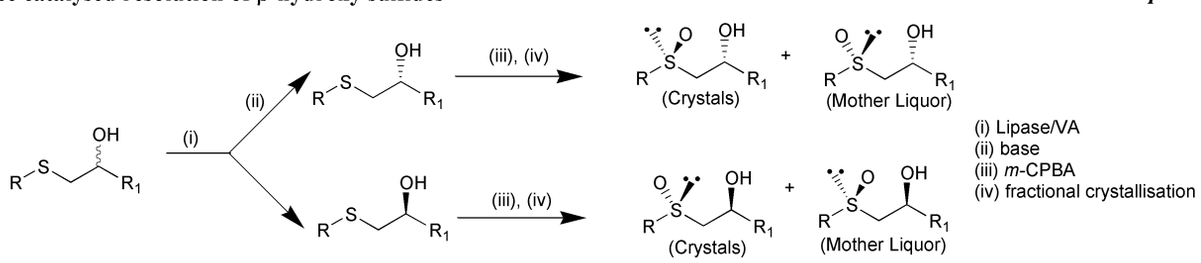


K. Shioji, Y. Ueno, Y. Kurauchi and K. Okuma, *Tetrahedron Lett.*, 2001, **42**, 6569.

A lipase catalysed kinetic resolution of *P*-chiral phosphine oxide containing a 1-hydroxymethyl group is reported. Acylation of **1** with an acyl donor in diisopropyl ether afforded the corresponding acylated phosphine oxide **3** and the unreacted alcohol **2**.

Lipase catalysed resolution of β-hydroxy sulfides

Lipase

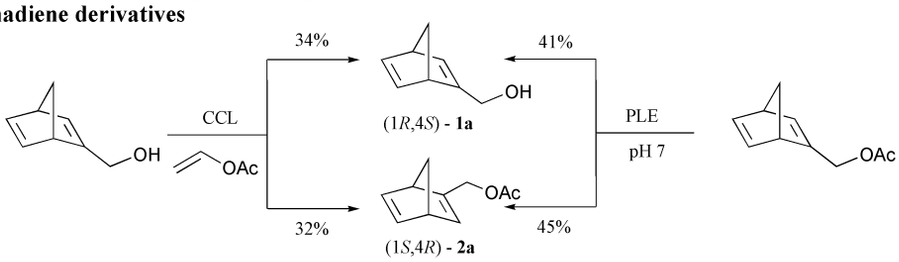


S. Singh, S. Kumar and S. S. Chimni, *Tetrahedron: Asymmetry,* 2001, **12**, 2457.

Humicola lanuginosa lipase-catalysed acylation of β-hydroxy sulfides provides both the (*R*)- and (*S*)-enantiomers in high enantiomeric purity. In two cases the resolved hydroxy sulfides were oxidised to give β-hydroxy sulfoxides in >99% ee.

Resolution of (±)-2-substituted norbornadiene and hexachloronorbornadiene derivatives

Lipase

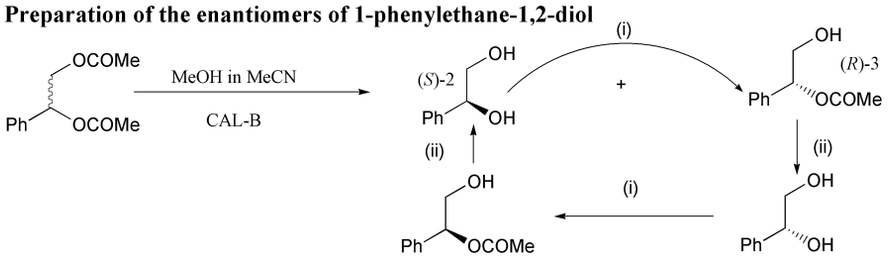


C. Tanyeli, G. Celikel and I. M. Akhmedov, *Tetrahedron: Asymmetry*, 2001, **12**, 2305.

A highly efficient resolution of (±)-2-hydroxymethylbicyclo[2.2.1]hepta-2,5-diene **1,** (±)-2-acetoxymethylbicyclo[2.2.1]hepta-2,5-diene **2**, and the corresponding hexachlorinated derivatives with catalytic amounts of CCL and PLE is reported.

Preparation of the enantiomers of 1-phenylethane-1,2-diol

Lipase

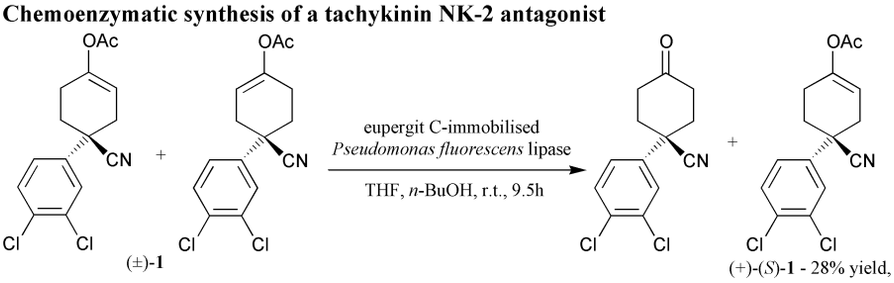


P. Virsu, A. Liljeblad, A. Kanerva and L.T. Kanerva, *Tetrahedron: Asymmetry,* 2001, **12**, 2447.

In the presence of CAL-B, the sequential one-pot methanolysis of the diacetate in acetonitrile allowed the preparation of (*S*-2)-diol (ee = 97%) and (*R*-3)-1-acetoxy-1-phenylethanol (ee = 94%). Mitsunobu esterification inverted the configuration of the diol (*S*-2) allowing transformation of the initial racemate to one enantiomer.

Chemoenzymatic synthesis of a tachykinin NK-2 antagonist

Lipase

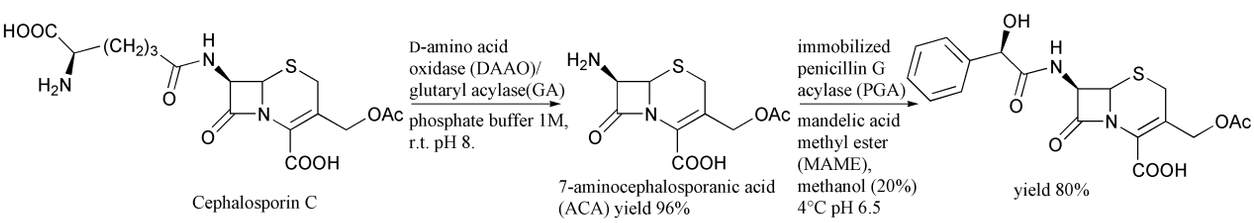


G. Allan, A. J. Carnell, M. L. E. Hernandez and A. Pettman, *Tetrahedron*, 2001, 8193.

The biotransformation was optimised in terms of solvent, temperature and immobilisation method used. The enantiomerically pure enol acetate **1** was used as an intermediate for the synthesis of a tachykinin NK-2 antagonist.

Modulation of PGA properties *via* immobilization techniques

Acylase

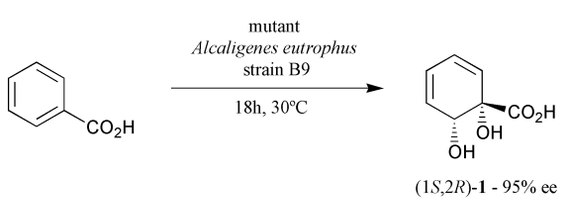


M. Terreni, G. Pagani, D. Ubiali, R. Fernández-Lafuente, C. Mateo and J. M. Guisán, *Bioorg. Med. Chem. Lett.*, 2001, **11**, 2429.

The PGA catalysed acylation was optimised. The enzyme was immobilised on agarose (with either limited or multipoint linkage) and Eupergit C. The efficiency of the enzymatic transformation was determined for each of these, with varying buffer and solvent concentration. A final chemical step completed the *one-pot* synthesis of Cephamandole.

Microbial dihydroxylation of benzoic acid

Alcaligenes eutrophus

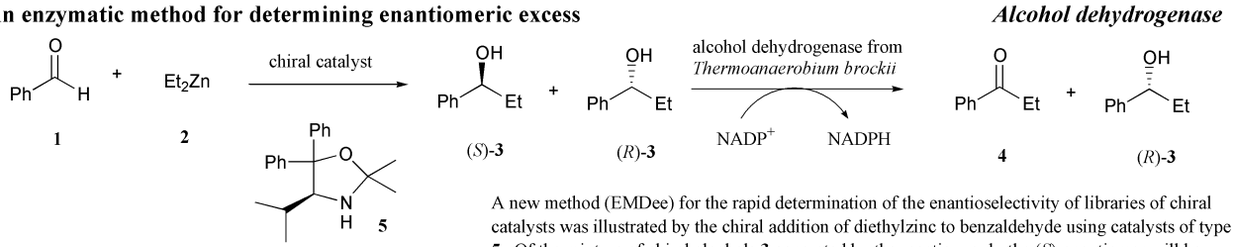


A. G. Myers, D. R. Siegel, D. J. Buzard and M. G. Charest, *Org. Lett.*, 2001, **3**, 2923.

The microbial dihydroxylation of benzoic acid was carried out on a >250 g scale. The product **1** undergoes many oxidative and rearrangement processes to produce many synthetic starting materials.

An enzymatic method for determining enantiomeric excess

Alcohol dehydrogenase

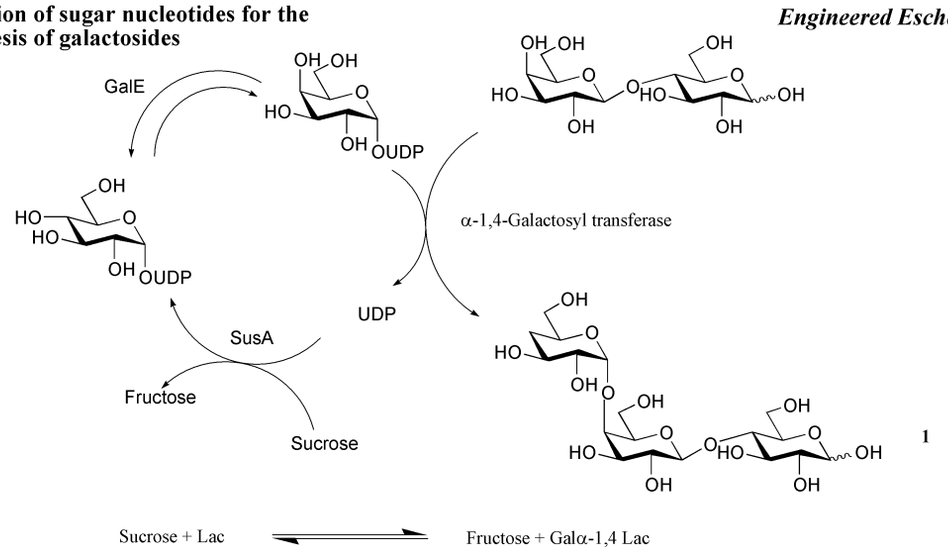


P. Abato and C. T. Seto, *J. Am. Chem. Soc.*, 2001, **123**, 9206.

A new method (EMDee) for the rapid determination of the enantioselectivity of libraries of chiral catalysts was illustrated by the chiral addition of diethylzinc to benzaldehyde using catalysts of type **5**. Of the mixture of chiral alcohols **3** generated by the reaction, only the (*S*)-enantiomer will be oxidised by the NADPH dependent alcohol dehydrogenase from *Thermoanaerobium brockii*. The rate of oxidation was monitored using spectrophotometric assay at 340 nm. As *KM* for the (*S*)-substrate is approximately equal to the value of *Ki* for the (*R*)-form as inhibitor, the rate of oxidation could be used as a direct measure of enantioselectivity, with an estimated error of +/- 10%.

In vivo regeneration of sugar nucleotides for the large scale synthesis of galactosides

Engineered Escherichia coli



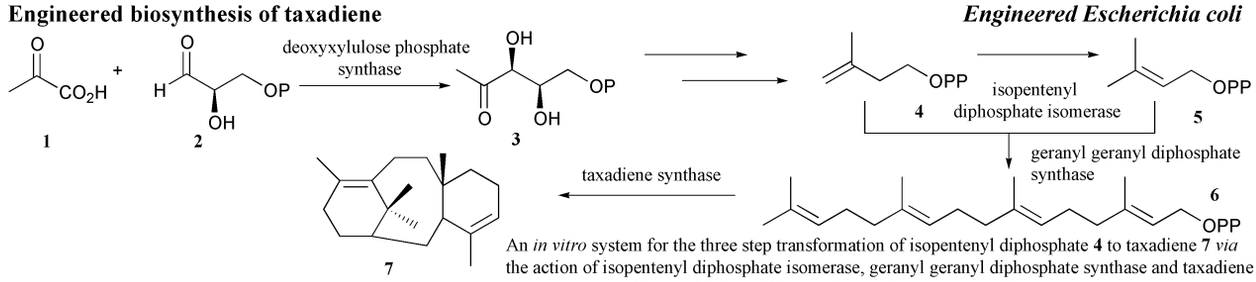
Sucrose + Lac ⇌ Fructose + Galα-1,4 Lac

The large scale synthesis of galactosides using glycosyl transferases requires the ready availability of sugar nucleotides such as uridine 5'-diphosphogalactose (UDP-Gal). A novel strain of *E. coli* is reported into which have been engineered genes encoding UDP-galactose-4-epimerase (GalE), sucrose synthase (SusA) and α-1,4-galactosyl transferase (α-1,4-GT). This allowed for the self-sufficient regeneration of UDP-galactose from sucrose and spent UDP as shown in the figure, coupled to the synthesis of trisaccharides such as Galα-1,4 Lac (globotriose) **1**.

X. Chen, J. Zhang, P. Kowal, Z. Liu, P. R. Andreana, Y. Lu and P. G. Wang, *J. Am. Chem. Soc.*, 2001, **123**, 8866.

Engineered biosynthesis of taxadiene

Engineered Escherichia coli

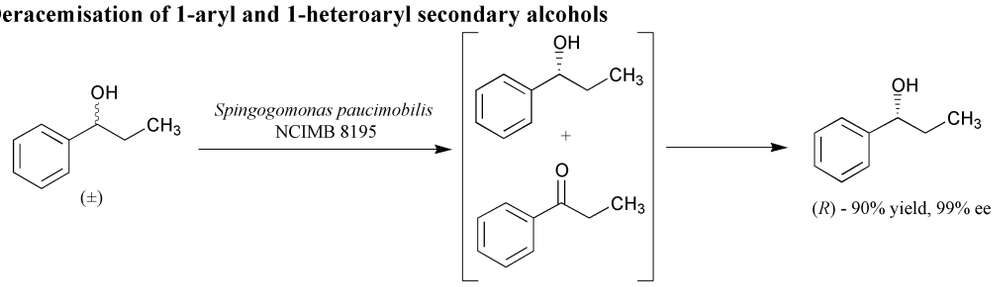


An *in vitro* system for the three step transformation of isopentenyl diphosphate **4** to taxadiene **7** *via* the action of isopentenyl diphosphate isomerase, geranyl geranyl diphosphate synthase and taxadiene synthase yielded 10 mg of **7** from 360 mg of **4**. An *in vivo* system for the conversion was also developed wherein the action of the above three co-expressed enzymes was preceded by generation of **4** from **1** and **2** *via* the action of overexpressed deoxyxylulose phosphate synthase. 1.3 mg taxadiene **7** was produced from a 1 L culture of the recombinant strain.

Q. Huang, C. A. Roessner, R. Croteau and A. I. Scott, *Bioorg. Med. Chem.*, 2001, **9**, 2237.

Deracemisation of 1-aryl and 1-heteroaryl secondary alcohols

Microorganism

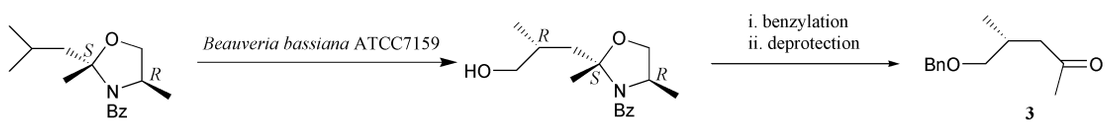


Spingogomonas paucimobilis NCIMB 8195 catalysed the deracemisation of a wide range of aryl and heteroaryl alcohols.

G. R. Allan and A. J. Carnell, *J. Org. Chem.*, 2001, **66**, 6495.

Biohydroxylation of ketones

Microorganism

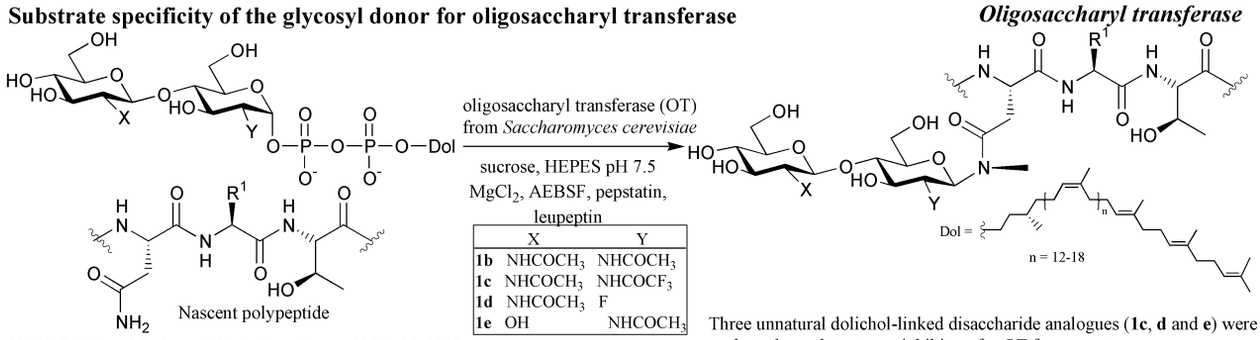


Hydroxylation of **1** with *B. bassiana* gave alcohol **2**. Subsequent benzylation of **2**, followed by removal of the docking/protecting group gave (*R*)-5-benzyloxy-4-methylpentan-2-one **3** (99% ee).

A. de Raadt, B. Fetz, H. Griengl, M. F. Klingler, B. Krenn, K. Mereiter, D. F. Münzer, P. Plachota, H. Weber and R. Saf, *Tetrahedron*, 2001, 8151.

Substrate specificity of the glycosyl donor for oligosaccharyl transferase

Oligosaccharyl transferase



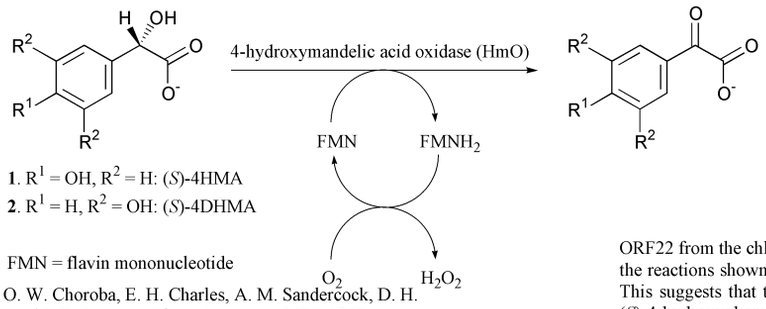
	X	Y
1b	$NHCOCH_3$	$NHCOCH_3$
1c	$NHCOCH_3$	$NHCOCF_3$
1d	$NHCOCH_3$	F
1e	OH	$NHCOCH_3$

V. W.-F. Tai and B. Imperiali, *J. Org. Chem.*, 2001, **66**, 6217.

Three unnatural dolichol-linked disaccharide analogues (**1c**, **d** and **e**) were evaluated as substrates or inhibitors for OT from yeast.

Characterisation of a hydroxymandelate oxidase

Oxidase



1. R^1 = OH, R^2 = H: (*S*)-4HMA
2. R^1 = H, R^2 = OH: (*S*)-4DHMA

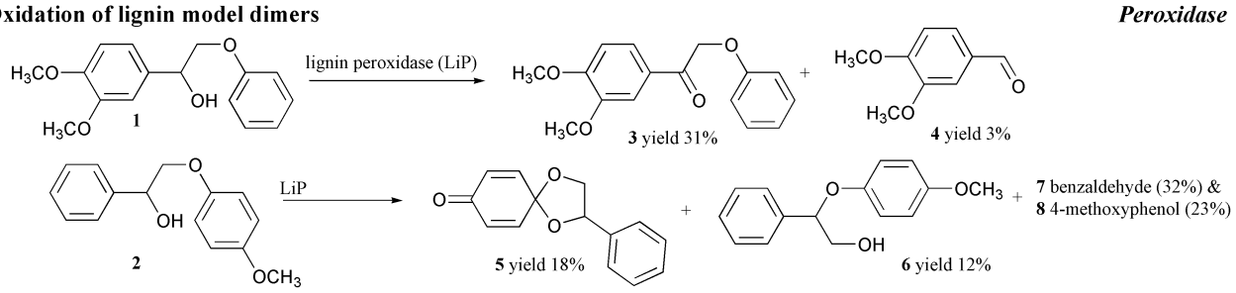
FMN = flavin mononucleotide

T.-L. Li, O. W. Choroba, E. H. Charles, A. M. Sandercock, D. H. Williams, and J. B. Spencer, *Chem. Commun.*, 2001, 1752.

ORF22 from the chloroeremomycin gene cluster was cloned, expressed and the reactions shown identified the enzyme as a hydroxymandelate oxidase. This suggests that the enzyme is involved in the biosynthetic pathway to (*S*)-4-hydroxyphenylglycine and (*S*)-3,5-dihydroxyphenylglycine.

Oxidation of lignin model dimers

Peroxidase

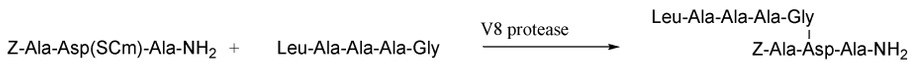


E. Baciocchi, M. Bietti, M. F. Gerini, O. Lanzalunga and S. Mancinelli, *J. Chem. Soc. Perkin Trans. 2*, 2001, 1506.

The reactions were also performed using the one-electron oxidant $K_5[Co(III)W_{12}O_{40}]$. The products were rationalised through mechanistic discussion. The proposed radical mechanism for the transfomation of **2** was verified by further LiP oxidations of suitable substrates.

Synthesis of isopeptides

Protease

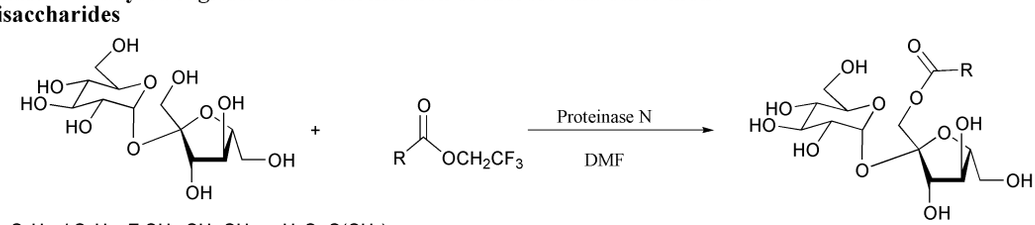


N. Wehofsky, M. Alisch and F. Bordusa, *Chem. Commun.*, 2001, 1602.

14 peptides containing either Asp or Glu were esterified with carboxymethylthiol (SCm) at their side-chain carboxylates. The resulting SCm functionalised side chains were accepted by V8 protease (from *Staphylococcus aureus*) in the synthesis of isopeptides. The peptides tested included variations in the position of the Glu/Asp moieties and peptide length. Each donor was coupled with 5 different acceptor peptides. Yields ranged from 39 to 68%. Chain elongation of the Glu containing peptides had little effect on yield, while for Asp, yields increased by 10-20%. Variation in chain length of acceptor peptides had little effect on yield.

Proteinase *N*-catalysed regioselective esterification of sucrose and other mono- and disaccharides

Proteinase

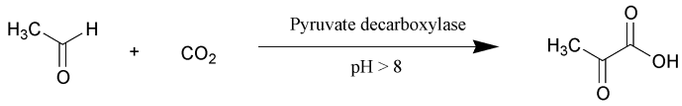


R = *n*-C_3H_7, *i*-C_3H_7, *E*-CH_3-CH=CH- or H_2C=C(CH_3)-

P. Potier, A. Bouchu, J. Gagnaire and Y. Queneau, *Tetrahedron: Asymmetry*, 2001, **12,** 2409.

Crude Proteinase N was used as a catalyst for the synthesis of carbohydrate (sucrose) esters by transesterification of activated esters in organic solvents. The influence of reaction parameters (*e.g.* temperature, solvent, pH, *etc.*) was studied.

Enzymatic synthesis of pyruvic acid ***Pyruvate decarboxylase***



M. Miyazaki, M. Shibue, K. Ogino, H. Nakamura and H. Maeda, *Chem. Commun.*, 2001, 1800.

The ligation of acetaldehyde and carbon dioxide to yield pyruvic acid has been performed in buffered solution using pyruvate decarboxylase as catalyst. Best results were obtained using a 100 μM solution of acetaldehyde in 500 mM $NaHCO_3$-Na_2CO_3 buffer at pH 11, giving a yield of 81%. This was much higher than the equivalent enzyme-catalysed reaction peformed under 20 atm CO_2 in DMF.

Synthesis of a trisaccharide on a homogeneously soluble PEG polymer ***Transferase, sucrose synthase and epimerase***

N. Brinkmann, M. Malissard, M. Ramuz, U. Römer, T. Schumacher, E. G. Berger, L. Elling, C. Wandrey and A. Liese, *Bioorg. Med. Chem. Lett.*, 2001, **11**, 2503.

The trisaccharide was synthesised on a homogeneously soluble polymeric support. The complete multi-enzyme system consisted of the enzymes shown as well as sucrose synthase (SuSy) and uridine 5'-diphosphoglucose (UDP-Glc) 4-epimerase for UDP-Gal (re)generation. The synthesis was monitored by HPLC. Cleavage by hydrogenation using palladium gave Galα(1→3)Galβ(1→4)GlcNAc in 23% yield from **1**.