ABSTRACTS PERKIN 1

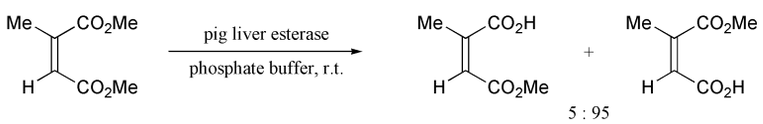
Perkin 1 Abstracts: Biocatalysis in Organic Synthesis

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.

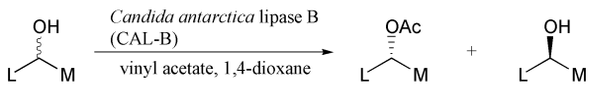
Regioselective hydrolysis of (*Z*)- and (*E*)- diesters ***Lipase***



Ethyl and propyl esters, and the (*E*)-isomer in each case were also tested. High selectivity was observed for the (*Z*)-isomers (with the least substituted environment preferred), although there was a large variation depending on the alcohol part. For the (*E*)-isomers regioselectivity was relatively low (typically 60:40) and there was little variation with the alcohol part.

R. Schmid, V. Partali, T. Anthonsen, H. W. Anthonsen and L. Kvittingen, *Tetrahedron Lett.*, 2001, **42**, 8543.

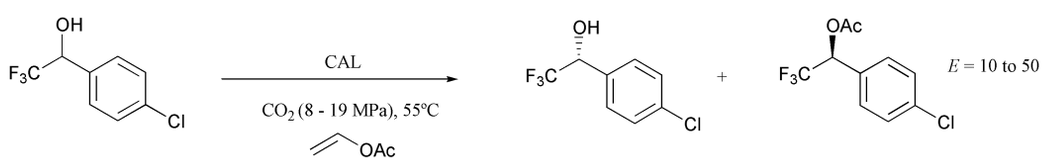
Enantioselective acetylation of α-substituted α-propylmethanols and α-substituted benzyl alcohols ***Lipase***



Initial study concerned the effect of the variation of L, with M = propyl. 5 substrates, all with six carbon groups as the L substituent were tested. Higher reaction rates were observed for those with no branching at the α- or β-positions, but higher e.e.'s were achieved with the more conformationally rigid groups (Ph and particularly cyclohexyl). The second study used fixed l (Ph) and varied M (*n*-propyl, methoxymethyl allyl and cyanomethyl). No direct correlation between the size of M and the *E* was established, suggesting that steric repulsions are not the only interactions responsible for CAL-B stereoselectivity.

E. García-Urdiales, F. Rebolledo and V. Gotor, *Tetrahedron: Asymmetry*, 2001, **12**, 3047.

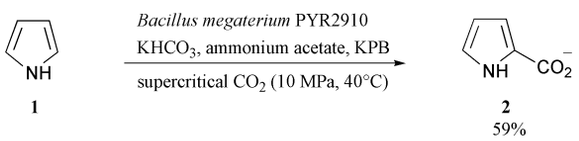
Control of enantioselectivity with pressure for lipase-catalysed esterification in supercritical carbon dioxide ***Lipase***



The enantioselectivity of esterification of **1** in supercritical carbon dioxide changed continuously from *E* = 10 to 50 by decreasing the pressure at 55°C. The effect of solvent on enantioselectivity was examined without changing the kind of solvent.

T. Matsuda, R. Kanamaru, K. Watanabe, T. Haradu and K. Nakamura, *Tetrahedron Lett.*, 2001, **42**, 8319.

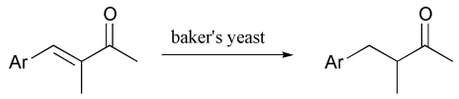
Conversion of pyrrole to pyrrole-2-carboxylate ***Bacillus megaterium***



A study of the use of cells of *Bacillus megaterium* PYR2910 for CO_2 fixation is reported. Pyrrole **1**, was converted to pyrrole-2-carboxylate **2**, in supercritical CO_2 using these cells and the yield of the carboxylation reaction in this medium was 12 times higher than under atmospheric pressure.

T. Matsuda, Y. Ohashi, T. Harada, R. Yanagihara, T. Nagasawa and K. Nakamura, *Chem. Commun.*, 2001, 2194.

Asymmetric reduction of enones ***Baker's yeast***

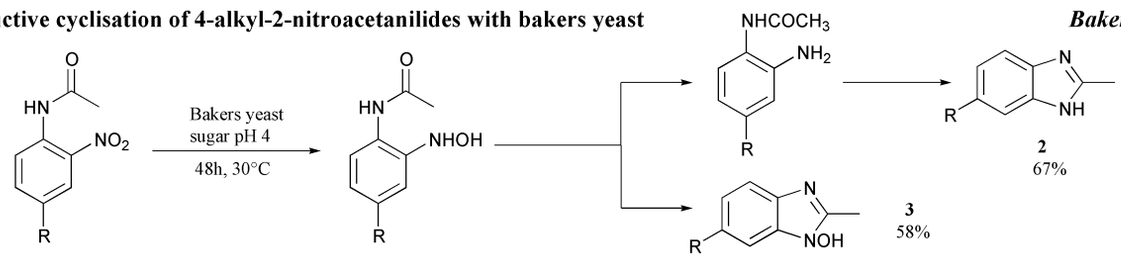


Ar = 2, 3 or 4-Py,
and *N*-O-2, 3 or 4-Py

Six enones were tested to determine the effect of electrostatic interactions on stereoselectivity. Only the carbon-carbon double bond was reduced. E.e.'s ranged from 65 - >95% with *S* configuration in all cases except Ar = *N*-O-2-Py. Highest e.e's were observed for substrates with the heteroatom in the *meta* position. The screen was repeated with an ethyl group in place of the α-methyl group. E.e.'s were in the range 96 - 99%. The larger alkyl group fixes the substrate more effectively, resulting in the enhanced e.e.'s observed.

Y. Kawai, M. Hayashi and N. Tokitoh, *Tetrahedron: Asymmetry*, 2001, **12**, 3007.

Reductive cyclisation of 4-alkyl-2-nitroacetanilides with bakers yeast ***Bakers yeast***



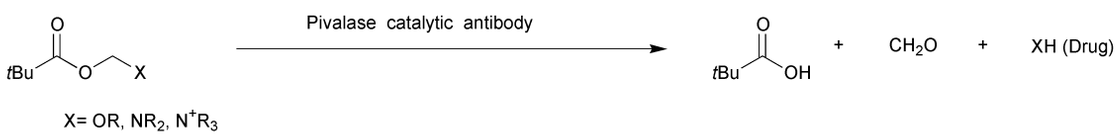
1

R = Cl, Br, NO_2, CF_3, CN

A series of substituted 2-nitroacetanilides, **1**, were regioselectively reduced by bakers yeast in acidic media to afford the corresponding 2-methylbenzimidazoles, **2**, and 6-substituted 1-hydroxy-2-methylbenzimidazoles, **3**.

A. Navarro-Ocaña, L. F. Olguín, H. Luna, M. Jiménez-Estrada and E. Bárzana, *J. Chem. Soc., Perkin Trans. 1*, 2001, 2754.

Pivalase catalytic antibodies: towards abzymatic activation of prodrugs ***Catalytic antibody***

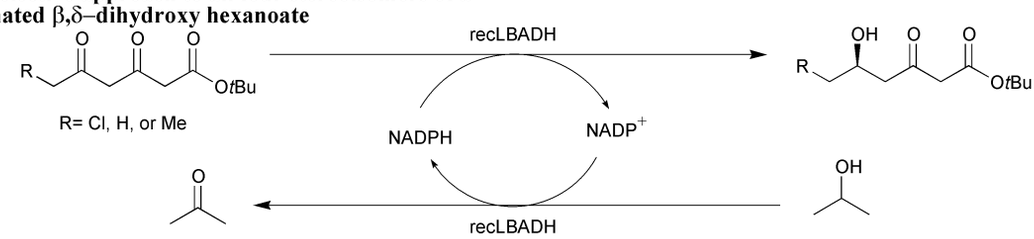


X= OR, NR_2, N^+R_3

Monoclonal antibody libraries were screened against the *tert*-butyl phosphonate hapten and the chloromethyl phosphonate hapten with pivaoyloxymethyl-umbelliferone as a fluorogenic substrate. Pivalase catalytic antibodies might be useful for activating orally available pivaloyloxymethyl prodrugs.

N. Bensel, M. T. Reymond and J. L. Reymond, *Chem. Eur. J.*, 2001, **7**, 4604.

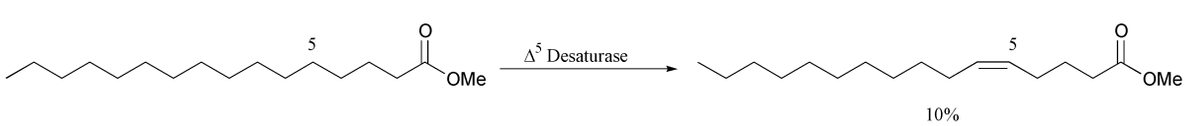
Biocatalytic reduction of β,δ–diketo esters: a highly stereoselective approach to all four stereoisomers of a chlorinated β,δ–dihydroxy hexanoate ***Dehydrogenase***



The key step of the sequence is a highly regio- and enantioselective single-site reduction of *tert*-butyl 6-chloro-3,5-dioxohexanoate by two enantiocomplementary biocatalysts.

M. Wolberg, W. Hummel and M. Müller, *Chem. Eur. J.*, 2001, **7**, 4562.

Cryptoregiochemical analysis of bacterial desaturation ***Desaturase***



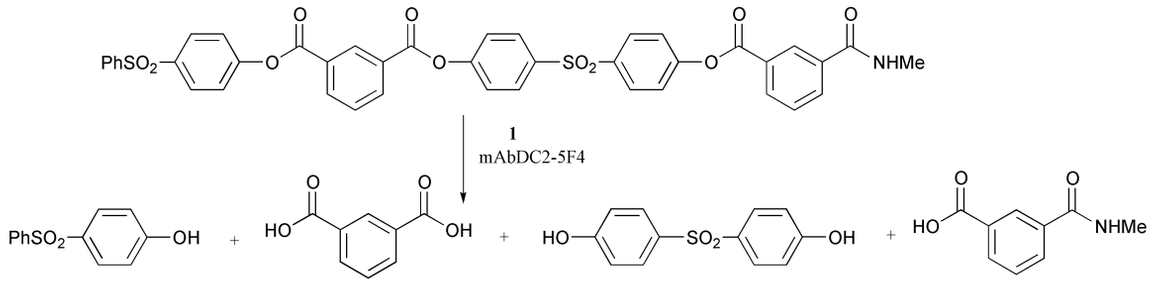
10%

A study of the cold-induced Δ^5 desaturation of long chain fatty acids in *Bacillus subtilis* is reported. Measurement of the individual primary deuterium kinetic isotope effects associated with C-H bond cleavage at C-5 and C-6 revealed C-5 as the site of initial oxidation in Δ^5 desaturation.

L. Fauconnot and P. H. Buist, *Bioorg. Med. Chem. Lett.*, 2001, **11**, 2879.

Reactive immunisation elicits catalytic antibodies for polyester hydrolysis

Esterase

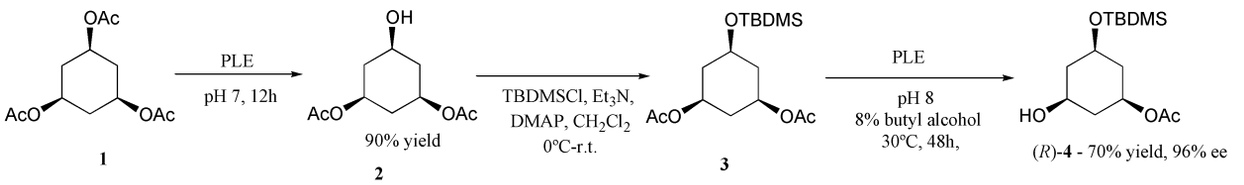


D.-W. Chen, R. J. Kubiak, J. A. Ashley and K. D. Janda, *J. Chem. Soc., Perkin Trans. 1,* 2001, 2796.

Reactive immunisation was used to produce catalytic antibodies that cleave oligomeric esters. The asymmetric triester **1**, was preferentially hydrolysed *via* an *endo*-cleavage pathway.

Chemoenzymatic synthesis of (*S*)-5-(*tert*-butyldimethylsilyloxy)-2-cyclohexenone

Esterase

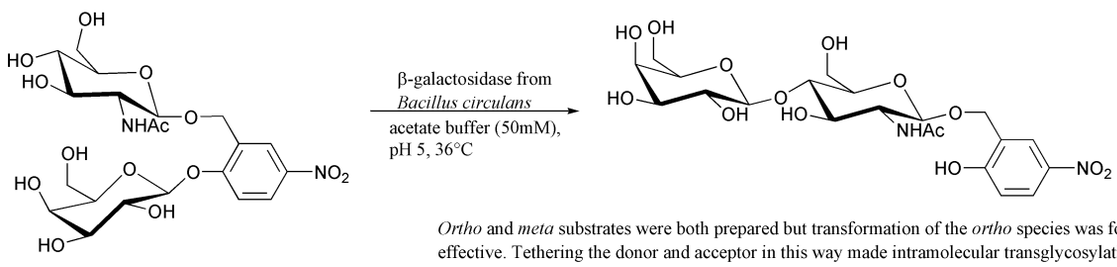


U. R. Kolkote, S. T. Ghorpade, S. P. Chavan and T. Ravindranathan, *J. Org. Chem*., 2001, **66**, 8277.

(*R*)-**4** is the key chiral intermediate in the synthesis of (*S*)-5-(*tert*-butyldimethylsilyloxy)-2-cyclohexenone and both enantiomers of chiral ketone intermediates of 19-nor-1α,25-dihydroxyvitamin D_3, a metabolite of vitamin D.

Intramolecular transglycosylation

Galactosidase

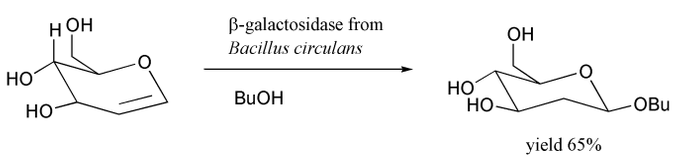


S. Komba and Y. Ito, *Tetrahedron Lett.*, 2001, **42**, 8501.

Ortho and *meta* substrates were both prepared but transformation of the *ortho* species was found to be more effective. Tethering the donor and acceptor in this way made intramolecular transglycosylation favourable, minimising the competing hydrolysis. In order to investigate the extent of post-transglycosylation hydrolysis, the galactosidase resistant product sialyl-α-(2→6)-LacNAc was obtained by adding sialyltransferase and CMP-sialic acid to the system. In this case, the yield increased to 39%.

Synthesis of 2-deoxy-β-glucosides

Glycosidase



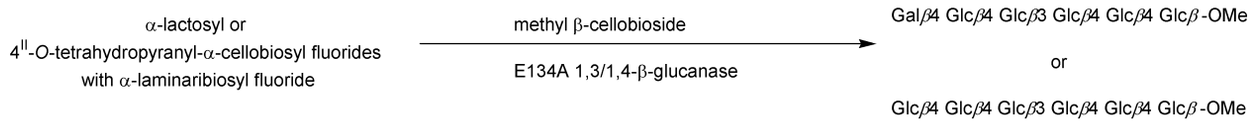
yield 65%

A. Trincone, E. Pagnotta, M. Rossi, M. Mazzone and M. Moracci, *Tetrahedron*: *Asymmetry*, 2001, **12**, 2783.

Other alcohols tested were methanol and pentan-2-ol. Furthermore, 2 pyranosidic acceptors were used, giving disaccharides. Methyl-α-D-glucopyranoside was unexpectedly β-linked *via* the O(2) and O(3) positions of the α-methyl glucoside. Mechanistic discussion based on labelling studies was reported.

Oligosaccharide synthesis by coupled *endo*-glycosynthases of different specificity

Glycosynthase

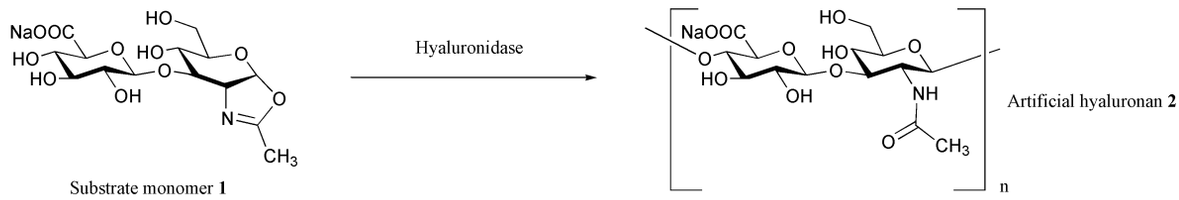


M. Faijes, J. K. Fairweather, H. Driguez and A. Planas, *Chem. Eur. J.,* 2001, **7,** 4651.

Two *endo*-glycosynthases, the E134A mutant of 1,3/1,4-β-glucanase from *Bacillus licheniformis* and the E197A mutant of cellulase Cel7B from *Humicola insolens*, were used in coupled reactions for the step-wise synthesis of hexasaccharide substrates of 1,3/1,4–β-glucanases.

Chemoenzymatic synthesis of artificial hyaluronan

Hyaluronidase



Substrate monomer **1**

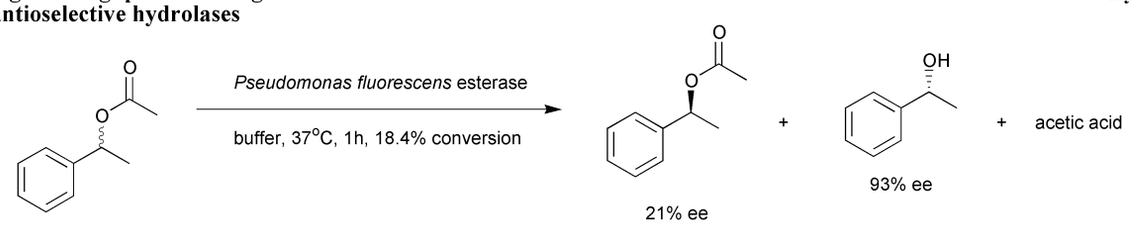
Artificial hyaluronan **2**

S. Kobayashi, H. Morii, R. Itoh, S. Kimura and M. Ohmae, *J. Am. Chem. Soc*., 2001, **123**, 11825.

A novel GlcAβ(1-3)GlcNAc disaccharide oxazoline derivative **1** was designed as a donor for *in vitro* synthesis of hyaluronan **2** using hyaluronidase from ovine testes. Polysaccharide was obtained in 52% yield. The oxazoline derivative was thought to serve as a transition state analogue substrate monomer for hyaluronidase catalysis.

A high-throughput-screening method for the identification of active and enantioselective hydrolases

Hydrolase

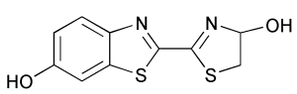


M. Baumann, R. Stürmer and U. T. Bornscheuer, *Angew. Chem., Int. Ed.*, 2001, **21,** 4201.

The hydrolase-catalysed reaction releases acetic acid which is stoichiometrically "transformed" into NADH which is quantified by UV measurement at 340nm.The enantioselectivity of the hydrolase was determined by GC analysis.

Identification of enzyme produced firefly oxyluciferin

Luciferase



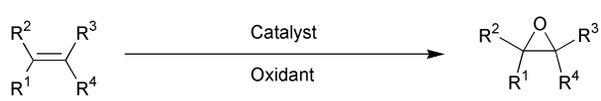
1

J. C. G. Esteves de Silva, J. M. C. S. Magalhães and R. Fontes, *Tetrahedron Lett*., 2001, **42**, 8173.

Firefly oxyluciferin **1** was enzymatically synthesised by firefly luciferases from firefly luciferin and ATP followed by oxidation of the enzyme bound adenylyl intermediate with release of AMP, CO_2 and oxyluciferin **1**. **1** was characterised by 13C and 1H NMR, UV-vis spectrometry and RP-HPLC using different pH elution conditions.

Artifical enzymes formed through directed assembly of molecular square encapsulated epoxidation catalysts

Oxidase

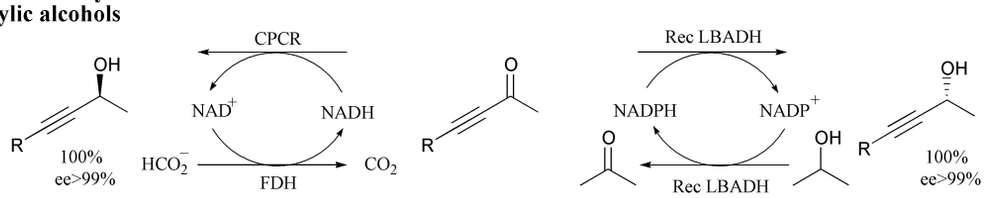


M. L. Merlau, M. del Pilar Mejia, S. T. Nguyen and J. T. Hup, *Angew. Chem., Int. Ed.,* 2001, **40,** 4239.

A manganese porphyrin was incorporated into a protein superstructure using a directed-assembly approach. Styrene epoxidation was increased ten fold following complex assembly. The catalysts lifetime was also extended from ten minutes to over three hours.

Enantioselective synthesis of both enantiomers of various propargylic alcohols

Oxidoreductase



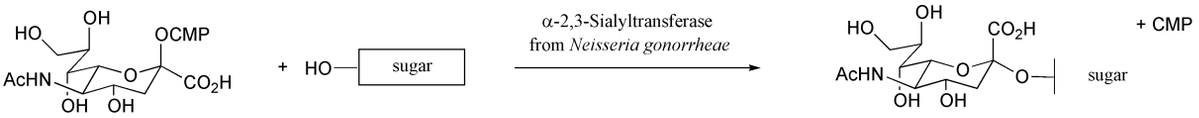
R = C_6H_5, 4-MeO-C_6H_4, 4-F-C_6H_4, 3-Br-C_6H_4

T. Schubert, W. Hummel, M.R. Kula and M. Müller, *Eur. J. Org. Chem.,* 2001, 4181.

A number of acetylenic carbonyl groups are reduced to the corresponding propargylic alcohols with high yield and excellent enantiomeric excess. These reactions are catalysed by the oxidoreductases *Lactobacillus brevis* alcohol dehydrogenase and *Candida parapsilosis* carbonyl reductase.

α-2,3-Sialyltransferase from *Neisseria gonorrheae*

α-2,3-Sialyltransferase



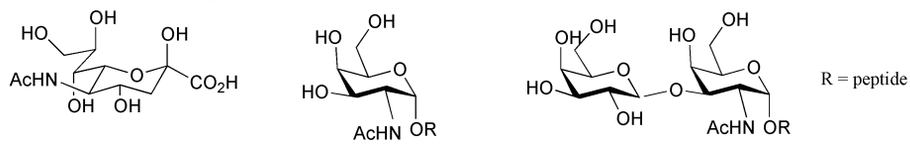
CMP = cytidine monophosphate

M. Izumi, G.-J. Shen, S. Wacowich-Sgarbi, T. Nakatani, O. Plettenburg and C.-H. Wong, *J. Am. Chem. Soc.*, 2001, **123**, 10909.

An extensive study of the potential of recombinant α-2,3-sialyltransferase (ST) from *Neisseria gonorrheae* for use in chemoenzymatic syntheses of sialosides was reported. Representative mono- and oligosaccharides, glycolipids and glycopeptides and their sulfate derivatives were synthesised and found to be acceptor substrates in preparative transformations. The enzyme form *N. gonorrheae* has the widest acceptor substrate specificity of of any ST yet reported.

Synthesis of sialylated glycopeptides

Sialyltransferase



1 **2** **3**

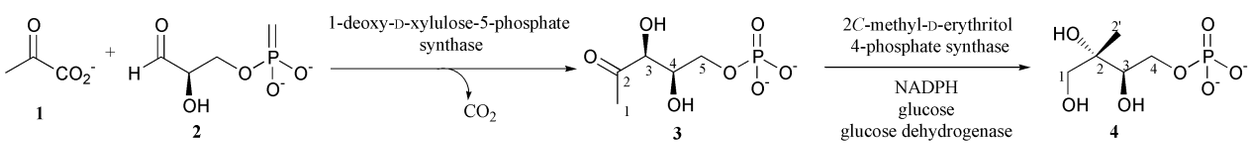
R = peptide

S. K. George, T. Schwietenk, B. Holm, C. A. Reis, H. Clausen and J. Kihlberg, *J. Am. Chem. Soc.*, 2001, **123**, 11117.

Sialic acid **1** was transferred from CMP-sialic acid to acceptor substrates **2** and **3** using α-2,6-sialyltransferase (ST6GalNAc-I) and α-2,3-sialyltransferase (ST3Gal-I) respectively to yield regioselectively sialylated glycopeptides in yields up to 74%. In addition, substrate **3** was transformed to its β-1,6- *N*-acetylglucosamine derivative using *N*-acetylglucosaminyltransferase. Products are representative of Tn and sialyl Tn tumor associated carbohydrate antigens.

Isotope-labelled 2*C*-methyl-D-erythritol 4-phosphate

Synthase



1 **2** **3** **4**

S. Hecht, J. Wungsintaweekul, F. Rohdich, K. Kis, T. Radykewicz, C. A. Schuhr, W. Eisenreich, G. Richter and A. Bacher, *J. Org. Chem.*, 2001, **66**, 7770.

Five biotransformation strategies were optimised for different isotope labelling patterns. ^{13}C and ^{14}C were introduced using D-glyceraldehyde 3-phosphate **2** prepared from dihydroxyacetone phosphate or glucose as the starting material. ^{3}H was incorporated into the 1*R* position of **4** using 1-deoxy-D-xylulose 5-phosphate **3** as the starting material.