Perkin 1 Abstracts: Biocatalysis in Organic Synthesis

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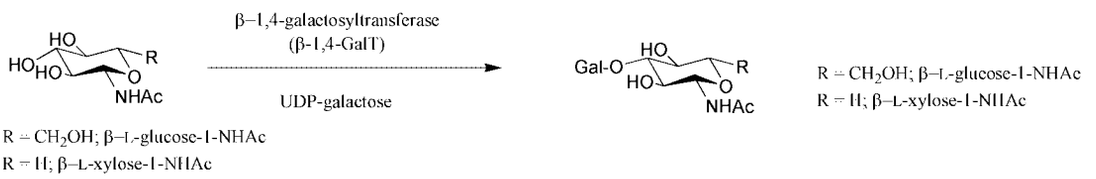
Perkin 1 Abstracts: Biocatalysis in Organic Synthesis aims to cover recent literature concerning the applications of enzymes and micro-organisms as catalysts in organic synthesis. The abstracts will emphasise the key synthetic step(s) that are mediated by the biocatalyst. Emerging technologies for biocatalyst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

β-Galactopyranosyl-(1, 3) linked disaccharides from L-sugars

Galactosyltransferase

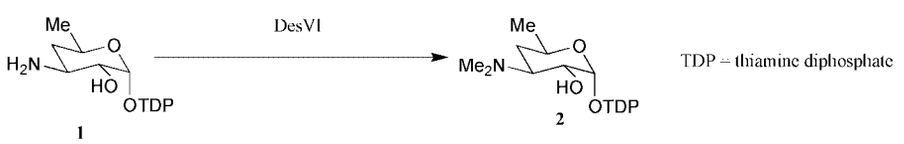


Y. Nishida, H. Tamakoshi, Y. Kitagawa, K. Kobayashi and J. Thiem, *Angew. Chem., Int. Ed.*, 2000, **39**, 2000.

β -1,4-GalT catalyses β -1,3 galactosyl transfer to L -sugars as substrates if an *N* -acetyl group is present at the β -anomeric position. Yields of 15-20% were obtained. 1-*N*-acetylation reduces enzyme activity toward D-sugars however.

Dimethylation of amino sugars

N-methylase

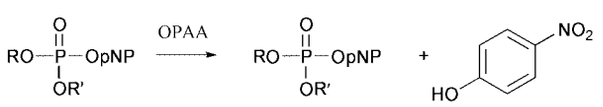


C.-W. Chang, L. Zhao, H. Yamase and H.-W. Liu, *Angew. Chem., Int. Ed.*, 2000 **39**, 2160.

The enzyme DesVI, which catalyses dimethylation of amino sugar **1** , was expressed in *E. coli* and purified.The enzymatic synthesis of the product **2** *in vitro* and HPLC enzyme assay confirmed the role of DesVI in the biosynthesis of desosamine in *Streptomyces venezuelae*.

Substrate and stereochemical specificity of an organophosphorus acid anhydrolase (OPAA)

Organophosphorus acid anhydrolase (OPAA)



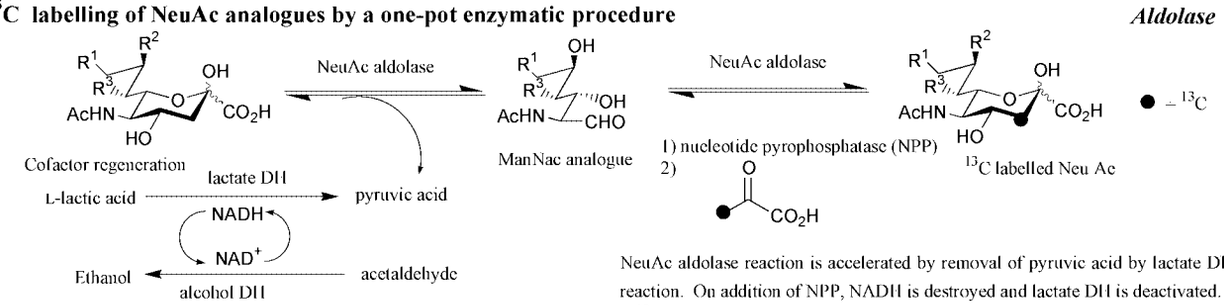
1) R = R′ = Me
2) R = R′ = Et (Paraoxon)
3) R = R′ = ^{i}Pr
4) R = R′ = Ph
5) R = Et, R′ = Me
6) R = ^{i}Pr, R′ = Me
7) R = Ph, R′ = Me
8) R = ^{i}Pr, R′ = Et
9) R = Ph, R′ = Et
10) R = Ph, R′ = ^{i}Pr

C. M. Hill, F. Wu, T.-C. Cheng, J. J. DeFrank and F. M. Raushel, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1285.

Preference for the S_p enantiomer is observed, most markedly with compounds **5** and **6** . Stereoselectivity is lost when no methyl group is present. Loss in selectivity is accompanied by decreased reaction rates, suggesting larger groups do not fit in the active site of the enzyme.

^{13}C labelling of NeuAc analogues by a one-pot enzymatic procedure

Aldolase

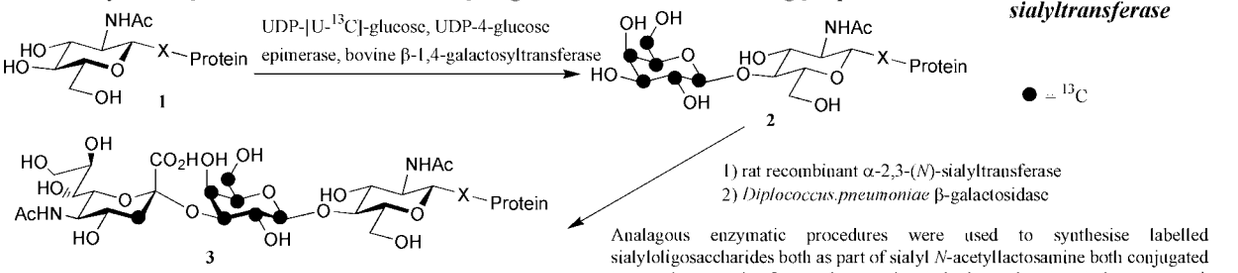


T. Miyazaki, H. Sato, T. Sakakibara and Y. Kajibara, *J. Am. Chem. Soc.*, 2000, **122**, 5678.

NeuAc aldolase reaction is accelerated by removal of pyruvic acid by lactate DH reaction. On addition of NPP, NADH is destroyed and lactate DH is deactivated. [3-^{13}C] pyruvic acid is then added, allowing label incorporation. The technique was applied to five NeuAc analogues in 46-76% yield.

Chemoenzymatic synthesis of ^{13}C labelled sialyloligosaccharides on an intact glycoprotein.

Galactosyltransferase/ sialyltransferase



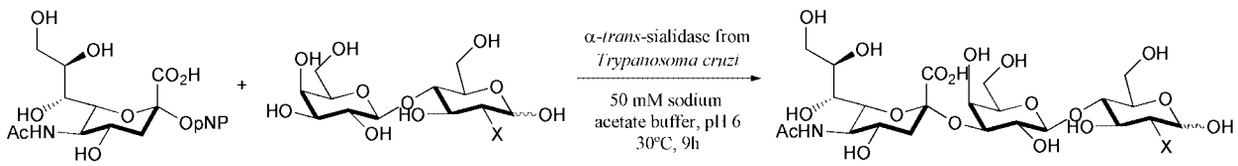
1) rat recombinant α-2,3-(*N*)-sialyltransferase
2) *Diplococcus.pneumoniae* β-galactosidase

T. Miyazaki, H. Sato, T. Sakakibara and Y. Kajihara, *J. Am. Chem. Soc.*, 2000, **122**, 5678.

Analagous enzymatic procedures were used to synthesise labelled sialyloligosaccharides both as part of sialyl *N*-acetyllactosamine both conjugated to a glycoprotein **3** and unconjugated, in order to study comparative conformational properties and dynamic behaviour of sialylgalactosides in both states.

Synthesis of sialyloligosaccharides

α-trans-sialidase



pNP = *para*-nitrophenyl

X = OH, NHAc

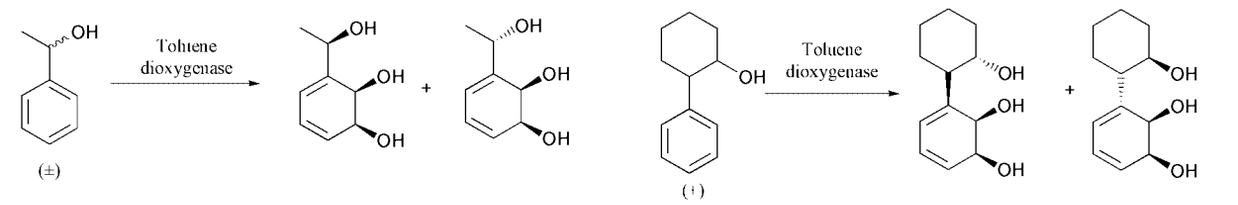
X = OH : 87%
X = NHAc : 85%

S. Singh, M. Scigelova, M. L. Hallberg, O. W. Howarth, S. Schenkman and D. H. G. Crout, *Chem. Commun.*, 2000, 1013.

It was also found that the enzyme was able to catalyse the sialylation of C-6-substituted internal β -galactose units, forming novel, branched polysaccharides.

Oxidation of aromatic compounds

Toluene dioxygenase



Escherichia coli JM109 (pDTG601)	1	:	1
Pseudomonas putida F39/D	4	:	6

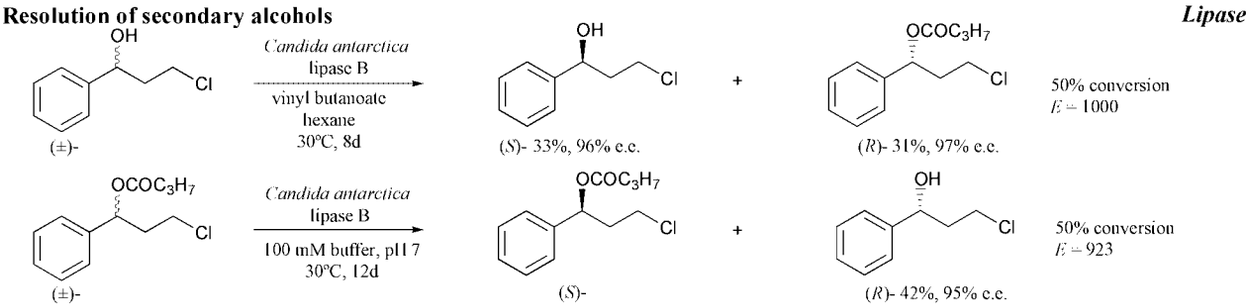
Escherichia coli JM109 (pDTG601)	1	:	1
Pseudomonas putida F39/D	no conversion		

V. Bui, T. V. Hansen, Y. Stenstrom, D. W. Ribbons and T. Hudlicky, *J. Chem. Soc., Perkin Trans. 1*, 2000, 1669.

Investigation into kinetic resolutions mediated by toluene dioxygenase. A range of substrates were tested with little or no success.

Resolution of secondary alcohols

Lipase

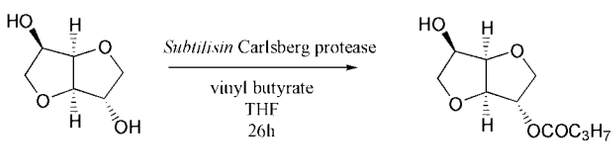


H.-L. Liu, B. H. Hoff and T. Anthonsen, *J. Chem. Soc., Perkin Trans. 1*, 2000, 1767.

The (*R*)- and (*S*)-alcohols were further reacted to give the non-tricyclic anti-depressants fluoxetine, tomoxetine and nisoxetine.

Regioselective acylation of isosorbide

Protease



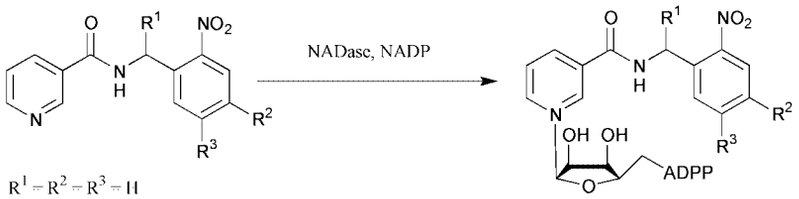
62%

C. Brown, R. W. Marston, P. F. Quigley and S. M. Roberts, *J. Chem. Soc., Perkin Trans. 1*, 2000, 1809.

A number of enzymes were screened for regioselective acylation at the 2-position. *Subtilisin* Carlsberg protease and pig liver esterase were found to carry out the desired transformation. The 2-acylated product was then nitrated at the 5-position and deprotected to give the vasodilator isosorbide 5-mononitrate.

Enzymatic synthesis of caged NADP cofactors.

Glycohydrolase (NADase)



1, $R^1 = R^2 = R^3 = H$
2, $R^1 = H$, $R^2 = R^3 = OMe$
3, $R^1 = CH_3$, $R^2 = CO_2H$, $R^3 = H$
4, $R^1 = CO_2H$, $R^2 = R^3 = H$
5, $R^1 = CH_3$, $R^2 = R^3 = H$

6, $R^1 = R^2 = R^3 = H$
7, $R^1 = H$, $R^2 = R^3 = OMe$
8, $R^1 = CH_3$, $R^2 = CO_2H$, $R^3 = H$
9, $R^1 = CO_2H$, $R^2 = R^3 = H$

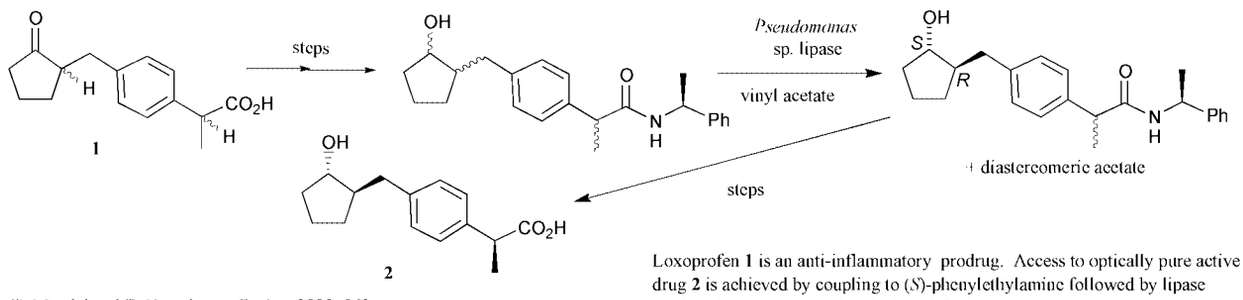
ADPP = adenine dinucleotide diphosphate

C. P. Salerno, D. Magde and A. Patron, *J. Org. Chem.*, 2000, **65**, 3971.

The synthesis of caged NADP analogues is reported. Products **6-9** were synthesised in 7-20% yield. No reaction occurred with **5**. NADP photorelease properties of **7-9** were tested. Superior release properties are conferred by α-methylation to the carboxamide. Product **8** showed a net photorelease of 75% with no fluorescent by-products.

Resolution of Loxoprofen analogue.

Lipase

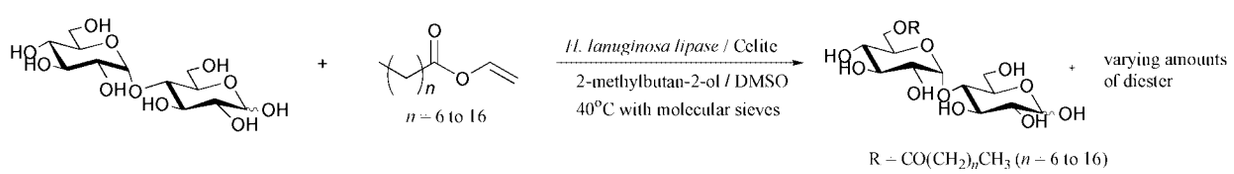


T. Mandai and T. Yamakawa, *Synlett*, 2000, 862.

Loxoprofen **1** is an anti-inflammatory prodrug. Access to optically pure active drug **2** is achieved by coupling to (*S*)-phenylethylamine followed by lipase catalysed resolution and chemical modification.

Regioselective synthesis of fatty acid esters of maltose and other saccharides

Lipase



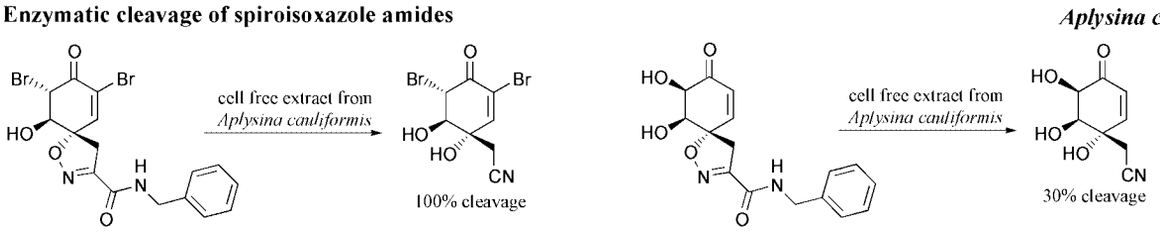
$R = CO(CH_2)_nCH_3$ ($n = 6$ to 16)

M. Ferrer, M. A. Cruces, F. J. Plou, M. Bernabé and A. Ballesteros, *Tetrahedron*, 2000, **56**, 4053.

The effects of DMSO concentration, type and amount of lipase, carbon chain length and activation of acyl donors were also studied. Yields of monoester formed ranged from 25 to 80%. α and β–Dodecyl glycosides of maltose, leucrose and maltotriose were also acylated giving yields of monoester ranging from 21 to 90%.

Enzymatic cleavage of spiroisoxazole amides

Aplysina cauliformis

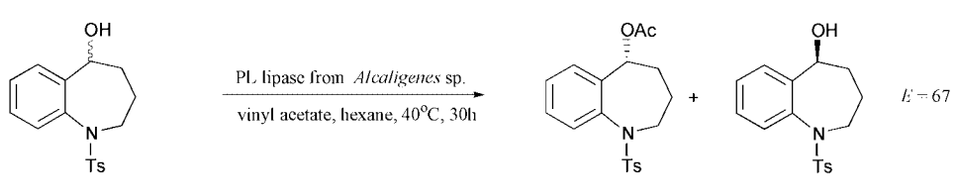


K. Goldenstein, T. Fendert, P. Proksch and E. Winterfeldt, *Tetrahedron*, 2000, **56**, 4173.

Other brominated and non-brominated, as well as methyl ester analogues, were screened to prove that the N-H bond, the hydroxy function and two bromoatoms were important for the enzymatic cleavage through deprotonation of the N-H group of the amide. These analogues were obtained *via* an enantiotopic double bond differentiating high pressure Diels-Alder cycloaddition.

Resolution of benzazepine alcohols

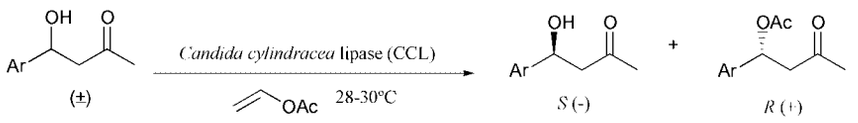
Lipase



J. Matsubara, K. Kitano, K. Otsubo, Y. Kawano, T. Ohtani, M. Bando, M. Kido, M. Uchida and F. Tabusa, *Tetrahedron*, 2000, **56**, 4667.

Various lipases were screened. Enzymatic transesterification of 4,5-diol analogues was also successful using mixed cosolvents: isopropyl ether/dimethylformamide.

Resolution of aryl β-hydroxy ketones ***Lipase***



55%yield, 70% e.e. 44%yield, >96% e.e.

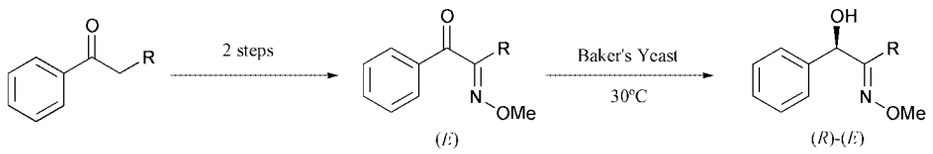
42% conversion

[Ar = 4-chlorophenyl]

The resolution of a variety of β-hydroxy ketones has been investigated. Of 3 lipases, CCL was shown to be most effective. Variations in Ar had little effect on e.e.s and yield.

M. S. Nair and S. Joly, *Tetrahedron: Asymmetry*, 2000, **11**, 2049.

Enantioselective reduction of keto oximes ***Reductase***

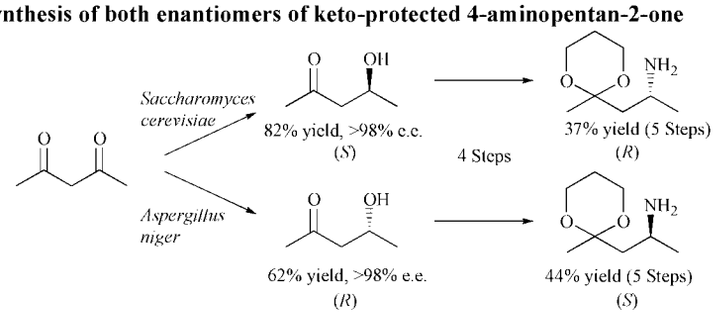


48% yield, >99% e.e.
[R = *n*-Bu]

A route to potential building blocks for the stereoselective synthesis of norephredrine analogues has been developed. e.e.s and yields dependant on R. Highest e.e. for R = *n*-Bu.

O. C. Krentz, R. C. M. Segura, J. A. R. Rodrigues and P. J. S. Moran, *Tetrahedron: Asymmetry*, 2000, **11**, 2107.

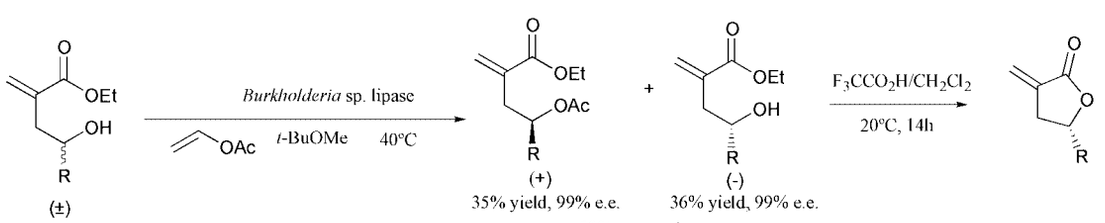
Synthesis of both enantiomers of keto-protected 4-aminopentan-2-one ***Reductase***



Both enantiomers of the amine have been obtained by microbial reduction of pentane-2,4-dione. Subsequent steps gave each keto-protected 4-aminopentan-2-one with e.e. > 98%.

P. Besse, S. Ciblat, J.-L. Canet, Y. Troin and H. Veschambre, *Tetrahedron: Asymmetry*, 2000, **11**, 2211.

Resolution of γ-hydroxy esters ***Lipase***



35% yield, 99% e.e. 36% yield, 99% e.e.

50% conversion

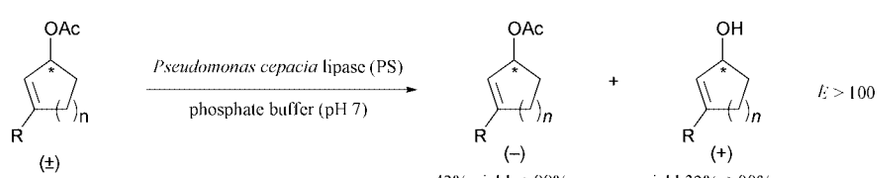
E > 200

R = Ph

A route to optically active α-methylene γ-lactones has been developed. Resolution prior to cyclisation was most effective. Higest e.e.s for R = Ph using *Burkholderia* sp. lipase. R = Me and various other lipases also investigated.

W. Adam, P. Groer, and C. R. Saha-Möller, *Tetrahedron: Asymmetry*, 2000, **11**, 2239.

Resolution of 3-nitrocyclopent(or hex)-2-en-1-yl acetates ***Lipase***



43% yield, >99% e.e. yield 32%, >99% e.e.

E > 100

n = 2

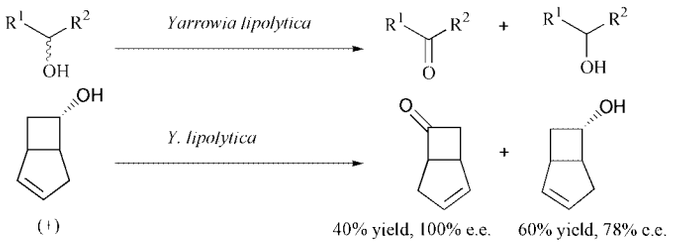
R = NO_2

Resolution studied for *n* = 1 & 2, and R = H & NO_2. Pig Liver acetone powder was also used. Highest yield and e.e. were achieved for *n* = 2, R = NO_2, using Lipase PS.

J. Doussot, A. Guy, R. Garreau, A. Falguières and C. Ferroud, *Tetrahedron: Asymmetry*, 2000, **11**, 2259.

Oxidative resolution of secondary alcohols

Oxido/Reductase

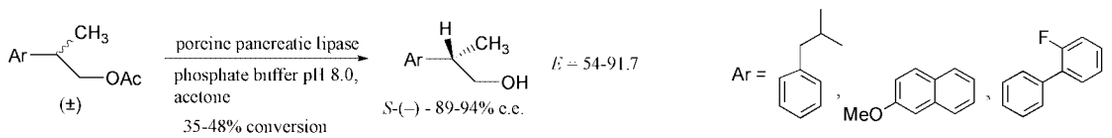


G. Fantin, M. Fogagnolo, A. Medici, P. Pedrini and S. Fontana, *Tetrahedron: Asymmetry*, 2000, **11**, 2367.

Oxidation of 8 cyclic and acyclic racemic secondary alcohols with ten *Y. lipolytica* strains has been studied. The corresponding reduction of the ketone was also investigated. Comparison of the enantio- preference of the oxidation and reduction suggests the presence of two alcohol dehydrogenases with opposite enantioselectivity.

Chemoenzymatic synthesis of chiral anti-inflammatory drugs

Lipase

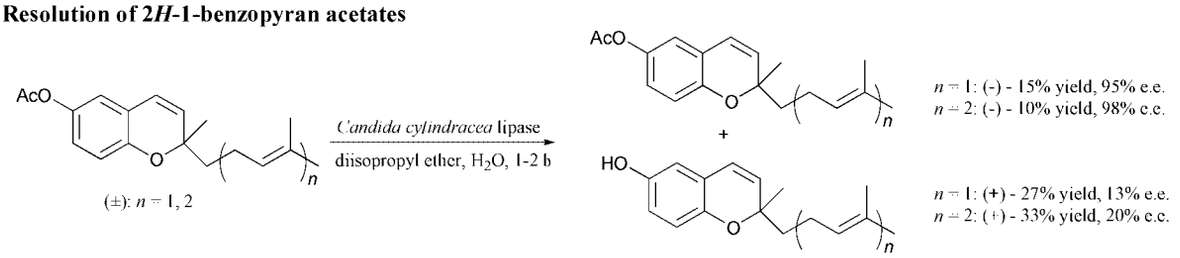


A. Basak, A. Nag, G. Bhattacharya, S. Mandal and S. Nag, *Tetrahedron: Asymmetry*, 2000, **11**, 2403.

An alternative route to chiral anti-inflammatory drugs, Ibuprofen, Naproxen and Flurbiprofen is reported. Absolute configuration of the alcohols determined by comparison with literature values.

Resolution of 2*H*-1-benzopyran acetates

Lipase

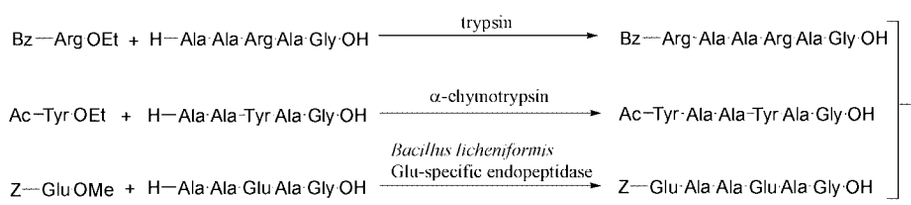


J. Y. Goujon, F. Zammattio and B. Kirschleger, *Tetrahedron: Asymmetry*, 2000, **11**, 2409.

Porcine pancreatic and *Candida rugosa* lipases gave reduced efficiency for the substrate.

Enzymatic coupling of highly specific amino acid-containing peptide fragments

Protease



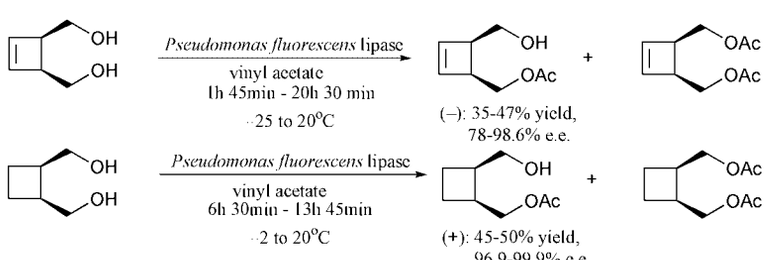
Conditions:
(a) at 25°C; 0.1M Hepes buffer pH 8.0, 0.2M NaCl, 0.02 $CaCl_2$
(b) at –15°C; distilled water pH 8.0 before freezing

N. Wehofsky, M. Haensler, S. W. Kirbach, J.-D. Wissmann and F. Bordusa, *Tetrahedron: Asymmetry*, 2000, **11**, 2421.

The effect of freezing on the enzymatic coupling of highly specific amino acid-containing peptide fragments was investigated. Freezing efficiently represses the cleavage of specific peptide bonds independent of their individual localisation and specificity achieving irreversible and efficient peptide bond formation without proteolytic side reactions.

Enzymatic acylation of cyclobutene and cyclobutane *meso*-diols

Lipase

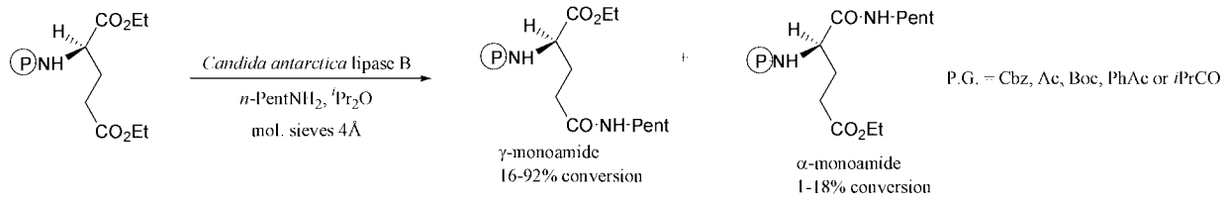


C. Pichon, C. Hubert, C. Alexandre and F. Huet, *Tetrahedron: Asymmetry*, 2000, **11**, 2429.

For both cyclobutene and cyclobutane, yields and e.e.s were significantly increased when enzymatic acylations were run below room temperature. Enzyme recycling is possible.

Regioselective enzymatic γ-monoamidation of D-glutamic acid diesters

Lipase

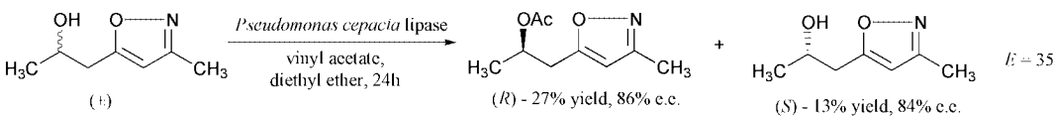


S. Conde, P. López-Serrano and A. Martínez, *Tetrahedron: Asymmetry*, 2000, **11**, 2537.

Regioselectivity depends on the enantiomer and the *N*-protecting group used: α-monoamides are exclusively obtained in the L-series. In the D-series, γ-monoamides are always the main product with γ:α ranging from 4.5 (Ⓟ = Cbz) to 35 (Ⓟ = isobutyryl). No diamides were detected.

Resolution of β-hydroxyisoxazoles

Lipase

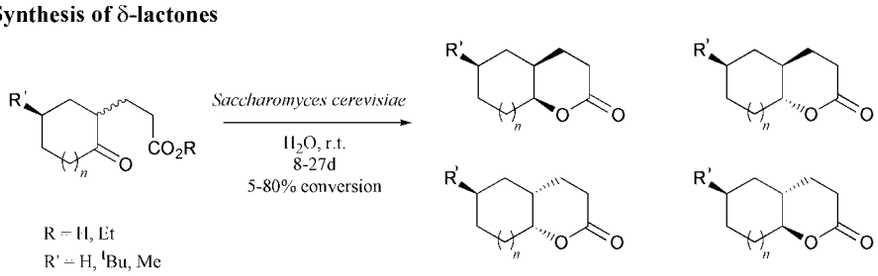


J. A. Fuentes, A. Maestro, A. M. Testera and J. M. Báñez, *Tetrahedron: Asymmetry*, 2000, **11**, 2565.

Various enzymes, acylating agents and solvents were studied with respect to the influence on the enantioselectivity of the reaction. The transesterification of a (±)-2-*p*-tolylethyl derivative was also studied using both Lipomod AC ($E = 110$) and *Candida antarctica* lipase ($E = 50$).

Synthesis of δ-lactones

Saccharomyces cerevisiae



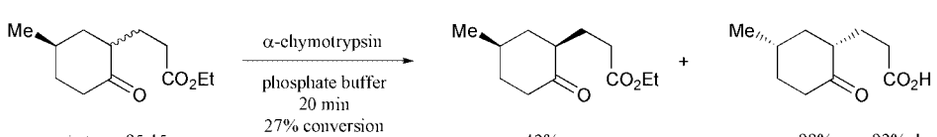
R = H, Et
R' = H, ^{t}Bu, Me
n = 1, 2

E. Fogal, C. Forzato, P. Nitti, G. Pitacco and E. Valentin, *Tetrahedron: Asymmetry*, 2000, **11**, 2599.

In general α -keto acids are less efficient than α -keto esters, with regards to conversion, e.e. and d.e.. The reduction by *Saccharomyces cerevisiae* often results in diastereomeric mixtures, especially when starting with diastereomers.

Resolution of α-ketoesters

Protease

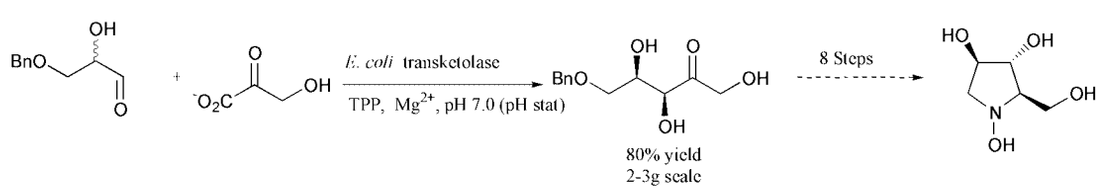


E. Fogal, C. Forzato, P. Nitti, G. Pitacco and E. Valentin, *Tetrahedron: Asymmetry*, 2000, **11**, 2599.

Other enzymes were screened with varying success *e.g. Pseudomonas fluroscens* lipase, porcine pancreatic lipase.

Synthesis of a novel *N*-hydroxypyrrolidine

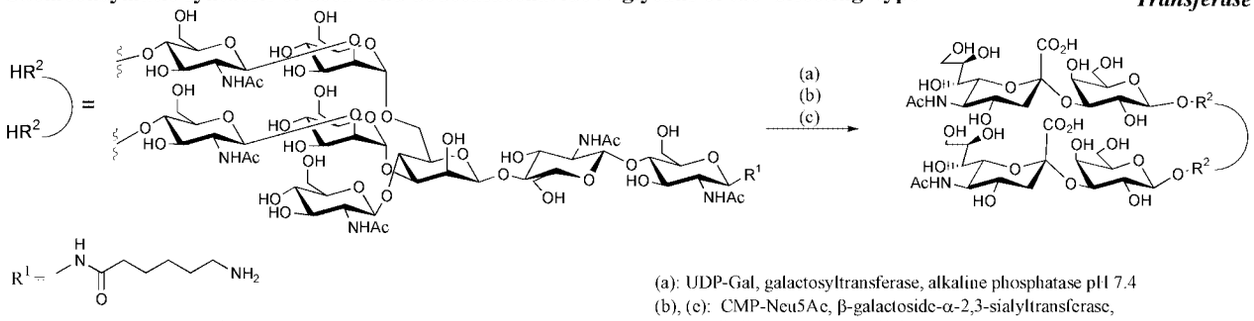
Transketolase



A. J. Humphrey, S. F. Parsons, M. E. B. Smith and N. J. Turner, *Tetrahedron Lett.*, 2000, **41**, 4481.

A novel *N*-hydroxypyrrolidine has been prepared using transketolase catalysed C-C bond formation in the key step.

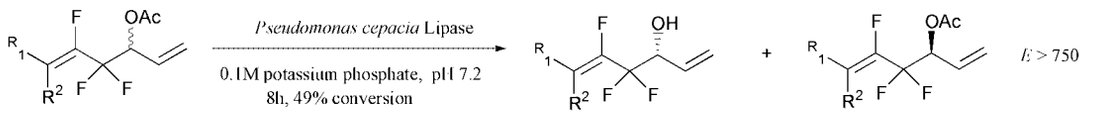
Chemoenzymatic synthesis of deca- and dodecasaccharide *N*-glycans of the 'bisecting' type ***Transferase***



(a): UDP-Gal, galactosyltransferase, alkaline phosphatase pH 7.4
(b), (c): CMP-Neu5Ac, β-galactoside-α-2,3-sialyltransferase, alkaline phosphatase pH 6.5

C. Unverzagt and J. Seifert, *Tetrahedron Lett.*, 2000, **41**, 4549.

Synthesis of optically active partly *gem*-difluorinated allylic alcohols ***Lipase***



Where R^1,R^2 = Et >99% e.e. (38% yield) Where R^1,R^2 = Et 94% e.e. (42% yield)

T. Itoh, K. Kudo, N. Tanaka, K. Sakabe, Y. Takagi and H. Kihara, *Tetrahedron Lett.*, 2000, **41**, 4591.

The 4,4,5-trifluoroalk-1,5-dien-3-ols were formed by [2,3]-Wittig rearrangement of 1,1,2-trifluoroallylic ethers. Four commerically available lipases were screened with *E* values ranging from 2 to >750.